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For Period Ending February 15, 1963

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director

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PUBLICATIONS

A total of 334 papers, abstracts, and book reviews by members of the Biology Division have been published during this report period or are in press. This figure compares with 337 for the same period in 1962 and with 357 for the August 1962 report period. The 81 published papers and 3 book reviews are listed below. The 50 published abstracts and the 200 items in press are not listed.

The proceedings of the 1962 Gatlinburg Symposium, *Specificity of Cell Differentiation and Interaction*, were published as a supplement to the October 1962 *Journal of Cellular and Comparative Physiology*.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	Analytical techniques for cell fractions. II. A spectrophotometric column monitoring system	*Anal. Biochem.* **4,** 269–83 (1962)
	The zonal ultracentrifuge: A new instrument for fractionating mixtures of particles	*J. Phys. Chem.* **66,** 1984–89 (1962)
Bender, M. A, and P. E. Eide	Separation of monkey (Ateles) leukocytes for culture	*Mammalian Chromosomes News Letter* **8,** 3 (1962)
Bender, M. A, P. Carolyn Gooch, and D. M. Prescott	Aberrations induced in human leukoctye chromosomes by ^{3}H-labeled nucleosides	*Cytogenetics* **1,** 65–74 (1962)
Bender, M. A, and D. M. Prescott	DNA synthesis and mitosis in cultures of human peripheral leukocytes	*Exptl. Cell Res.* **27,** 221–29 (1962)
Benedict, W. H.	Development of x ray-induced lamellar cataract in the newborn mouse in relation to age at time of irradiation	*Trans. Am. Ophthalmol. Soc.* **60,** 373–407 (1962)
Bertsch, W. F.	Two photoreactions in photosynthesis: Evidence from the delayed light emission of *Chlorella*	*Proc. Natl. Acad. Sci. U.S.* **48,** 2000–2004 (1962)
Bezkorovainy, Anatoly, and D. G. Doherty	Comparison of bovine and human orosomucoids	*Nature* **195,** 1003 (1962)
Bond, H. E.	A sex-associated protein in liver tissue of the rat and its response to endocrine manipulation	*Nature* **196,** 242–44 (1962)
Boyce, R. P., and R. B. Setlow	A simple method of increasing the incorporation of thymidine into the deoxyribonucleic acid of *Escherichia coli*	*Biochim. et Biophys. Acta* **61,** 618–20 (1962)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Brockman, H. E.	Viability of *Neurospora crassa* ascospores after heat inactivation	*Neurospora Newsletter* **2**, 16–18 (1962)
Celada, Franco, and Takashi Makinodan	Radiosensitivity of homograft-rejecting and antibody-synthesizing capacities of isolated cells: Its significance to tolerance	pp 157–62 in *Mechanisms of Immunological Tolerance, Proceedings of Symposium Held at Liblice Near Prague, November 8–10, 1961* (ed. by M. Hašek, A. Lengerová, and M. Vojtíšková), publishing house of the Czechoslovak Academy of Sciences, Prague, 1962
Chu, E. H. Y., and M. A Bender	Cytogenetics and evolution of primates	*Ann. N.Y. Acad. Sci.* **102**, 253–66 (1962)
Clayton, R. K.	Evidence for the photochemical reduction on coenzyme Q in chromatophores of photosynthetic bacteria	*Biochem. Biophys. Research Communs.* **9**, 49–53 (1962)
	Primary reactions in bacterial photosynthesis. I. The nature of light-induced absorbancy changes in chromatophores; evidence for a special bacteriochlorophyll component	*Photochem. Photobiol.* **1**, 201–10 (1962)
	Primary reactions in bacterial photosynthesis. II. The quantum requirement for bacteriochlorophyll conversion in the chromatophore	*Photochem. Photobiol.* **1**, 305–11 (1962)
	Primary reactions in bacterial photosynthesis. III. Reactions of carotenoids and cytochromes in illuminated bacterial chromatophores	*Photochem. Photobiol.* **1**, 313–23 (1962)
Clayton, R. K., and P. S. Rudolph	The lethal effects of alpha irradiation on wild-type and on a high-catalase mutant of *Rhodopseudomonas spheroides*	*Radiation Res.* **17**, 839–46 (1962)
Congdon, C. C	*Diagnosis and Treatment of Acute Radiation Injury. Proceedings of a Scientific Meeting Jointly Sponsored by the International Atomic Energy Agency and the World Health Organization, Geneva, October 17–21, 1960,* International Documents Service (a division of Columbia University Press), New York, 1961, 425 pp, illustrations	*Science* **137**, 123–24 (1962) (book review)
	Remarks in round-table discussion: Embryological development of the immune system	*J. Cellular Comp. Physiol.* **60** (Suppl. 1), 159–72 (1962)
de Serres, F. J.	Heterokaryon-incompatibility factor interaction in tests between Neurospora mutants	*Science* **138**, 1342–43 (1962)
de Serres, F. J., and Rosalie S. Osterbind	Estimation of the relative frequencies of x-ray-induced viable and recessive lethal mutations in the *ad-3* region of *Neurospora crassa*	*Genetics* **47**, 793–96 (1962)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Dolin, M. I.	Effect of near-ultraviolet irradiation on the peroxide content of solutions of oxidized or reduced diphosphopyridine nucleotide	*Biochim. et Biophys. Acta* **63**, 219–21 (1962)
Doria, Gino	Homograft reaction in mouse radiation chimeras. I. Indirect evidence for graft-anti-host activity	*J. Immunol.* **89**, 453–58 (1962)
	Homograft reaction in mouse radiation chimeras. II. Direct evidence for graft-anti-host activity	*J. Immunol.* **89**, 459–62 (1962)
Duplan, J. F., and N. S. Wolf	Age-related factors which influence the value of the mouse embryo for post-irradiation restoration of the adult	*Intern. J. Radiation Biol.* **5**, 597–607 (1962)
Ehling, U. H., and D. G. Doherty	AET protection of the reproductive capacity of irradiated mice	*Proc. Soc. Exptl. Biol. Med.* **110**, 493–94 (1962)
Ehling, U. H., and M. L. Randolph	Skeletal abnormalities in the F_1-generation of mice exposed to ionizing radiations	*Genetics* **47**, 1543–55 (1962)
Finamore, F. J., and A. H. Warner	The occurrence of P^1P^4 diguanosine 5′-tetraphosphate in brine shrimp eggs	*J. Biol. Chem.* **238**, 344–48 (1963)
Ginsberg, D. M., and John Jagger	Possible errors arising from the use of fritted-glass filters for bubbling of cell suspensions, especially in irradiation experiments	*J. Bacteriol.* **83**, 1361–62 (1962)
Grell, E. H.	Melanogaster — New Mutants (nw^D)	*Drosophila Info. Serv.* **36**, 37 (1962)
Grell, Rhoda F.	A new model for secondary nondisjunction: The role of distributive pairing	*Genetics* **47**, 1737–54 (1962)
Grell, Rhoda F., and E. H. Grell	A correction to the cytology of the rearrangement associated with Glazed	*Drosophila Info. Serv.* **36**, 71 (1962)
Haber, A. H.	Morphogenesis and the cell theory	pp 79–88 in *Plant Tissue Culture and Morphogenesis* (ed. by J. C. O'Kelley), Scholar's Library, New York
Hollaender, Alexander	Symposium on Specificity of Cell Differentiation and Interaction — Introduction	*J. Cellular Comp. Physiol.* **60** (Suppl. 1), 211 pages, October 1962
Hollaender, Alexander, and S. F. Carson	Biology Division Semiannual Progress Report for Period Ending August 15, 1962	ORNL-3352
Jacobson, K. B.	Ribonucleotides of RNA: Separation by chromatography on sheets of diethylaminoethylcellulose	*Science* **138**, 515–16 (1962)
Jagger, John	Photoreactivation	pp 406–07 in *McGraw-Hill Yearbook of Science and Technology: 1962* (W. H. Crouse, Editor-in-Chief), McGraw-Hill, New York, 1962
Jagger, John, and R. S. Stafford	Biological and physical ranges of photoprotection from ultraviolet damage in microorganisms	*Photochem. Photobiol.* **1**, 245–57 (1962)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Kenney, F. T.	Induction of tyrosine-α-ketoglutarate transaminase in rat liver. IV. Evidence for an increase in the rate of enzyme synthesis	*J. Biol. Chem.* **237**, 3495–98 (1962)
Kimball, R. F.	Chromosome duplication and mutation	pp 116–22 in *Strahlenwirkung und Milieu (Radiation Effect and Milieu), Internationales Radiobiologisches Symposion in Montreux, 28 May–3 June 1961* (ed. by H. Fritz-Niggli), Verlag von Urban & Schwarzenberg, Munich-Berlin, 1962 (Supplement to Strahlentherapie)
Kimball, R. F., and Stella W. Perdue	Quantitative cytochemical studies on *Paramecium.* V. Autoradiographic studies of nucleic acid syntheses	*Exptl. Cell Res.* **27**, 405–15 (1962)
	Studies on the refractory period for the induction of recessive lethal mutation by x rays in Paramecium	*Genetics* **47**, 1595–1607 (1962)
Kimball, R. F., and Lore Vogt-Köhne	Effects of radiation on cell and nuclear growth in *Paramecium aurelia*	*Exptl. Cell Res.* **28**, 228–38 (1962)
Kirby-Smith, J. S.	*Radiobiology – Proceedings of the Third Australasian Conference on Radiobiology Held at the University, Sydney, 15–18 August, 1960 by the Australian Radiation Society* (ed. by P. L. T. Ilbery), Butterworths, London, 1961, xi, 315 pp, illustrations	*Science* **137**, 219 (1962) (book review)
Löbbecke, E.-A., and R. C. von Borstel	Mutational response of Habrobracon oocytes in metaphase and prophase to ethyl methanesulfonate and nitrogen mustard	*Genetics* **47**, 853–64 (1962)
Magni, G. E., and R. C. von Borstel	Different rates of spontaneous mutation during mitosis and meiosis in yeast	*Genetics* **47**, 1097–1108 (1962)
Makinodan, Takashi, and J. F. Albright	Cellular variation during immune response; one possible model of cellular differentiation	*J. Cellular Comp. Physiol.* **60** (Suppl. 1), 129–44 (1962)
Makinodan, Takashi, Franco Celada, and E. E. Capalbo	An immunological approach to the study of radiation-induced blood chimeras	In *Proceedings of the VIIIth International Congress of Hematology, Tokyo, September 4–10, 1960, vol 1, Nucleonics and Leukemia* (ch.-ed.-comm., Seizo Katsunuma), pp 155–61, Pan-Pacific Press, Tokyo, 1962
Mazur, Peter	*Biological Effects of Freezing and Supercooling*, by Audrey U. Smith (Monograph No. 9 of the Physiological Society), Williams & Wilkins, Baltimore, 1961, 462 pp	*Am. Scientist* **50**, 418A (1962) (book review)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Monesi, Valerio	Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis, using tritiated thymidine	*J. Cell Biol.* **14**, 1–18 (1962)
	Relation between x-ray sensitivity and stages of the cell cycle in spermatogonia of the mouse	*Radiation Res.* **17**, 809–38 (1962)
Novelli, G. D.	Mechanisms involved in the control of enzyme synthesis	pp 435–60 in *The Molecular Basis of Neoplasia* (ed. by R. W. Cumley, Marilyn Abbott, *et al.*), University of Texas Press, Austin, 1962
Oakberg, E. F.	The effect of low radiation doses on spermatogonia and oocytes of the mouse	pp 103–11 in *Strahlenwirkung und Milieu (Radiation Effect and Milieu), Internationales Radiobiologisches Symposion in Montreux, 28 May–3 June 1961* (ed. by H. Fritz-Niggli), Verlag von Urban & Schwarzenberg, Munich-Berlin, 1962 (Supplement to Strahlentherapie)
Peck, H. D., Jr.	Metabolism of inorganic sulfur compounds	p 312 in *McGraw-Hill Yearbook of Science and Technology: 1962* (W. H. Crouse, Editor-in-Chief), McGraw-Hill, New York, 1962
Popp, R. A.	Studies on the mouse hemoglobin loci. II. Position of the hemoglobin locus with respect to albinism and shaker-1 loci	*J. Heredity* **53**, 73–75 (1962)
	Studies on the mouse hemoglobin loci. III. Heterogeneity of electrophoretically indistinguishable single-type hemoglobins	*J. Heredity* **53**, 75–77 (1962)
	Studies on the mouse hemoglobin loci. IV. Independent segregation of *Hb* and *Sol*: Effect of the loci on the electrophoretic and solubility properties of hemoglobins	*J. Heredity* **53**, 77–80 (1962)
	Studies on the mouse hemoglobin loci. V. Differences among tryptic peptides of the β-chain governed by alleles at the *Hb* locus	*J. Heredity* **53**, 142–46 (1962)
	Studies on the mouse hemoglobin loci. VI. A third allele, Sol^3, at the *Sol* locus	*J. Heredity* **53**, 147–48 (1962)
	Studies on the mouse hemoglobin loci. VII. Differences among tryptic peptides of the α-chain governed by alleles at the *Sol* locus	*J. Heredity* **53**, 148–51 (1962)
Popp, R. A., and Diana M. Popp	Inheritance of serum esterases having different electrophoretic patterns among inbred strains of mice	*J. Heredity* **53**, 111–14 (1962)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Rabson, Robert, N. E. Tolbert, and P. C. Kearney	Formation of serine and glyceric acid by the glycolate pathway	*Arch. Biochem. Biophys.* **98**, 154–63 (1962)
Russell, Liane B.	Chromosome aberrations in experimental mammals	pp 230–94 in *Progress in Medical Genetics*, vol II (ed. by A. G. Steinberg and A. G. Bearn), Grune & Stratton, New York, 1962
Russell, W. L.	An augmenting effect of dose fractionation on radiation-induced mutation rate in mice	*Proc. Natl. Acad. Sci. U.S.* **48**, 1724–27 (1962)
	Genetic hazards of radiation	*Proc. Am. Phil. Soc.* **107**, 11–17 (1963)
Schwartz, Drew	Genetic studies on mutant enzymes in maize. III. Control of gene action in the synthesis of pH 7.5 esterase	*Genetics* **47**, 1609–15 (1962)
	Report for 1962 Maize Cooperation News Letter: (1) Synthesis of hybrid esterase enzymes in *E* heterozygotes, (2) Regulatory mutant at the *E* locus, (3) Two new alleles of the *E* gene found in teosinte	*Maize Genet. Coop. News Letter* **36**, 68–69 (1962)
Setlow, Jane K., and R. B. Setlow	Nature of the photoreactivable ultra-violet lesion in deoxyribonucleic acid	*Nature* **197**, 560–62 (1963)
Smith, L. H., and T. W. McKinley, Jr.	Erythrocyte survival in guinea pigs	*Proc. Soc. Exptl. Biol. Med.* **111**, 768–71 (1962)
Smith, L. H., and Otto Vos	Sensitivity and protection of mouse bone marrow cells x-irradiated *in vitro*	*Intern. J. Radiation Biol.* **5**, 461–70 (1962)
Stulberg, M. P., and G. D. Novelli	Amino acid activation	pp 401–32 in *The Enzymes*, vol 6, 2d ed. (ed. by P. D. Boyer, Henry Lardy, and Karl Myrbäck), Academic Press, New York, 1962
Upton, A. C.	Comments on Report of the United Nations Scientific Committee on the effects of atomic radiation	*Cancer Res.* **22**, 1139–41 (1962)
Volkin, Elliot	Biosynthesis of RNA in relation to genetic coding problems	pp 271–89 in *Molecular Genetics* (ed. by J. H. Taylor), Academic Press, New York, 1963
Volkin, Elliot, and Anna Ruffilli	Dissociation of macromolecular synthetic processes in T2-infected *E. coli*	*Proc. Natl. Acad. Sci. U.S.* **48**, 2193–2200 (1962)
von Borstel, R. C. (editor)	Carbondale Yeast Genetics Conference (November 16–18, 1961)	*Microbial Genetics Bulletin*, Supplement to No. 19, January 1963
Warkany, Josef, E. H. Y. Chu, and Emanuel Kauder	Male pseudohermaphroditism and chromosomal mosaicism	*Am. J. Diseases Children* **104**, 172–79 (1962)
Whiting, Anna R.	Contrasts in radiation-induced mutation rates at different meiotic stages	pp 183–95 in *Strahlenwirkung und Milieu (Radiation Effect and Milieu), Internationales Radiobiologisches Symposion in Montreux, 28 May–3 June 1961* (ed. by H. Fritz-Niggli), Verlag von Urban & Schwarzenberg, Munich-Berlin, 1962 (Supplement to Strahlentherapie)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Williams, D. B.	Sensitivity of *Spathidium spathula* to low doses of x radiation	*J. Protozool.* **9**, 119–22 (1962)
Wolff, Sheldon	Biochemical aspects of chromosome breakage and rejoining (intergenic mutations)	pp 1436–60 in *Recent Advances in Botany, Lectures and Symposia Presented to the IX International Botanical Congress, Montreal, 1959*, vol II, University of Toronto Press, Toronto, 1961
	The kinetics for two-break chromosome exchanges	*J. Theoret. Biol.* **3**, 304–14 (1962)
Yamada, Tuneo	The inductive phenomenon as a tool for understanding the basic mechanism of differentiation	*J. Cellular Comp. Physiol.* **60** (Suppl. 1), 49–64 (1962)
(Members of the Biology Division)	*Mammalian Cytology and Somatic Cell Genetics – Abstracts of Conference Held November 15–17, 1962, at Gatlinburg, Tennessee* (M. A Bender and E. H. Y. Chu, co-chairmen)	Reprint No. 2304
	Report of Conference on Recent Developments in Studies of Blood Platelets	Reprint No. 2176
	Summary of Reports of Bone Marrow Conferences Organized by Members of the Biology Division, ORNL	Reprint No. 2305

LECTURES

During the reporting period August 1962 to February 1963, members of the Biology Division presented 198 lectures and speeches, compared with 135 for the corresponding period of 1962. Of these, 96 were before professional societies in the United States and abroad, 21 were given as part of the Traveling Lecture Program, and 81 were presented on invitation of universities, laboratories, and scientific organizations.

Details on speaking activities outside the continental United States will be found in the section "Foreign Travel." Educational activities are described later (see p 25).

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Adler, H. I.	Genetic analysis of radiation and sensitivity in *Escherichia coli*	1. Columbia University, New York 2. Yale University School of Medicine, New Haven, Conn.
	Radiation microbiology	1. David Lipscomb College, Nashville, Tenn. 2. George Peabody College for Teachers, Nashville, Tenn. 3. University of Chattanooga, Chattanooga, Tenn.
	Some aspects of microbial genetics	University of Chattanooga, Chattanooga, Tenn.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Adler, H. I.	Space biology	1. David Lipscomb College, Nashville, Tenn. 2. George Peabody College for Teachers, Nashville, Tenn. 3. University of Chattanooga, Chattanooga, Tenn.
Albright, J. F.	Current work in radiation immunology	St. Jude Hospital, Memphis, Tenn.
Albright, J. F. (E. E. Capalbo, and Takashi Makinodan)	Some quantitative properties of antibody-producing cells	IX International Congress of Hematology, Symposium on Cytokinetics, Mexico City, Mexico
Anderson, N. G.	An approach to molecular pathology	National Arthritis Institute, Bethesda, Md.
	Basic mechanisms in cell division	Gordon Research Conference on Cancer, New London, N.H.
	Integrated bioanalytical systems	National Food and Drug Administration, Washington, D.C.
	Molecular scans of cells and tissues	Florida State University, Tallahassee
	Theory and operation of the zonal ultracentrifuge	National Cancer Institute, Bethesda, Md.
	Viral isolation in zonal ultracentrifuges	Lilly Research Laboratories, Indianapolis, Ind.
	Virus isolation in the zonal ultracentrifuge	National Institute of Infectious Diseases, Bethesda, Md.
	The zonal ultracentrifuge — a new basic biophysical tool	UNESCO Symposium on Amplification Techniques in Cell Biology, University of Buffalo, Buffalo, N.Y.
Anderson, N. G. (P. Barringer, R. E. Canning, C. L. Burger, and C. T. Rankin)	Banding of DNA and viruses in a new zonal ultracentrifuge	Biophysical Society, New York
Barnett, W. E. (and F. J. de Serres)	Evidence for nitrous acid—induced nontransition mutation [*Genetics* **47**, 941 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Barnett, W. E. (C. J. Wust, and G. D. Novelli)	Amino acid incorporation in *Neurospora* [*The Branched Chain* (program and abstr. issue) **18**, 45 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.
Bell, Sandra (and Sheldon Wolff)	Studies of the mechanism of the effect of FUdR on chromosomes	Am. Soc. Cell Biol., San Francisco, Calif.
Bender, M. A	Chromosome breakage *in vitro*	Symposium on Mammalian Tissue Culture and Cytology, São Paulo, Brazil
	Discussion following paper "Chromosomal Aberrations," by C. E. Ford	New York Academy of Sciences Conference on Physical Factors Modifying Response to Radiation, New York

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Bender, M. A (and P. E. Eide)	The karyotype of the wooley monkey (*Lagothrix*) [*Mammalian Chromosomes News Letter* **9**, 40 (1963)]	Conference on Mammalian Cytology and Somatic Cell Genetics, Gatlinburg, Tenn.
Bender, M. A (and P. Carolyn Gooch)	Somatic chromosome aberration analyses in cases of accidental whole-body irradiation [*Mammalian Chromosomes News Letter* **9**, 41 (1963)]	*Ibid.*
Bollum, F. J.	Enzymic replication of DNA	American Chemical Society, SE Regional Meeting, Symposium on the Biosynthesis of DNA, RNA, and Protein, Gatlinburg, Tenn.
	Enzymic synthesis of DNA (2 lectures)	University of Chicago, Chicago, Ill.
	Nature of DNA-polymerase products	Case Institute of Technology, Cleveland, Ohio
Bertsch, W. F.	Delayed light emission of photosynthetic organisms	1. Carnegie Institution of Washington, Stanford, Calif. 2. University of California, Los Angeles 3. Scripps Institution of Oceanography, University of California, La Jolla
	Delayed light emission of plants	University of Tennessee, Knoxville
	Solid state phenomena and photosynthesis	Reed College, Portland, Ore.
Carson, S. F.	1. Biotin and B_{12} coenzyme catalyzed reactions in the interconversion of propionate and succinate: Propionyl carboxylase and the transcarboxylases 2. Biotin and B_{12} coenzyme catalyzed reactions in the interconversion of propionate and succinate: Methylmalonyl isomerase and racemose	University of Georgia, Athens
Cattanach, B. M.	Chemical mutagenesis in mice and tests of the inactivated-X hypothesis	1. California Institute of Technology, Pasadena 2. City of Hope Medical Center, Durate, Calif.
Chu, E. H. Y.	Abnormalities of sex chromosomes	Symposium on Mammalian Tissue Culture and Cytology, São Paulo, Brazil
	Mammalian chromosome cytology	AIBS, Am. Soc. Zool., Corvallis, Ore.
	Mammalian cytogenetics	University of Chile, Santiago
	Photoreactivation and action spectrum of ultraviolet-induced mammalian chromosome aberrations [*Genetics* **47**, 948 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
	Ultraviolet-induced aberrations of mammalian chromosomes [*Mammalian Chromosomes News Letter* **9**, 46 (1963)]	Conference on Mammalian Cytology and Somatic Cell Genetics, Gatlinburg, Tenn.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Cohn, W. E (Keiichi Kusama, L. O. Frøholm, D. M. Prescott, and W. E Cohn)	Pseudouridine metabolism in *Tetrahymena* [*Abstr. of Papers, 142nd Mtg., Am. Chem. Soc (1962)*, pp 39C–40C]	142nd Meeting, American Chemical Society, Atlantic City, N.J.
Congdon, C. C	Biologic mechanisms of protection against radiation	International Congress of Dermatology, Symposium on Radiobiology, Washington, D.C.
Congdon, C. C (Joan W. Goodman, and J. W. Ferrebee)	The thymus in radiation chimeras	Conference on Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection, New York
Cudkowicz, Gustavo	A non-codominant histocompatibility factor in the C57BL mouse	Roswell Park Memorial Institute, Buffalo, N.Y.
	A recessive histocompatibility factor in the mouse	University of Milan, Milan, Italy
	Specific histocompatibility requirements for cellular grafts in the mouse	Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.
Cudkowicz, Gustavo (A. C. Upton, L. H. Smith, W. L. Hughes, and M. A. Kastenbaum)	An approach to the characterization of stem cells in the mouse bone marrow	Conference on Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection, New York
Dekaban, A. S. (and M. A Bender)	Chromosome studies in mongoloids and their families [*Mammalian Chromosomes News Letter* **9**, 50 (1963)]	Conference on Mammalian Cytology and Somatic Cell Genetics, Gatlinburg, Tenn.
de Serres, F. J.	Factors influencing recombination and chromosome interference in *Neurospora* [*Genetics* **47**, 950 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
de Serres, F. J. (Giorgio Morpurgo, and F. J. de Serres)	Factors influencing allelic complementation among *ad-3B* mutants of *Neurospora* [*Genetics* **47**, 972–73 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Doherty, D. G.	Chemical protection against ionizing radiation	1. Am. Soc. Pharmacol. and Exptl. Therapeutics, Symposium on Radiation and Radioisotopes in Pharmacology, Nashville, Tenn. 2. MEND Lecture, University of Virginia School of Medicine, Charlottesville
Doherty, D. G. (and J. A. Duke)	Pepsin catalyzed hydrolysis of synthetic substrates [*The Branched Chain* (program and abstr. issue) **18**, 41–42 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.
Doria, Gino	Studies on the ontogeny of transplantation antigens in mice	Conference on Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection, New York
Finamore, F. J.	A new pyrophosphate ester in brine shrimp eggs	Louisiana State University, New Orleans

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Fritz, P. J. (and K. B. Jacobson)	Studies on lactic dehydrogenase isozymes [*The Branched Chain* (program and abstr. issue) **18**, 42 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.
Glassman, E. (and S. F. Carson)	Production of xanthine dehydrogenase activity in combined extracts of mutants of *Drosophila melanogaster* deficient in this enzyme [*The Branched Chain* (program and abstr. issue) **18**, 42 (1962)]	*Ibid.*
P. Carolyn Gooch (and M. A Bender)	Chromosome aberrations induced by 14 Mev neutron irradiation of human blood *in vitro* [*Mammalian Chromosomes News Letter* **9**, 53 (1963)]	Conference on Mammalian Cytology and Somatic Cell Genetics, Gatlinburg, Tenn.
Goodman, Joan W.	Transplantation of peritoneal cells	Conference on Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection, New York
Grell, E. H.	The dose effect of ma-1$^+$ and ry$^+$ on xanthine dehydrogenase activity in *Drosophila melanogaster* [*Genetics* **47**, 956 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
	Gene dosage and enzyme activity in *Drosophila*	California Institute of Technology, Pasadena, Calif.
Grell, Rhoda F.	A new hypothesis for meiosis in the female of *Drosophila melanogaster*	California Institute of Technology, Pasadena, Calif.
	The role of distributive pairing in secondary nondisjunction [*Genetics* **47**, 956 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Gude, W. D.	Spontaneous glomerulosclerosis	Alpha Epsilon Delta, Western Kentucky State Teachers College, Bowling Green
Haber, A. H.	Gibberellin actions on plants and their general relevance for growth studies	University of Alabama, Tuscaloosa
	Inadequacy of the cell theory for interpreting morphogenesis	1. Rutgers University, New Brunswick, N.J. 2. University of Alabama, Tuscaloosa
	Roles of cell division in plant differentiation	Research Institute for Advanced Study, Baltimore, Md.
	Roles of cell division in plant growth and differentiation	1. Harvard University, Cambridge, Mass. 2. Brown University, Providence, R.I. 3. University of Georgia, Athens
	Roles of cell division in plant morphogenesis	Yale University, New Haven, Conn.
Haber, A. H. (and D. E. Foard)	Cell expansion, differentiation, and nuclear functions in gamma-irradiated wheat growing without tissue-cell division [*Plant Physiol.* **37** (suppl), liv (1962)]	AIBS, Am. Soc. Plant Physiol., Corvallis, Ore.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Jacobson, K. B. (and Anna-Lisa Dopirak)	RNA chromatography on DEAE-cellulose paper sheets [*The Branched Chain* (program and abstr. issue) **18,** 44 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.
Jagger, John	Light and life	1. Morehouse College, Atlanta, Ga. 2. Agnes Scott College and Oglethorpe University, Decatur, Ga.
	Photoprotection from far ultraviolet effects in cells	Pennsylvania State University, University Park
Jagger, John (and R. S. Stafford)	Biological effects of vacuum ultraviolet radiation	National Institutes of Health Symposium on Recent Developments in Research Methods and Instrumentation, Washington, D.C.
Jagger, John (W. C. Wise, and R. S. Stafford)	Initial steps in the mechanism of photoprotection in bacteria	Biophysical Society, New York
James, T. W.	Synchronization of cell growth and division	UNESCO Symposium on Amplification Techniques in Cell Biology, University of Buffalo, Buffalo, New York
Kaji, Hideko	Mechanisms of insulin action on transport through membrane	University of Pennsylvania, Philadelphia
Karasaki, Shuichi	Ultrastructure of yolk platelets in the amphibian egg [p T7 in *Electron Microscopy: Fifth International Congress for Electron Microscopy Held in Philadelphia, Pennsylvania, August 29th to September 5th, 1962,* vol 2 (Biology), ed. S. S. Breese, Jr., Academic Press, New York, 1962]	Fifth International Congress for Electron Microscopy, Philadelphia, Pa.
Kenney, F. T.	Immunochemical analysis of the induction of tyrosine-α-ketoglutarate transaminase in rat liver	New York Academy of Sciences Symposium on Antibody to Enzymes — A Three Component System, New York
	Mechanism of hormonal control of rat liver tyrosine transaminase	International Symposium on Regulation of Liver Enzyme Activity and Synthesis, Indiana University School of Medicine, Indianapolis
Kenney, F. T. (and F. J. Kull)	Studies on the mechanism of hormonal induction of hepatic enzymes [*The Branched Chain* (program and abstr. issue) **18,** 42 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.
Khym, J. X.	The reaction of methylamine with periodate-oxidized adenosine 5′-phosphate [*The Branched Chain* (program and abstr. issue) **18,** 43–44 (1962)]	*Ibid.*
Kimball, R. F.	Cell growth and the retention of RNA labeling over a series of cell generations in *Paramecium aurelia*	Am. Soc. Cell Biol., San Francisco, Calif.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Krieg, D. R.	Ethyl methanesulfonate—induced reversion of bacteriophage T4rII mutants [*Genetics* **47**, 965–66 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
	Ethylation-induced mutagenesis of phage T4	University of Chicago, Chicago, Ill.
Lindsley, D. L.	Preliminary investigations on the genetics of male sterility in *Drosophila*	University of Wisconsin, Madison
Löbbecke, E.-A. (and D. R. Krieg)	Nitrogen mustard—induced mutation of bacteriophage [*Genetics* **47**, 968 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Long, T. J.	Metabolic approaches to morphogenesis	Rutgers University, New Brunswick, N.J.
Makinodan, Takashi	Current work on immunology	Oregon State University, Corvallis
Makinodan, Takashi (and J. F. Albright)	Cytokinetics of antibody response	Third International Symposium on Immunopathology, La Jolla, Calif.
Mans, R. J.	The evolution of an amino acid incorporating system from maize	University of Georgia, Athens
Mans, R. J. (and G. D. Novelli)	Relationship of various nucleic acid fractions to amino acid incorporation in the maize system [*The Branched Chain* (program and abstr. issue) **18**, 46 (1962)]	American Chemical Society, 14th SE Regional Meeting, Gatlinburg, Tenn.
Marin, Guglielmo	The lethal effect of incorporation of tritium-labeled nucleic acid precursors in mammalian cells grown *in vitro*	Am. Soc. Cell Biol., San Francisco, Calif.
Mattingly, Sister M. Augustine	Metabolic inhibition by actinomycin-D in *Vicia faba*	*Ibid.*
Mazur, Peter	Kinetics of water loss from cells during freezing	Biophysical Society, New York
	Mechanisms of injury in frozen and frozen-dried cells	Specialists' Conference on Culture Collections, Ottawa, Canada
Mead, C. G.	The relationship of a DNA-associated RNA with euchromatic and heterochromatic derived DNA of *Drosophila melanogaster* [*Genetics* **47**, 970 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Miller, O. L., Jr.	Development of the multinucleolar condition in amphibian oocytes	Am. Soc. Cell Biol., San Francisco, Calif.
	Studies on the ultrastructure and metabolism of nucleoli in amphibian oocytes [p NN 8 in *Electron Microscopy: Fifth International Congress for Electron Microscopy Held in Philadelphia, Pennsylvania, August 29th to September 5th, 1962*, vol 2 (Biology), ed. by S. S. Breese, Jr., Academic Press, New York, 1962]	Fifth International Congress for Electron Microscopy, Philadelphia, Pa.
Nishimura, Susumu (and G. D. Novelli)	The inactivation of S-RNA by ribonuclease from *Bacillus subtilis* [*The Branched Chain* (program and abstr. issue) **18**, 45–46 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Novelli, G. D.	Control mechanisms in the cell-free synthesis of β-galactosidase	1. University of Oklahoma Medical Research Institute, Oklahoma City 2. Los Alamos, N. Mex.
	Protein synthesis (3 lectures)	University of Georgia, Athens
	Protein synthesis in bacteria	1. Biochemistry Club of St. Louis, St. Louis, Mo. 2. Indiana University School of Medicine, Indianapolis 3. Columbia University, New York
	The role of template RNA in β-galactosidase synthesis	American Chemical Society, SE Regional Meeting, Symposium on the Biosynthesis of DNA, RNA, and Protein, Gatlinburg, Tenn.
Novelli, G. D. (and J. M. Eisenstadt)	Specific template RNA for β-galactosidase	Symposium on Informational Macromolecules, New Brunswick, N.J.
Novelli, G. D. (Susumu Nishimura, and Audrey N. Best)	Observations on the mechanism of amino acid incorporation in a cell-free system from *Escherichia coli* [*The Branched Chain* (program and abstr. issue) **18**, 46 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.
Oakberg, E. F.	Influence of germ cell stage on reproductive and genetic effects of radiation in mammals	Columbia University, New York
	Relationship between histological and cytological damage to the gonads and subsequent genetic effects of radiation	Annual Meeting of the Eastern Psychiatric Research Association, New York
Oakberg, E. F. (and Evelyn Clark)	Species comparisons in radiation response of the gonads	Symposium on Effects of Ionizing Radiation on the Reproductive System, Fort Collins, Colo.
	Species differences in radiation response of the mammalian primary oocyte [*Genetics* **47**, 974 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Odell, T. T., Jr.	Delayed somatic effects of radiation in mammals	ORNL-ORINS Short Course in Health Physics, Oak Ridge, Tenn.
	Studies on production and regulation of rat blood platelets	IX International Congress of Hematology, Mexico City, Mexico
Pal, B. C. (and C. A. Horton)	The structure of 3-methyladenine and methylation of 6-dimethylaminopurine [*Abstr. of Papers, 142nd Mtg., Am. Chem. Soc.* (1962), p 39C]	142nd Meeting, American Chemical Society, Atlantic City, N.J.
Peck, H. D., Jr. (and S. F. Carson)	Properties of adenosine 5′-phosphosulfate reductase from *Desulfovibrio desulfuricans* [*The Branched Chain* (program and abstr. issue) **18**, 46 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Phares, E. F. (Mary V. Long, and S. F. Carson)	Negative ion mass analysis of succinic acid in studies on the mechanism of the methylmalonyl isomerase reaction [*The Branched Chain* (program and abstr. issue) **18,** 41 (1962)]	*Ibid.*
Popp, R. A.	Growth and differentiation of donor hematopoietic tissues in irradiated mammals	University of Florida, Gainesville
	Inherited differences in the primary structure of mouse hemoglobins	1. Sigma Xi Club, Coral Gables, Fla. 2. University of Georgia, Athens
	Physiopathology of radiation in mammals	University of Miami, Coral Gables, Fla.
Popp, R. A. (and G. E. Cosgrove)	Relative ability of parental marrows to repopulate lethally irradiated F_1 hybrids	Conference on Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection, New York
Prescott, D. M.	Biochemical studies on nuclear function and nuclear replication	Purdue University, Lafayette, Ind.
	Nuclear function and replication	1. Princeton University, Princeton, N.J. 2. Harvard Medical School, Boston, Mass.
	The problems of measurement in the cell cycle	UNESCO Symposium on Amplification Techniques in Cell Biology, University of Buffalo, Buffalo, N.Y.
Prescott, D. M. (and M. A Bender)	Turnover of chromosomal protein and RNA during growth and division	Am. Soc. Cell Biol., San Francisco, Calif.
Randolph, M. L.	General radiobiological aspects of variation of LET	Conference on Advances in Meson and Nuclear Research Below 1 Bev, Gatlinburg, Tenn.
	Genetic damage as a function of LET	New York Academy of Sciences Conference on Physical Factors Modifying Response to Radiation, New York
Roesel, Hilde A. (and A. H. Haber)	Effects of light on growth pattern of wheat coleoptiles	Am. Soc. Plant Physiol., Memphis, Tenn.
Russell, Liane B.	Chromosomal aberration in experimental mammals	Symposium on Mammalian Tissue Culture and Cytology, São Paulo, Brazil
	Genetic activity of the mammalian X chromosome	1. AIBS, Genetics Society, Symposium on the Mammalian X Chromosome, Corvallis, Ore. 2. University of Buenos Aires, Buenos Aires, Argentina
	The relative sensitivity of various germ-cell stages of the mouse to radiation-induced nondisjunction, chromosome losses and deficiencies	University of Chile, Santiago

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Russell, Liane B. (Jean W. Bangham, and Clyde L. Saylors)	Delimitation of chromosomal regions involved in V-type position effects from X-autosome translocations in the mouse [*Genetics* **47**, 981–82 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Russell, W. L.	Genetic effects of radiation	1. Laboratorio de Investigacion de Cancer, Lima, Peru 2. University of Buenos Aires, Buenos Aires, Argentina
	Genetic effects of radiation – implications of mouse data	Tenth International Congress of Radiology, Symposium on Genetic and Somatic Implications of Radiation and Radiation Protection, Montreal, Canada
	Mammalian radiation genetics	1. Symposium on Specific Topics in Radiobiology, Rio de Janeiro, Brazil 2. University of Chile, Santiago 3. Comisión Nacional de Energía Atómica, Buenos Aires, Argentina
Schuel, Herbert	Distribution of acid phenyl phosphatase activities in rat liver brei fractionated in the zonal ultracentrifuge	Biophysical Society, New York
Schuel, Herbert (S. R. Tipton, and N. G. Anderson)	Automatic recording of cytochrome oxidase activity in rat liver brei fractions separated in the zonal ultracentrifuge [*Am. Zool.* **2**, 453 (1962)]	AIBS, Am. Soc. Zool., Corvallis, Ore.
Schwartz, Drew	Analysis of mutations in the regulatory system which alter the timing of gene action in esterase enzyme syntheses [*Genetics* **47**, 982–83 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Setlow, Jane K.	Photoreactivable and non-photoreactivable damage in DNA	Yale University, New Haven, Conn.
	Wavelength-dependent fraction of biological damage due to thymine dimers	Biophysical Society, New York
Setlow, R. B.	The effects of UV light on viruses	University of Chile, Santiago
	Molecular changes responsible for ultraviolet inactivation of the biological activity of DNA	Symposium on Specific Topics in Radiobiology, Rio de Janeiro, Brazil
	Molecular changes responsible for the UV inactivation of DNA	Sloan-Kettering Institute, New York
	Replication of DNA	1. Bowman-Gray School of Medicine, Winston-Salem, N.C. 2. University of São Paulo, São Paulo, Brazil 3. University of Chile, Santiago

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Setlow, R. B. (W. L. Carrier, and F. J. Bollum)	Ultraviolet irradiated primer DNA: nearest neighbor frequencies in the product	Biophysical Society, New York
Smith, L. H.	Uptake of Fe^{59} by spleens of lethally irradiated mice given isologous bone marrow	Conference on Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection, New York
Stapleton, G. E.	Repair processes and radiation damage in microorganisms	1. Brescia College, Owensboro, Ky. 2. University of Louisville, Louisville, Ky. 3. Vanderbilt University, Nashville, Tenn.
Suzuki, D. T.	A possible role of asynapsis in interchromosomal effects on crossing over in *Drosophila melanogaster* [*Genetics* **47**, 989 (1962)]	AIBS, Genetics Society, Corvallis, Ore.
Swenson, P. A. (and R. B. Setlow)	Formation and splitting of uracil dimers in poly U by ultraviolet light	Biophysical Society, New York
Upton, A. C.	Experimental radiation leukemogenesis in the mouse	Brookhaven National Laboratory, Upton, L.I., N.Y.
	The nucleus of the cancer cell: Effects of ionizing radiation	American Cancer Society Conference on the Nucleus of the Cancer Cell, Rye, N.Y.
	Observations on the pathogenesis of radiation-induced myeloid leukemia	Argonne National Laboratory, Argonne, Ill.
	Observations on the pathogenesis of radiogenic neoplasia	University of Pittsburgh, Pittsburgh, Pa.
	The problem of radiation-induced neoplasia	University of Chicago, Chicago, Ill.
Upton, A. C. (V. K. Jenkins, and J. W. Conklin)	Myeloid leukemia in the mouse	New York Academy of Sciences Conference on Physical Factors Modifying Response to Radiation, New York
von Borstel, R. C.	Dominant lethality and genetically nontransmissible damage induced by radiation	University of Chicago, Chicago, Ill.
	Preface and postscript to the symposium — recent advances in cytogenetics and developmental genetics	AIBS, Am. Soc. Zool., Corvallis, Ore.
Wallace, R. A.	Studies on amphibian yolk	Duke University, Durham, N.C.
Wallace, R. A. (and Shuichi Karasaki)	The isolation of morphologically intact amphibian yolk platelets and their role in developmental processes [*Am. Zool.* **2**, 456 (1962)]	AIBS, Am. Soc. Zool., Corvallis, Ore.
Wells, Carolyn (read by title)	An analysis of the RNA and DNA nucleotides of three strains of *Tetrahymena pyriformis* [*Am. Zool.* **2**, 457 (1962)]	*Ibid.*

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Welshons, W. J.	Cytological contributions to mammalian genetics	AIBS, Am. Soc. Zool., Corvallis, Ore.
	Detection and use of cytological anomalies in the mouse	Symposium on Mammalian Tissue Culture and Cytology, São Paulo, Brazil
	Use of chromosome anomalies in the mouse	University of Wisconsin, Madison
Williams, A. K. (and G. D. Novelli)	Incorporation into protein of C^{14} labeled amino acids by a cell-free preparation from *Saccharomyces fragilis* [*The Branched Chain* (program and abstr. issue) **18**, 45 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.
Wolff, Sheldon	Chromosome splitting as revealed by combined x-ray and labeling experiments	Am. Soc. Cell Biol., San Francisco, Calif.
Wust, C. J.	Biochemical studies on the mechanism of antibody synthesis	1. Duke University, Durham, N.C. 2. Florida State University, Tallahassee 3. University of Miami, Miami, Fla.
	Interference with antibody neutralization by coenzyme and reducing agents	New York Academy of Sciences Symposium on Antibody to Enzymes – A Three Component System, New York
	The use of enzyme-antigens in the study of antibody synthesis	1. Tulane University, New Orleans, La. 2. Tuskegee Institute, Tuskegee, Ala.
Wust, C. J. (and G. D. Novelli)	Amino acid incorporation by rat spleen ribosomes [*The Branched Chain* (program and abstr. issue) **18**, 45 (1962)]	American Chemical Society, 14th Annual SE Regional Meeting, Gatlinburg, Tenn.
Yamada, Tuneo	An analysis of the differentiation process	Rice University, Houston, Tex.
	Studies on cellular differentiation	1. University of Connecticut, Storrs 2. University of Massachusetts, Amherst 3. Vanderbilt University, Nashville, Tenn.

VISITING LECTURERS

During the six months preceding this report, 35 lectures were given on the Biology Seminar program by guest speakers from scientific institutions and universities in this country and abroad. Visiting lecturers included scientists from Czechoslovakia, England, France, Germany, Italy, Japan, The Netherlands, and Switzerland.

SPEAKER	AFFILIATION	SUBJECT
Werner Arber	Institute of Biophysics, University of Geneva, Geneva, Switzerland	Host-controlled modification of phage and host specificity of DNA
Corrado Baglioni	International Laboratory of Genetics and Biophysics, Naples, Italy	Molecular genetics of human hemoglobins
G. W. Barendsen	Radiobiological Institute, Rijswijk, The Netherlands	Survival of human cells in tissue culture after irradiation with ionizing radiations of different LET

SPEAKER	AFFILIATION	SUBJECT
Hans Becker	University of Marburg, Marburg, Germany	Gene action and cell differentiation
W. R. Bryan	National Institutes of Health, Laboratory of Viral Oncology, National Cancer Institute, Bethesda, Md.	Some biological considerations of the tumor viruses
H. G. Callan	Department of Biology, University of Minnesota, Minneapolis	Protein and RNA syntheses in lampbrush chromosomes
Arthur L. Colwin and Laura Hunter Colwin	Department of Biology, Queens College, Flushing, N.Y.	The behavior of the gamete membranes in fertilization
R. E. Ecker	J. Hillis Miller Health Center, University of Florida, Gainesville	Ribosome concentration and the rate of growth of bacteria
R. A. Flickinger	University of California, Davis	Control of cellular differentiation by regulation of protein synthesis
C. E. Ford	Medical Research Council, Radiobiological Research Unit, Harwell, Berkshire, England	Recent developments in cytogenetic studies
I. C. Gunsales	University of Illinois, Urbana	Inductive and action specificity of oxidases and dehydrogenases for terpenes
John B. Hanson	Department of Agronomy, University of Illinois, Urbana	The impairment of respiration in senescing cells by the high content of RNase and other basic proteins
A. P. Harrison	Vanderbilt University, Nashville, Tenn.	Phenotypic, genotypic, and chemical changes in starving populations of *Aerobacter aerogenes*
Milan Hašek	Institute of Experimental Biology and Genetics, Czechoslovak Academy of Sciences, Prague, Czechoslovakia	Quantitative aspects of immunological tolerance
Peter Hans Hofschneider	Institute of Biophysics, University of Geneva, Geneva, Switzerland	Studies on the DNA of bacteriophage 174
H. E. M. Kay	Department of Clinical Pathology, The Royal Marsden Hospital, London, England	The development of immunological potentiality in man
Sohei Kondo	National Institute of Genetics, Misima, Sizuoka-ken, Japan	Multi-target model for RBE-LET relationships
E. L. Kuff	Protein Chemistry Section, Laboratory of Biochemistry, National Cancer Institute, Bethesda, Md.	Cytochemical studies of mouse plasma cell tumors
Max Lauffer	Division of Natural Sciences, University of Pittsburgh, Pittsburgh, Pa.	The thermodynamics of membrane equilibria in multicomponent systems
M. C. Bessis	Centre National de Transfusion Sanguine, Paris, France	Preliminary results of thermal micropuncture of cells with a LASER microbeam
G. E. Magni	University of Pavia, Pavia, Italy	On the mechanism of spontaneous mutation during meiosis in yeast
George Mathé	Hospital Saint-Louis, Paris, France	Total body irradiation and bone marrow transfusion in man

SPEAKER	AFFILIATION	SUBJECT
Werner Maurer	University of Cologne, Cologne, Germany	Autoradiographic results with labeled thymidine and labeled amino acids Special discussion of self-absorption and absorption in tritium autoradiography
Gunther Meyer	Max Planck Institute, Tübingen, Germany	Specific functional structures in spermatocyte nuclei of difficult *Drosophila* species
George Némethy	Polymer & Interface Studies Chemistry Research, General Electric Co., Schenectady, N.Y.	Hydrophobic bonding in proteins and its effects on secondary structure
Ludvik Novak	Academy of Sciences, Brno, Czechoslovakia	Program of the Institute of Biophysics, Czechoslovak Academy of Sciences, Brno, Czechoslovakia
Alex B. Novikoff	Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, New York	Enzymic activities of cytomembranes
Harvey Patt	Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Ill.	An evaluation of granulocyte production
J. R. Postgate	Department of Microbiology, University of Illinois, Urbana	The senescence and death of *Aerobacter aerogenes*
Alan Richter	The Wistar Institute, Philadelphia, Pa.	Stages in the maturation of equine encephalitis virus
Arthur Rörsch	Medical Biological Laboratory of the National Defense Research Council, T.N.O., Rijswijk, The Netherlands	Gene control radiation sensitivity in bacteria
Karl Sax	Institute of Radiation Biology, University of Tennessee, Knoxville	Spontaneous and x-ray induced aging in plants and animals
Kendric Smith	Department of Radiology, Stanford University School of Medicine, Palo Alto, Calif.	Studies on the apparent *in vivo* cross-linking of DNA and protein by uv
A. A. van Soestbergen	Mercy Hospital, Toledo, Ohio	The oxygen effect in experiments with ionizing radiation of microorganisms

SPEAKERS AT PROFESSIONAL MEETINGS, SPRING–SUMMER 1963

In the period between this and the next semiannual report, 52 Biology Division staff members are scheduled to present papers or to participate in symposia at 13 professional society meetings. As usual, more speeches will be set later in the period. The society meetings are given below in calendar order, followed by the names of currently scheduled speakers and coauthors, listed alphabetically.

Biophysical Society – N. G. Anderson (coauthors, H. P. Barringer, R. E. Canning, C. L. Burger, and C. T. Rankin, Jr.), J. Jagger (coauthors, W. Curtis Wise and R. S. Stafford), Peter Mazur, H. Schuel, Jane K. Setlow, and P. A. Swenson (coauthor, R. B. Setlow)

Seventh Annual Southeastern Developmental Biology Conference – S. Karasaki and H. Schuel (coauthors, N. G. Anderson and S. R. Tipton)

144th National Meeting of the American Chemical Society – J. X. Khym

Sixteenth Annual Biology Research Conference – D. M. Prescott and F. J. Bollum

Federation of American Societies for Experimental Biology – J. F. Albright (coauthors, T. Makinodan and Rachel Carter), N. G. Anderson, R. R. Becker (coauthor, F. Sawada), F. J. Bollum (coauthor, G. E. Houts), Piero Cammarano (coauthor, G. D. Novelli), C. C Congdon, D. G. Doherty, M. I. Dolin (coauthor, K. B. Jacobson), P. J. Fritz, D. L. Greenman (coauthor, F. T. Kenney), K. B. Jacobson, A. Kaji (coauthor, Hideko Kaji), A. L. Kretchmar (coauthor, C. C Congdon), S. Nishimura, H. D. Peck, Jr. (coauthor, T. E. Deacon), E. H. Perkins, and H. Schuel (coauthor, Regina Schuel)

Symposium on "Use and application of radioisotopes and radiation in the control of plant and animal insect pests" (sponsored by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations) – R. C. von Borstel

Symposium on "Human chromosome abnormalities" (sponsored by the American Association of Pathologists and Bacteriologists) – M. A Bender

63rd Annual Meeting of the American Society for Microbiology – H. I. Adler, Robert H. Baum (coauthor, M. I. Dolin), Sister M. Regina Lanigan (coauthor, H. D. Peck, Jr.), E. F. Phares (coauthors, Mary V. Long and S. F. Carson), G. E. Stapleton, and R. L. Tyndall (coauthors, K. B. Jacobson and Ernestine Teeter)

24th Annual Meeting of the American Industrial Hygiene Association (Joint Medical-Radiation Session) – W. L. Russell

54th Annual Meeting of the American Association for Cancer Research – V. K. Jenkins (coauthors, A. C. Upton and N. G. Anderson)

Radiation Research Society – G. E. Cosgrove (coauthors, A. C. Upton, C. C Congdon, D. G. Doherty, and D. G. Gosslee), E. B. Darden, Jr. (coauthors, Margaret Bradley and A. C. Upton), John Jagger (coauthors, R. S. Stafford and R. J. Mackin, Jr.), R. F. Kimball, Toshihiko Sado (coauthor, Martha R. Leonard), Frank Seto, L. H. Smith, and W. B. Stallcup (coauthors, G. Cudkowicz and W. L. Hughes)

International Conference on Cell Division and Induction of Cancer by Radiation – C. C Congdon (summary), Joan Wright Goodman, Alexander Hollaender (chairman of session), D. M. Prescott, A. C. Upton

Sixth International Embryological Conference and Business Meeting of Editorial Board of Journal of Embryology and Experimental Morphology – Tuneo Yamada

FOREIGN TRAVEL

Seven Biology Division members attended a two-part symposium held in São Paulo and Rio de Janeiro, Brazil: Alexander Hollaender, M. A Bender, E. H. Y. Chu, W. L. Russell, Liane B. Russell, R. B. Setlow, and W. J. Welshons.

Drs. Bender, Chu, Liane B. Russell, and Welshons gave papers at the first conference on October 22–25 in São Paulo, "Mammalian Tissue Culture and Cytology." Dr. Hollaender was president of that symposium and chairman of the closing session. W. L. Russell organized a round-table discussion at São Paulo on "The Future Development of Research on Human and Mammalian Cytogenetics."

W. L. Russell and R. B. Setlow presented papers at the October 29–31 Rio de Janeiro meeting, "Specific Topics in Radiobiology." Dr. Russell was round-table discussion chairman at this meeting, and Dr. Hollaender was session chairman on October 30. After the conference, visits and consultations were made at several laboratories and the Universities of São Paulo, Chile, and Buenos Aires. R. B.

Setlow gave a seminar at The Institute of Physics and Mathematics at the University of Chile, "Replication of DNA." Dr. Chu also gave a seminar at the University of Chile. W. L. and Liane B. Russell also gave seminars in Buenos Aires, Santiago, and Lima.

Alexander Hollaender attended a meeting of the executive committee of the Comité International de Photobiologie in Paris and discussed molecular biophysics with Edouard Kellenberger at the University of Geneva in September.

J. F. Albright and T. T. Odell, Jr., attended the IX International Congress of Hematology, September 8–15 in Mexico City. Albright spoke on "Some quantitative properties of antibody-producing cells," and Odell on "Studies on production and regulation of rat blood platelets."

Three Division members traveled to Canada in the period covered by this report. W. L. Russell gave a paper on implications of mouse data at the X International Congress of Radiology in September at Montreal, "Genetic and Somatic Implications of Radiation and Radiation Protection." Peter Mazur attended a specialist's conference on culture collections and presented a paper, "Mechanisms of injury in frozen and frozen-dried cells," August 26–29 in Ottawa. The meeting was sponsored by the Canadian Committee on Culture Collections of Microorganisms (National Research Council of Canada). R. A. Wallace attended a National Research Council meeting in January and consulted with W. H. Cook at the Division of Applied Biology in Ottawa.

Gustavo Cudkowicz spoke on "A recessive histocompatibility factor in the mouse" on February 14 at the University of Milan, Milan, Italy.

W. E Cohn and W. J. Welshons are spending a year abroad (see p 26).

SIXTEENTH ANNUAL BIOLOGY RESEARCH CONFERENCE

The 1963 Annual Research Conference, sponsored by the Biology Division of Oak Ridge National Laboratory, will be held April 8–11 at the Mountain View Hotel, Gatlinburg, Tenn. The organizing committee, composed of D. M. Prescott, chairman, R. F. Kimball, Tuneo Yamada, F. J. Bollum, N. G. Anderson, and R. C. von Borstel, announced the following program:

Monday, April 8

Opening Remarks – Alexander Hollaender

Welcome Address – Alvin M. Weinberg, Director, Oak Ridge National Laboratory

Introduction – D. M. Prescott, Chairman, Organizing Committee

Morning

Chairman – Herman Kalckar, Massachusetts General Hospital and Harvard University, Boston, Mass.

Patterns of synthesis of RNA and other cell components during the cell cycle of Schizosaccharomyces pombe – J. M. Mitchison, Department of Zoology, University of Edinburgh, Edinburgh, Scotland

The cycle of bacterial duplication – A. B. Pardee and Peter Kuempel, Department of Biology, Princeton University, Princeton, N.J.

Afternoon

Chairman – Max Alfert, Department of Zoology, University of California, Berkeley

Role of RNA and protein synthesis in the DNA replication cycle in bacteria – Ole Maaløe, University Institute of Microbiology, Copenhagen, Denmark

Experiments on the extranuclear control of gene activity in dipteran polytene chromosomes – Heinrich Kroeger, Zoological Institute, Eidgenössische Technische Hochschule, Zürich, Switzerland

Smoker – Host: Union Carbide Corporation

Tuesday, April 9

Morning

Chairman – Erik Zeuthen, Biological Institute, Carlsberg Foundation, Copenhagen, Denmark

Intermediate states in enzymatic DNA synthesis – F. J. Bollum, Biology Division, Oak Ridge National Laboratory

DNA synthesis in relation to chromosome reproduction and the reunion of breaks – J. Herbert Taylor, Department of Botany and Zoology, Columbia University, New York.

Afternoon

(Free)

Evening

Reception and dinner

Wednesday, April 10

Morning

Chairman – V. R. Potter, Department of Oncology, University of Wisconsin Medical School, Madison

Genetic implications of histone behavior – D. P. Bloch, Department of Botany, University of Texas, Austin

Biochemistry of histones and the cell cycle – Harris Busch and L. S. Hnilica, Department of Pharmacology, Baylor University College of Medicine, Houston, Tex.

Afternoon

Chairman – J. F. Danielli, Department of Medicinal Chemistry, School of Pharmacy, University of Buffalo, Buffalo, N.Y.

Synthetic activities leading to mitosis – Daniel Mazia, Department of Zoology, University of California, Berkeley

Nucleic acids and information transfer – Liebe F. Cavalieri, Cornell University Medical College, N.Y.

Evening

Round-table discussion: *Differential sensitivity of the cell life cycle*

Chairman – Hans Ris, Department of Zoology, University of Wisconsin, Madison

Participants: L. G. Lajtha, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, England
Nirmal K. Das, Department of Zoology, University of California, Berkeley
C. L. Smith, Department of Radiotherapeutics, University of Cambridge, Cambridge, England
L. J. Tolmach, Washington University School of Medicine, St. Louis, Mo.
Erik Zeuthen, Biological Institute, Carlsberg Foundation, Copenhagen, Denmark

Thursday, April 11

Morning

Chairman – Rollin D. Hotchkiss, The Rockefeller Institute, New York, N.Y.

Control of cell division in microalgae – Hiroshi Tamiya, The Tokugawa Institute for Biological Research, Tokyo, Japan

Synthesis and behavior of nuclear proteins during the cell life cycle – D. M. Prescott and M. A Bender, Biology Division, Oak Ridge National Laboratory

ANNUAL INFORMATION MEETING

The Oak Ridge National Laboratory Advisory Committee for Biology visited the Biology Division November 7–9. The committee is composed of Edwin Chargaff, Columbia University; Rollin D. Hotchkiss, The Rockefeller Institute; Curt Stern, University of California; Maxwell M. Wintrobe, University of Utah; and Paul C. Zamecnik, Massachusetts General Hospital.

Division investigators summarized their work at a conference in the Division on November 8, and the committee consulted with individual investigators on the other two days.

BONE MARROW CONFERENCES

Bone marrow transplantation and chemical protection conferences were first started in 1957 in Oak Ridge. These conferences provide a means for prompt exchange of the latest experimental information between the clinical and animal researchers. In addition to the Biology Division staff, many people and organizations participate in arranging for these meetings.

Proceedings of the meetings usually are published in *Blood, The Journal of Hematology,* by the Biology Division, or by the sponsoring organization.

Biology Division members took active part in a *Conference on Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection,* December 1–3, 1962, in New York City. R. A. Popp, L. H. Smith, Joan W. Goodman, G. Cudkowicz, and C. C Congdon presented papers.

Other meetings being planned are:

April 16, 1963, Atlantic City, N.J. – Round table discussion on *New Research Developments in Bone Marrow Transplantation and Chemical Protection.* Chairman, L. M. Tocantins.

September 7–9, 1964, Paris, France. – Conference, tentative topic – *Bone Marrow Transplantation and Secondary Disease.*

CONFERENCE ON AMPHIBIAN YOLK PLATELETS

Twenty scientists from the United States and Canada gathered at the Biology Division on October 12–13 to participate in the Conference on Amphibian Yolk Platelets, the first of its kind ever held. Nine members of the Biology Division took part in the informal conference. Dr. Robin Wallace organized the conference.

The one and one-half day program was divided into three sections:

"Cytological and Biochemical Features of Amphibian Yolk"
Chairman – Paul Gross, Brown University, Providence, Rhode Island

"Processes Related to Yolk Formation and Utilization"
Chairman – Reed A. Flickinger, Department of Zoology, University of California, Davis, Calif.

"The Role of Yolk Platelets in Embryological Processes"
Chairman – J. K. F. Holtfreter, University of Rochester, Rochester, New York

This conference will be held regularly. In addition, conferences on other specialized branches of developmental biology will be organized in the near future.

MAMMALIAN CYTOLOGY AND SOMATIC CELL GENETICS

On November 15–17, about 75 scientists attended the meeting on Mammalian Cytology and Somatic Cell Genetics held in Gatlinburg. The program consisted of four sessions. M. A Bender and E. H. Y. Chu were cochairmen of this meeting.

AMERICAN CHEMICAL SOCIETY MEETING

Twenty-seven members of the Biology Division attended the Southeastern Regional Meeting of the American Chemical Society held in Gatlinburg on November 1–3. Of these, 20 were authors and co-authors of papers that were presented, and 5 presided over various sessions. G. D. Novelli was program chairman for the Biochemistry Section.

EDUCATIONAL ACTIVITIES

Student Trainee Program. – Applications for the Summer Student Trainee Program have been reviewed, and 15–20 of these undergraduates will be selected to participate in 1963. In addition, a new program will be inaugurated within the Division under which students who have just received a bachelor's degree and have been accepted by a graduate school in the autumn will be selected to work in the Division for the summer. About six students will participate in this new program.

Small College Program. – The Small College Program, set up by the Biology Division, is basically an effort by the Division to arrange visits and lectures at small colleges. H. I. Adler gave four lectures at two colleges during this report period. He spoke on "Space Biology" and "Some Aspects of Microbial Genetics" to the Department of Biological Sciences at the University of Chattanooga in October. In November he presented lectures on "Space Biology" and "Radiation Microbiology" at David Lipscomb College and the Peabody College in Nashville.

John Jagger gave two lectures in November on "Light and Life" at Morehouse College and Oglethorpe University in Atlanta and at Agnes Scott College, Decatur, Ga.

University Cooperative Program. – S. F. Carson gave two lectures on "Chemical Microbiology" at the University of Georgia, Athens, in December, as part of the cooperative graduate and postdoctoral teaching program between that university and the Biology Division. In January he gave two lectures on "Biotin and B_{12} Coenzyme-Catalyzed Reactions in the Interconversion of Propionate and Succinate: (1) Propionyl Carboxylase and the Trans-Carboxylase, (2) Methylmalonyl Isomerase and Racemase" at the Bacteriology Department, University of Georgia.

G. D. Novelli presented three lectures on protein synthesis as part of a graduate course in biochemistry at the University of Georgia in December.

In February R. J. Mans gave a lecture at the Biochemistry Department of the University of Georgia, "The Evolution of an Amino Acid Incorporating System from Maize."

Courses. – Three Biology Division investigators gave courses in October. G. E. Cosgrove spoke on "Acute Injury and Recovery in Mammals" at an ORNL Health Physics course. T. T. Odell gave a lecture on "Delayed Somatic Effects of Radiation in Mammals" as part of the ORNL-ORINS Short Course in Health Physics. G. E. Stapleton spoke on "Protection and Recovery Against Radiation at the Cell Level" at ORINS.

H. I. Adler and G. E. Stapleton presented a series of weekly seminars beginning February 9 in radiation biology as a part of the Institute of Radiation Biology program at the University of Tennessee. The last seminar was March 2.

A. C. Upton taught a course on biological effects of radiation at the Department of Pathology of the University of Pittsburgh School of Medicine in February.

Training Programs. – A. C. Upton spoke on "Biological Effects of Radiation – New Concepts" on October 16 at the 11th Annual Convention of the U.S. Civil Defense Council at the James White Memorial Auditorium-Coliseum, Knoxville.

DIVISION MEMBERS ON LEAVES OF ABSENCE

Four staff members are away from the Division temporarily.

J. S. Kirby-Smith, Biophysics Group, has been associated with the Division of Biology and Medicine of the Atomic Energy Commission, Washington, D.C., since January. He will remain there for one year.

D. R. Krieg of the Phage Genetics Group is on leave of absence for a year, beginning on January 1, 1963, during which he will be with the Department of Chemistry, Stanford University, Palo Alto, California. He has been granted a special United States Public Health Service fellowship to support his work at Stanford.

W. J. Welshons left the Division in February for a one-year stay at the University of Chile, Santiago, on a National Science Foundation Senior Postdoctoral Fellowship. He will work with Gustavo Hoecker in immunogenetics. Dr. Welshons plans to attend the "Symposium on the Control of Cell Division and the Induction of Cancer" which will be held in Lima, Peru, and Cali, Colombia, in July 1963.

W. E Cohn left the Division September 24 to spend a year abroad. Dr. Cohn will conduct research on viral nucleic acid problems with Professor Roy Markham at the Agricultural Research Council, Virus Research Unit, Cambridge, England, and with Marianne Grunberg-Manago at the Institute of Biology, Paris, France, under grants from the Guggenheim Foundation and the National Institutes of Health. Dr. Cohn will spend August and September of this year visiting laboratories in Europe and Japan and will return to the Laboratory in October.

COOPERATIVE PROGRAMS WITH NATIONAL INSTITUTES OF HEALTH

A cooperative study on chemical carcinogenesis has been initiated with the National Cancer Institute of the National Institutes of Health. The purpose of this program is to study the mechanism of induction of malignancy by chemicals, and the possible synergistic action between chemical carcinogens and radiation.

In another cooperative program with the National Institutes of Health, a high-speed zonal ultracentrifuge is being developed for the separation of subcellular particles. This program is under the direction of N. G. Anderson. A progress report on this study has been issued (ORNL-3415).

Cytology and Genetics

Effects of Radiation on Paramecium		Chromosome Cytology	
R. F. Kimball	Stella W. Perdue H. Marine Scandlyn	Sheldon Wolff Sandra Bell[c]	H. E. Luippold Judith M. Pier
Phage Genetics		**Fungal Genetics**	
D. R. Krieg[a] C. M. Steinberg Jane K. Setlow[b]	M. E. Boling Claire J. Witten	F. J. de Serres B. B. Webber W. E. Barnett[b] H. E. Brockman[b]	Ida R. Cox Arlee P. Teasley
[a]Leave of absence, Stanford University, Palo Alto, Calif. [b]USPHS Postdoctoral Fellow. [c]Consultant.		**Mammalian Genetic Cytology**	
		E. H. Y. Chu	Patricia G. Lankford Nancy H. Robinson

REPARABLE PREMUTATIONAL DAMAGE: DOSE RELATIONS AND PHENOTYPES

R. F. Kimball Stella W. Perdue
H. Marine Scandlyn

Introduction. – We have shown[1] that x-ray–induced lethal and slow growth mutations in *Paramecium aurelia* are of at least two origins. One type arises from lesions (class 1) that lead to one-hit mutations only, are reparable until the chromosomes replicate, and only then cause mutations. Quite possibly these lesions are alterations of DNA bases and cause mutation by misreplication. The second type arises from lesions (class 2) that can interact to form two-hit mutations quite independently of chromosome replication. Presumably these are chromosomal breaks. The present report concerns the class 1 lesions. This class produces nearly two-thirds of the mutations when log phase paramecia are irradiated but a much smaller part when stationary phase paramecia are irradiated,[2] especially if they are kept for a time afterward in stationary phase before adding food. Class 2 lesions,[1] and as far as we know any unidentified classes that might exist, contribute about equally to mutations whatever the growth phase of the irradiated paramecia. Consequently, the excess of mutations from log phase irradiations compared to stationary phase ones must come essentially entirely from class 1 lesions and can be used to study this class by itself. We are reporting here an investigation of the dose relations and phenotypic effect of mutations of this origin.

[1]R. F. Kimball, Stella W. Perdue, and H. Marine Scandlyn, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 33–34; R. F. Kimball, *Genetics* (in press).

[2]R. F. Kimball, Nenita Gaither, and Stella W. Perdue, *Proc. Natl. Acad. Sci. U.S.* **45**, 833 (1959).

Methods. – The paramecia were irradiated with 250-kvp x rays at about 1.6 kr/min. Stationary phase paramecia were retained for a day after irradiation before feeding. The methods for detecting mutations have been given in many earlier reports and publications, and only a few features need be detailed here. A large number of homozygous segregants were obtained from the irradiated paramecia, and a record was kept of the frequencies with which these segregants produce clones that (1) survive for 4 days with normal growth (no detectable mutations), (2) survive for 4 days but with distinctly less growth than normal (4-day viable, slow growth mutations), and (3) fail to survive for 4 days (4-day lethal mutations). Many of the second kind of clone fail to survive in further culture but some do with continued slow growth. The frequency of category (1) clones gives the probability of segregants without detectable mutation; the sum of the frequencies of categories (1) and (2) gives the probability of segregants without rapidly lethal mutations. On fairly simple theory, the semilog plot of these frequencies should have a curvilinear relation to dose approaching a linear one as the dose increases. There is no simple theory relating the transformed variable that was used in some of our other work to dose; consequently, it has not been used here.

Results and Discussion. – The data are plotted in Fig. 1. The dashed lines are the ratios between the smoothed forms of the other two curves and show the dose relations for mutations of class 1 origin. Obviously, these mutations make approximately as much contribution to the rapidly lethal class (Fig. 1*b*) as to all detectable mutations (Fig. 1*a*). There is no evidence from this comparison that mutations of different origins produce a markedly different spectrum of phenotypes.

Secondly, the dose curves for class 1 mutations are curvilinear, showing that at least some of the factors responsible for this curvilinearity, that is, gene interaction, selection of meiotic products, and two-hit mutations, are important for this class. The first two items result from the phenotypic expression of the mutations and, from the previous paragraph, might be expected to act on mutations of any origin.

The greater departure from linearity of the curve in Fig. 1*b* suggests that an appreciable fraction of the clones that die within 4 days do so because of multiple mutations with individually small effects

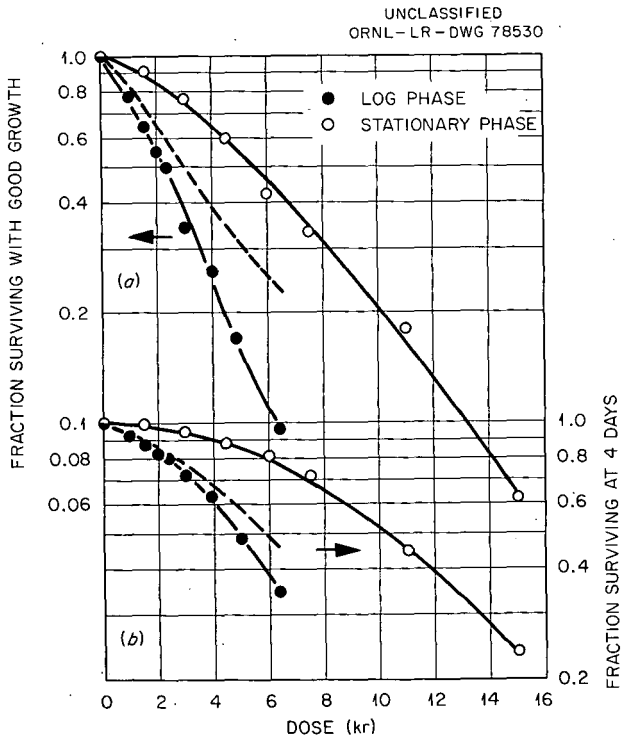

Fig. 1. Survival of Homozygous Segregants. *a*, the frequency of homozygous segregants that survive for 4 days with good growth (left-hand scale). *b*, the frequency of segregants that survive 4 days regardless of growth (right-hand scale). The dashed lines are the ratios of the frequencies for log and stationary phase calculated from the smoothed curves.

or of selection against meiotic products having mutations. Class 1 mutations do not produce two-hit aberrations, of course. Such aberrations should contribute relatively more to the stationary phase curve than to the log phase one and would be expected to produce grossly deficient segregants that ought to die early. Thus they would be expected to have their greatest effect on the stationary phase curve in Fig. 1*b*, and indeed this curve shows the greatest departure from linearity.

The apparent slight decrease in slope in the class 1 curve of Fig. 1*a* may not be significant. If it is, we may be encountering an effect of the higher doses of radiation on repair. If so, it is quite small within the range of doses used.

Thus these studies have established that (1) many of the mutations of class 1 origin have the rapidly killing phenotype, (2) they are subject to at least some of the factors that cause curvilinear

relations to dose, and (3) there may be some effect of the radiation on repair during log phase, but, if so, it is small.

STUDIES ON THE CONSERVATION OF MATERIALS IN *PARAMECIUM*

R. F. Kimball Stella W. Perdue
H. Marine Scandlyn

Introduction. – In the previous report[3] it was shown that the tritium label from uridine and cytidine was conserved during at least six cell generations of log phase growth. Similar findings have now been made with a tritiated amino acid, leucine. It has been shown, furthermore, that a considerable part of the tritium label from the pyrimidine ribonucleosides is lost if log phase paramecia are transferred to a nonnutritive salt solution for about 12 hr.

Methods and Results. – The method for studying conservation of the labeled amino acid was the same as that for the ribonucleosides. Paramecia were put into the label for a brief time early in interphase and then washed free. One product of each of several successive divisions was dried on a slide shortly after division; the other product was kept to continue the line of descent. A group of paramecia was also dried shortly before the estimated time of the first postlabeling division. Autoradiographs were made and developed, and the number of grains per unit area was determined for each specimen.

The results are shown in Fig. 2. The number of grains per unit area is halved during each cell generation, which would be expected if the label were conserved.

In another set of experiments paramecia were labeled with tritiated uridine and cytidine and allowed to divide. One product of division was kept for 12 hr in salt solution, the other in culture medium. Products of each of these two lines of descent were fixed after the third and the fourth cell division following labeling, autoradiographs were made, and the number of grains per unit area was counted. The lines of descent kept 12 hr in salt solution between the first and second postlabeling divisions had about half as much labeling as those that were continuously in culture medium, indicating a considerable loss of label during the period in salt solution.

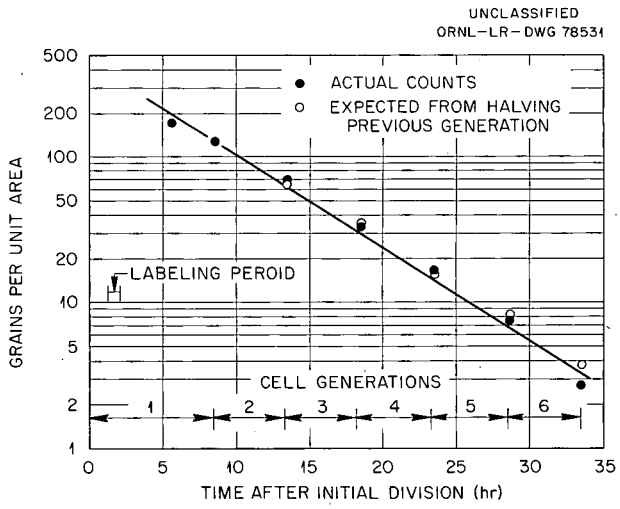

Fig. 2. Conservation of Label from H^3-Leucine in *Paramecium*. Grains per unit area plotted against time after the prelabeling division for paramecia fixed just before the first postlabeling division and immediately after several successive divisions.

Discussion. – The amino acid experiment shows that not only ribonucleosides but also amino acids are essentially completely conserved during log phase growth. The question remains whether they are conserved in macromolecular form as RNA and protein or by way of a pool of breakdown products that is conserved and reutilized. The idea of conservation of the macromolecules is supported by the demonstration in bacteria[4] and ascites tumor cells[5] that RNA is conserved. In any case, there seems to be no less perfect conservation of amino acids than of ribonucleosides. The finding that the label from ribonucleosides is lost during a period of starvation shows that even if macromolecular RNA is conserved under some conditions, it is not under others.

[3] R. F. Kimball, Stella W. Perdue, and H. Marine Scandlyn, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 29–31.

[4] C. I. Davern and M. Meselson, *J. Mol. Biol.* **2**, 153 (1960).

[5] J. F. Scott, E. B. Taft, and N. W. Letourneau, *Biochim. Biophys. Acta* **61**, 62 (1962).

THE FRACTION OF BIOLOGICAL DAMAGE DUE TO THYMINE DIMERS PRODUCED IN DNA BY VARIOUS WAVELENGTHS OF UV RADIATION

Jane K. Setlow

Introduction. – It was previously shown[6] that the *in vitro* photoreactivation of transforming DNA is correlated with the splitting of ultraviolet(uv)-induced thymine dimers. There is evidence that all the biological change in uv-irradiated DNA caused by a photoreactivating yeast extract may be accounted for by this one photochemical reaction.[6] Because the fraction of the damage that is not photoreactivable must be damage caused by some photochemical process or processes other than thymine dimer formation, a study has been made of how photoreactivability varies with the wavelength of inactivating radiation. The efficiency of different wavelengths in causing nonphotoreactivable damage should be a clue to the nature of the uv lesions that are not thymine dimers.

Results and Discussion. – Inactivation curves of *Hemophilus influenzae* transforming DNA with 11 different wavelengths of uv radiation, as well as corresponding inactivation curves following maximum photoreactivation have been obtained. At short wavelengths there is saturation of thymine dimer formation[7] at doses that have not yet removed all the detectable biological activity of the transforming DNA. At these doses there is observed a change in the dose-survival relation, such that further irradiation is much less effective in reducing biological activity. In this dose region, inactivation is similar to that found for non-thymine-dimer damage.

The photoreactivable sector at each wavelength represents the fraction of the biological damage due to thymine dimer formation. It may be calculated from the slopes of the inactivation curves with and without maximum photoreactivation. The photoreactivable sector is largest for the intermediate wavelengths, and decreases at the extremes of the spectrum. This variation may be explained by the difference in the absorption coefficients of nucleosides. The absorption of thymidine falls off more rapidly on either side of its maximum than does that of cytosine deoxyriboside. It is known that cytosine deoxyriboside, like thymidine, is more sensitive to *chemical* changes produced by uv irradiation.[8] The quantum yield for the thymine dimerization reaction apparently decreases in the long wavelength region,[9] which would also tend to decrease the fraction of the biological damage due to thymine dimerization.

The shape of the action spectrum for non-thymine-dimer damage (obtained by plotting the slopes of the inactivation curves with maximum photoreactivation vs wavelength of inactivating radiation) also suggests that cytosine deoxyriboside alteration is important in nonphotoreactivable damage.

[6] Jane K. Setlow and R. B. Setlow, *Nature* (in press).

[7] R. B. Setlow and W. L. Carrier, *Photochem. Photobiol.* (in press).

[8] D. Shugar and K. L. Wierzchowski, *Postery Biochem.* **4**, 243 (1958).

[9] R. B. Setlow, personal communication.

THE RATE OF PHOTOREACTIVATION OF TRANSFORMING DNA AS A FUNCTION OF INACTIVATING WAVELENGTH AND DOSE

Amir Muhammed Jane K. Setlow

Introduction. – Since there is evidence that the photoreactivable uv lesion is the thymine dimer,[6] we would expect the time rate of *in vitro* photoreactivation of transforming DNA with yeast extract to depend mostly on two parameters (with constant yeast extract concentration and photoreactivating illumination intensity): (1) the number of thymine dimers in the irradiated DNA (concentration of substrate), and (2) the fraction of the biological damage due to thymine dimers. Since these two factors vary with wavelength of the inactivating uv radiation as well as the dose, the rate of photoreactivation should also depend on the inactivating wavelength and the dose. The observed variation of the measured rate of *Hemophilus influenzae* transforming DNA photoreactivation in accordance with prediction based on known variation in the number of thymine dimers and the fraction of photoreactivable damage is further evidence that the thymine dimer is indeed the photoreactivable lesion.

Results and Discussion. – The rate of photoreactivation after 2390-A inactivation goes through a maximum at a 2390-A dose of 5×10^3 ergs/mm^2.

This is a reasonable result, since the number of thymine dimers increases with dose, until an equilibrium is reached, beyond which there is no further increase in dimers. However, we may suppose that the other, nonphotoreactivable, types of lesions continue to be made beyond the equilibrium point of the thymine dimers, and thus the fraction of biological damage due to thymine dimers decreases at large doses. The dose of 2390-A irradiation which corresponds to the largest photoreactivation rate is approximately the dose at which thymine dimer formation begins to slow down at this wavelength.[10]

The rate of photoreactivation has been measured after large doses of eight different wavelengths which produce about the same survival level. The rate varies by a factor of 6 from the low to the high wavelengths. After inactivation by a large dose at the low wavelengths, the fraction of biological damage due to thymine dimers is relatively low,[11] and hence the rate of photoreactivation is low. The rate increases with increasing wavelength to 2650 A and then levels off. For wavelengths higher than 2650 A there are somewhat more thymine dimers produced, but the fraction of damage that is photoreactivable decreases in this region of the spectrum.[11] These two effects apparently cancel each other, so that the photoreactivation rate remains fairly constant.

STUDIES ON THE NATURE OF THE RADIATION SENSITIVITY OF DESICCATED SEEDS

Sheldon Wolff H. E. Luippold

Introduction. – If normally dried seeds of barley are irradiated with x rays, it is found that they are less sensitive than are wet, growing seeds. This is true if the criterion of radiation damage is either seedling height or chromosome aberrations that have been induced in the nuclei of the cells. If, however, the dry seeds are desiccated, it is now found that the desiccated seeds are more sensitive than those of normal dryness.[12]

An equation has been formulated that describes the kinetics for two-break chromosome aberrations.[13] The equation, $Y = N(1 - e^{-kD})^2$, has two unknowns, one of which, N, represents the effective sites within the nucleus where two chromosomes come close enough to one another to form a two-break exchange if broken. The other parameter, k, represents the sensitivity of the system and may be defined as the average number of breaks per rad in a chromosome in a site.

Changes in either N or k can affect the yield of aberrations induced in cells. For instance, if the site number increases or if k increases, the yield, too, will increase.

Dose curves were obtained for two-hit aberrations in both normal and desiccated seeds and the curves analyzed to find the values of N and k that give the best fits. This was done in an attempt to find which of these two parameters, the site number or the sensitivity, changed when the seeds became radiosensitive following desiccation.

Results. – The dose curves for normal and desiccated seeds are presented in Fig. 3. The best fits of these curves to the equation were obtained for normal seeds when $N = 0.550$ and $k = 0.0442$, and for desiccated seeds when $N = 0.369$ and $k = 0.0811$.

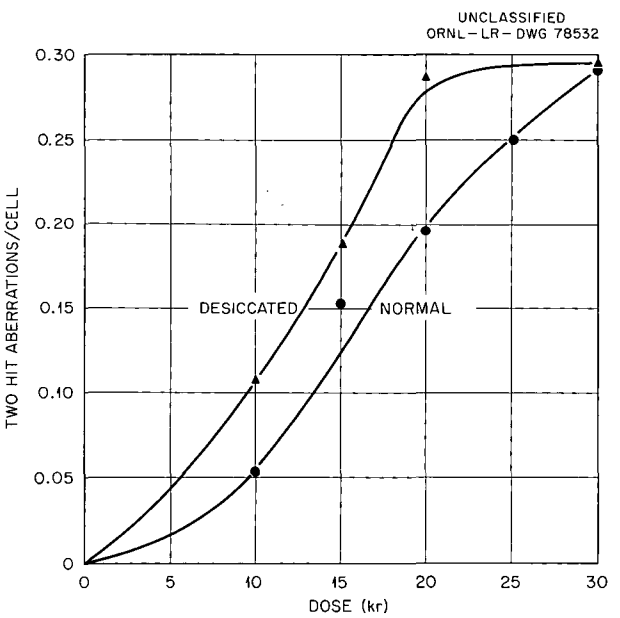

Fig. 3. Dose Curves for Normal (●) and Desiccated (▲) Seeds.

[10] R. A. Deering and R. Setlow, *Biochim. Biophys. Acta* (in press).

[11] Jane K. Setlow, this report.

[12] R. S. Caldecott, *Radiation Res.* **2**, 339 (1955).

[13] S. Wolff, *J. Theoret. Biol.* **3**, 304 (1962).

Discussion. – The results indicate that if there is any change in site number, it was a decrease following desiccation, whereas the sensitivity, *k*, increased; that is, the slope for the curve of desiccated seeds is greater than that for normal seeds and the curve for desiccated seeds saturates earlier than that for normal seeds. There is no a priori reason for suspecting that this would be a result of the experiment. As a matter of fact, one might have expected just the opposite; that is, that as the seeds became desiccated, the chromosomes would be drawn closer together and the site number for exchanges would increase, and that, conversely, as water was drawn out of the cell the indirect effects of radiation would become lessened and the sensitivity, *k*, would be decreased. The results in the present experiment show that this is not the case. Following desiccation the site number did not increase and the sensitivity did.

Such a finding is consistent with the concept developed by Powers *et al.*[14] that radiation produces radicals that in moist systems interact with water and are dissipated harmlessly. Under conditions of extreme desiccation, however, such radicals are long-lived and can cause biological damage. In desiccated systems, therefore, there would be an apparent increase in sensitivity caused by these radicals.

PRELIMINARY EXPERIMENTS ON THE CHEMISTRY OF CROSSING OVER

Sheldon Wolff F. J. de Serres
J. M. Pier

Introduction. – One way to study the chemistry of crossing over is to use specific metabolic inhibitors to inhibit chemical processes that occur at the time of genetic recombination. Similar techniques have been useful in the studies of radiation-induced aberrations in which it was found that protein synthesis inhibitors prevent the repair or rejoining of radiation-induced chromosome breaks.[15] Although the chromosome consists, in the main, of both nucleic acids and proteins, it has been postulated that the bonds formed in such aberration studies are protein. Crossing over has been likened to the breakage and rejoining of chromosomes.[16] There are, however, some differences that exist; for instance, crossing over is highly polarized whereas rejoining of radiation-induced breaks is not and either dicentrics or reciprocal translocations can be produced.

Experiments were carried out using various metabolic inhibitors in an attempt to see if crossing over could be modified when certain metabolic processes were inhibited. In particular, the experiments were designed to see whether or not protein synthesis, which can inhibit the rejoining of radiation-induced breaks, can affect crossing over.

Three different experiments were performed with the mold *Neurospora crassa*, which has the type of chromosomes found in higher forms; that is, they consist of nucleoproteins rather than just DNA as does the phage chromosome. The amount of recombination was measured when the cell was treated with metabolic inhibitors.

In all experiments the female parent was inoculated on paper cones in flasks containing Westergaard's medium. After the protoperithecia had formed, the conidia from the male parent and the chemical to be tested were added. Two to three flasks were used for each treatment. In the first experiment the marker Asco, which gives rise to white ascospores, was used. The asci produced were dissected in a very viscous methyl cellulose solution, and the percentage of second division segregations for the Asco character were scored visually under a dissecting microscope. Four hundred asci were scored in each treatment. In the second group of experiments the stocks 39113A (*nic-1*) and 1325a (*nic-2*) were used. In the third experiment stocks 74-OR33-5a (*bist-2*) and 39113A (*nic-1*) were used.

Results. – Chloramphenicol at a concentration of 600 μg/ml, which inhibits protein synthesis and has been found in our hands to inhibit by 25% the growth of *Neurospora*, does not cause any effect on recombination although it has been found to inhibit the rejoining of radiation-induced breaks in *Vicia faba*. We interpret this to mean that protein synthesis is not involved in recombination.

The only inhibitor that gave consistently higher recombination values when added at the time of

[14] E. L. Powers, R. B. Webb, and C. J. Ehret, *Radiation Res.* (suppl. 2), 94 (1960).

[15] S. Wolff, *Am. Naturalist* **94**, 85 (1960).

[16] K. Sax, *J. Cellular Comp. Physiol.* **45** (suppl. 2), 243 (1955).

meiosis was 5-fluorodeoxyuridine (FUdR), a compound which has been shown in some systems to be a specific inhibitor of DNA synthesis in that it inhibits the reaction by which thymidylate synthetase converts uridylate to thymidylate.[17]

In the first experiment, FUdR increased the percentage of second division segregation of the asco character by 26–50% (control values were 23.3–28.0%; FUdR-treated, 35.3%).

In the second experiment, FUdR increased recombination between *nic-1* and *nic-2* in the stocks used from 2.20 to 3.37%, a 53% increase.

In the third experiment, FUdR consistently increased the percentage of recombination between *bist-2* and *nic-1*. Recombination increased from 41.8% in the controls up to 46.5%. The results of this last experiment are not significantly higher than the controls. We suspect the reason for this is that the markers are very far apart. At large distances, even very great increases in crossing over do not increase the amount of recombination by very much in that recombination approached 50% asymptotically according to the formula, $R = \frac{1}{2}(1 - e^{-2x})$.

A compound that is structurally similar to FUdR, bromodeoxyuridine (BUdR), was also used. BUdR, which is a thymidine analog that, in some organisms, becomes incorporated into the DNA, did not have any effect on the recombination nor did uridine, which is usually added with FUdR to prevent any fluorouracil (a breakdown product of FUdR) from becoming incorporated into RNA.

Discussion. – The simplest explanation for these results is that genetic recombination, which entails the exchange of chromatids, is specific for DNA and that a functional mechanism for DNA synthesis is required when the bonds rejoining the two pieces are synthesized. This is unlike the rejoining or repair of x-ray–induced breaks that are nonpolarized and are inhibited by chloramphenicol.

STUDIES OF THE MECHANISM OF THE EFFECT OF FUdR ON CHROMOSOMES

Sandra Bell Sheldon Wolff

Introduction. – Fluorodeoxyuridine (FUdR) has been found to be a specific inhibitor of DNA synthesis in virus-infected *Escherichia coli.*[17] In experiments[18,19] with FUdR involving lateral roots of *Vicia faba*, chromosome gaps and shattering have been found. Because of the effect of FUdR on DNA synthesis in *E. coli*, and because the time involved in the appearance of chromosome damage after FUdR treatment began was equal to the time of G_2 in untreated cells, it was originally postulated that the chromosome damage occurred at the end of the DNA synthetic (S) portion of the cell cycle.[18] This has subsequently been shown to be incorrect.[19] The chromosomes are broken in the period following DNA synthesis (G_2). Exogenous thymine will reverse the effect of FUdR on *E. coli*,[17] and the addition of thymidine will apparently reverse the damage caused by FUdR on the chromosomes of *V. faba*.[18]

Since FUdR has never been shown to be an inhibitor of DNA synthesis in *V. faba* and since it now seems that FUdR affects chromosomes in G_2 rather than in S, a series of experiments was undertaken to see if the mechanism of the effect of FUdR on chromosomes could be found.

Results. – In experiments in which roots were treated with 10^{-5} *M* FUdR and an equal concentration of thymidine, the chromosomes showed an almost equal amount of damage as roots grown in 10^{-5} *M* FUdR alone. On the other hand, when the concentration of thymidine is 100 times greater than the concentration of FUdR, the amount of chromosome damage in the roots is greatly decreased and is only slightly higher than in control cells grown in thymidine alone.

Since increases in concentration of FUdR from 10^{-7} *M* to 10^{-5} *M* will decrease the time required for chromosome damage to become evident in cells reaching division,[18] an experiment was undertaken to see if chromosome damage could be obtained within 1 to 2 hr after FUdR treatment had begun by increasing FUdR concentration to 10^{-3} *M*. Since G_2 in *Vicia* has never been reported to be less than 2 hr, it was felt that this further decrease in the amount of time to obtain chromosome damage would be additional evidence that the chromosomes are broken in G_2 rather than in S. However, it was found that an increase of FUdR concentration to 10^{-3} *M* did not significantly decrease the amount of time required to obtain chromosome breakage.

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[17] S. S. Cohen *et al.*, *Proc. Natl. Acad. Sci. U.S.* **44**, 1004 (1958).

[18] J. H. Taylor, W. F. Haut, and Jeanne Tung, *Proc. Natl. Acad. Sci. U.S.* **48**, 190 (1962).

[19] Sandra Bell, Abstract for *Am. Soc. Cell Biol.* (November 1962).

Discussion. – Although FUdR causes its effect in some systems by inhibiting DNA synthesis, no proof has been put forward that this is true in *Vicia*. As a matter of fact, the mechanism of FUdR action that results in chromosome gaps and fragments in *Vicia* has been shown to be independent of the S period of the cell cycle. It has clearly been shown that thymidine will reverse or competitively inhibit the effect of FUdR. While the direct FUdR chromosome effect is presumably not on DNA synthesis, the thymidine results indicate that DNA may be at least indirectly involved in the chromosome damage found in *V. faba*. This does not exclude an additional effect of FUdR on proteins. Lindner and Kutkam[20] have reported an effect on histone content of tumor cells by fluorouracil, and FUdR is known to break down and produce fluorouracil. It seems likely that FUdR has multiple effects in the cell.

GENETIC ANALYSIS OF FORCED DIKARYONS BETWEEN STRAINS CARRYING X-RAY–INDUCED VIABLE OR RECESSIVE LETHAL MUTATIONS IN THE *ad-3* REGION OF *NEUROSPORA CRASSA*

F. J. de Serres

Homology tests on x-ray–induced *ad-3* mutants induced in a balanced heterokaryon showed complex interaction patterns described in terms of a complementation map in a previous report.[21] These tests and studies on HNO_2-induced *ad-3* mutations in the same system[22] suggest that the recessive lethal *ad-3* mutations induced by x rays must result from deletion of all or part of the *ad-3A* and *ad-3B* loci in addition to genetic material in immediately adjacent regions. *Ad-3* mutations recovered in such experiments now include both inter- and intragenic alterations at the *ad-3A* and *ad-3B* loci. Because of the diversity in the type of genetic damage at each locus in an essentially isogenic genetic system, the interaction of such mutations in forced dikaryons on minimal medium is of particular interest. Tests on forced dikaryons, or heterokaryons between unmarked strains, of viable *ad-3A* and *ad-3B* mutants have never shown growth at less than the wild-type rate. In the present experiments a comparison of the growth rates of various combinations of *ad-3* viable ($ad\text{-}3^{V}$) and *ad-3* recessive lethal ($ad\text{-}3^{RL}$) mutations has shown marked diversity in growth rates.

Results. – All possible dikaryons of between 5 *ad-3A* and 7 *ad-3B* mutations were recovered from the trikaryons used in the homology tests discussed in a previous report.[21] Such dikaryons are homokaryotic for the markers *al-2 pan-2 cot* and will grow rapidly on pantothenate-supplemented minimal medium at 23°C. Average linear growth rates for each dikaryon are given in Table 1. Genetic tests on mutants 9-1 and 17-1 showed that these were viable mutants with recessive lethal damage elsewhere in the genome ($ad\text{-}3B^{V}$ + RL). In 9-1 this damage is reparable ($ad\text{-}3B^{V}$ + RRL) on complete medium and is unlinked; in 17-1 this damage is irreparable ($ad\text{-}3B^{V}$ + IRL) on complete medium and is closely linked. None of the damage in the remaining *ad-3* mutants is reparable on complete medium ($ad\text{-}3^{IRL}$).

Discussion. – The linear growth rates of $ad\text{-}3^{V}$ + $ad\text{-}3^{IRL}$ combinations can be compared with that of the two viable mutants 11-0 + 1-1. Dikaryons involving mutants 13-0, 122-0, 10-1 and 121-1 (represented as extending into the "*X* region" between the *ad-3A* and *ad-3B* loci on the complementation map of the *ad-3* region[21]) show significant reductions in linear growth rate and provide evidence for a type of interaction not found in dikaryons involving two viable mutants. The extent of the genetic damage in these mutants, to the left of the *ad-3A* locus and to the right of the *ad-3B* locus, has not as yet been determined. More extensive damage in these regions, in mutants 123-1 and 122-0, than is apparent from present tests may be the basis for the greater reduction in growth rate found with 122-0 than 13-0 and 123-1 than 121-1 or 10-1. All $ad\text{-}3A^{IRL}$ + $ad\text{-}3B^{IRL}$ dikaryons show marked reductions in linear growth rate. Furthermore, there is a correlation between the rate of growth and the extent of functional damage in the "*X* region" on the complementation map.[21]

In each of the $ad\text{-}3A^{IRL}$ and $ad\text{-}3B^{IRL}$ mutations the *ad-3B* and *ad-3A* loci, respectively, are apparently genetically undamaged (damage extending into these areas should result in classification of such mutations as *ad-3A ad-3B* double

[20] A. Lindner and T. Kutkam, Abstract for *Am. Soc. Cell Biol.* (November 1962).

[21] F. J. de Serres, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 42–43.

[22] E. B. Lewis, *Advan. Genetics* **3**, 73 (1950).

Table 1. Mean Linear Growth Rates (mm/hr[a]) of Forced Dikaryons Between Strains Carrying Viable or Recessive Lethal Mutations (on Minimal Medium + 2 μg/ml Calcium Pantothenate ~23°C)[b]

	IRL 2-1	IRL 7-1	V + RRL 9-1	IRL 10-1	V + IRL 17-1	IRL 121-1	IRL 123-1	V 1-1
IRL 5-0	2.26 ± 0.03	2.10 ± 0.05	2.85 ± 0.09	1.70 ± 0.02	2.37 ± 0.02	1.99 ± 0.02	1.73 ± 0.06	2.80 ± 0.04
IRL 6-0	2.29 ± 0.08	2.19 ± 0.03	2.83 ± 0.13	1.52 ± 0.10	2.39 ± 0.04	1.79 ± 0.08	1.00 ± 0.20	2.83 ± 0.05
IRL 8-0	2.18 ± 0.04	1.62 ± 0.12	2.75 ± 0.16	1.58 ± 0.04	2.04 ± 0.09	1.41 ± 0.04	1.94 ± 0.13	2.80 ± 0.04
IRL 13-0	2.24 ± 0.06	1.56 ± 0.31	2.80 ± 0.14	c	2.30 ± 0.06	c	1.81 ± 0.16	2.77 ± 0.04
IRL 122-0	2.31 ± 0.06	1.42 ± 0.07	2.61 ± 0.09	c	2.16 ± 0.06	0.32 ± 0.02	1.08 ± 0.04	2.58 ± 0.14
V 11-0	2.86 ± 0.05	2.80 ± 0.07	2.83 ± 0.07	2.72 ± 0.05	2.79 ± 0.08	2.59 ± 0.18	2.55 ± 0.26	2.91 ± 0.08

[a]Quadruplicate growth tubes for each dikaryon, 95% confidence limits given for each estimate.

[b]Abbreviations:

IRL, irreparable recessive lethal mutation on minimal + adenine or complete medium.

RRL, irreparable recessive lethal mutation on minimal + adenine but reparable on complete medium.

V, viable mutation on minimal + adenine or complete medium.

[c]Combination not viable as a dikaryon.

mutants). Since these two loci are closely linked, dikaryons involving *ad-3*^{V} + *ad-3*^{IRL} mutations should be particularly sensitive to various types of position-effect phenomena[22] that would bring about changes in the activity of loci in immediately adjacent regions. If this is the basis for the marked reduction in the growth rates found in these dikaryons, the presence of *ad-3*^{IRL} mutations in forced heterokaryons with unlinked markers should be without effect, and combinations involving mutations 121-1, 123-1, and 122-0 should grow at the wild-type rate. Strains of the appropriate genotype are being constituted for a more complete analysis on the physiological effects of these *ad-3*^{IRL} mutations in forced dikaryons.

EVIDENCE FOR RECESSIVE LETHAL MUTATIONS RESULTING FROM GROSS CHROMOSOME DELETIONS IN BALANCED HETEROKARYONS OF *NEUROSPORA*

F. J. de Serres B. B. Webber

Genetic analyses of recessive lethal mutations induced in a heterokaryon suggest that the majority of x-ray–induced *ad-3* mutations are the result of deletions involving one or both loci.[23] The presence of closely linked biochemical markers in the adenine-requiring component of the dikaryon permitted the recovery only of those genetic alterations confined to the *ad-3* region. In theory, other events such as terminal or gross deletions involving many loci could be recovered with the appropriate supplementation of the incubation medium. The ability to recover and maintain such intergenic alterations in a balanced heterokaryon may be inferred from the experiments of Atwood and Mukai.[24] In their tests, recessive lethal mutations showing high homology were assumed to represent gross chromosomal deficiencies.

Results. – Recovery of recessive lethal *ad-3* mutations resulting from terminal or gross deletion of LGI markers was attempted by incubation of untreated and x-irradiated conidia in an adenine and niacin-supplemented medium. The location of genetic markers in each component of the balanced dikaryon used in this experiment was as follows:

	LGI						LGIII	LGIV	LGV	LGVI
1.	*A*	*hist-2*	*ad-3A*	*ad-3B*	*nic-2*	+ ;	*ad-2*;	+ ;	*inos*;	+
2.	*A*	+	+	+	+	*al-2*;	+ ;	*cot*;	+ ;	*pan-2*

Since the *hist-2* locus is very closely linked distally to the centromere, almost any intergenic alteration in the *ad-3* region induced or occurring spontaneously in component (2) should be recovered as a dikaryon in such a medium. In such an experiment 8/72 *ad-3* mutations had a requirement for both adenine and niacin; two mutations occurred spontaneously and six after x-ray treatment. The extent of the genetic damage in component (2) can be determined by making trikaryons with strains of the following composition: as component (3) *A* (LGI marker); +; *cot*; *inos*; +, and screening for *cot* colonies at 37°C by plating conidia of the trikaryon on minimal medium. Such tests with a *nic-1* marker located ~25 units distal to *nic-2* have shown that the six x-ray–induced recessive lethal mutations do not include the *nic-1* locus. Tests on the remaining two mutations are incomplete.

Discussion. – Present data from studies on spontaneous and x-ray–induced *ad-3* mutations induced in a heterokaryon have shown that a wide range of deletions of genetic material on the right arm of LGI can be recovered and maintained in a forced dikaryon. The results of the present experiments in combination with the results of homology tests on unselected samples of recessive lethal mutations[24] suggest that, with the appropriate markers, dikaryons can be obtained carrying deletions covering the entire genome. Mapping of any recessive lethal mutations induced in component (2) could be accomplished by a limited number of homology tests with tester dikaryons carrying deletions for all or part of each of the seven chromosomes.

NITROUS ACID–INDUCED RECESSIVE LETHALS IN *NEUROSPORA CRASSA*

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W. E. Barnett

Introduction. – A heterokaryon has been developed which permits the detection of both viable

[23] F. J. de Serres and Rosalie S. Osterbind, *Genetics* **47**, 793 (1962).

[24] K. C. Atwood and F. Mukai, *Proc. Natl. Acad. Sci. U.S.* **39**, 1027 (1953).

and recessive lethal mutations in the *ad-3* region of *Neurospora crassa.*[25] In this heterokaryon, 76% of the x-ray–induced *ad-3* mutants were recessive lethal, and 30% were *ad-3A ad-3B* double mutants. The *ad-3A ad-3B* double mutants were always recessive lethal. Since x rays induce intergenic as well as intragenic changes, the high incidence of recessive lethality and of *AB* doubles in the *ad-3* region may result from deletions. We have studied in the same system the effects of a chemical mutagen, nitrous acid, which is believed to induce primarily intragenic alteration.

Results. – The nitrous acid–induced *ad-3* mutants were classified as *ad-3A*, *ad-3B*, or *ad-3A ad-3B* double, and in addition as a *nic-2*–requiring mutant (if the defective region also covered the *nic-2* locus, which is approximately 2.5 crossover units from *ad-3*), by analyzing them with a set of tester strains. The mutants were further classified as to whether the component of the heterokaryon bearing the *ad-3* mutation was homokaryotic viable (V) or homokaryotic inviable (RL). These results are given in Table 2. The 70 mutants that were homokaryotic inviable were further tested to determine whether the recessive lethality was in the *ad-3* region or whether it was located elsewhere in the genome of that component. The *ad-3A ad-3B* double mutant carried its recessive lethality at the *ad-3* region, as did one or two *ad-3B* mutants. Since *ad-3A ad-3B* double mutants and *ad-3B*^{RL} mutants occur spontaneously, this low incidence cannot be attributed to nitrous acid treatment.

Discussion. – In contrast to x rays, nitrous acid does not induce mutational events at the *ad-3* region which result in *ad-3A ad-3B* double mutants or which simultaneously induce both an adenine requirement and recessive lethality in the *ad-3* region. These results can be interpreted on the basis that nitrous acid induces only intragenic changes, while x rays induce predominantly intergenic alterations.

[25] F. J. de Serres and Rosalie S. Osterbind, *Genetics* **47**, 793 (1962).

KINETICS OF THE INDUCTION OF *ad-3* MUTATIONS IN A BALANCED HETEROKARYON FOLLOWING X IRRADIATION

B. B. Webber F. J. de Serres

Following x irradiation of a balanced heterokaryon with a dose of 30 kr, one-third of the mutations were *ad-3A ad-3B* double mutants, and the majority (76%) of all mutants were recessive lethal.[25] Genetic analyses of such recessive lethal mutations suggest that they arise from intergenic alterations and represent a series of deletions of varying size.[26] One might expect this class of mutation to result from two-hit events and to increase exponentially with dose. A study on the kinetics of induction is being made to elucidate the mechanism of induction of *ad-3* mutations with x rays and to serve as a basis for similar studies with HNO_2 and 20-Mev protons.

[26] F. J. de Serres, this report.

Table 2. Nitrous Acid–Induced Mutation in *Neurospora*

HNO_2 Exposure (min)	Survival (%)	*ad-3* Mutants (per 10^6 survivors)	Number *ad-3* Mutations Tested							
			Total	*ad-3A*		*ad-3B*		*ad-3A*	*ad-3B*	*nic-2*
				V	RL	V	RL	V	RL	
0	100	0.5	4	0	0	4	0	0	0	0
30	79	55.6	112	20	1	85	6	0	0	0
60	44	78.4	100	14	1	73	12	0	0	0
90	14	113.9	98	19	2	60	16	0	1	0
120	2	139.5	108	20	9	57	22	0	0	0

Results. – A series of experiments was carried out involving irradiation (with varying doses of x rays; 250 kvp, 30 ma, 3-mm Al filter) of conidia of a heterokaryon with the following two components:

	LGI					LGIII	LGIV	LGV	LGVI
1.	*A*	*hist-2*	*ad-3A*	*ad-3B*	*nic-2*	+	*ad-2*;	+;	*inos*;	+
2.	*A*	+	+	+	+	*al-2*;	+	*cot*;	+;	*pan-2*

The average percentage survival of the heterokaryotic conidia from this dikaryon on minimal medium is presented in Table 3, along with the number of *ad-3* mutations per 10^6 survivors. The data on the survival of the heterokaryotic fraction fits a simple exponential or single-target curve, as expected from similar studies by Atwood and Mukai.[27] However, the frequency of *ad-3* mutations increases nonlinearly with dose, indicating that a substantial fraction of the mutants recovered result from multihit events.

Discussion. – If the total number of *ad-3* mutations recovered includes a one-hit component that increases linearly with dose in addition to a multicomponent that increases as the square of the dose, present data indicate the relative percentages of the *ad-3* mutants attributed to two-hit phenomena would be as follows: 2 kr, 15%; 10 kr, 47%; and 30 kr, 73%. Genetic analysis of approximately 100 *ad-3* mutations from each of four x-ray doses has been initiated to attempt to determine the class or classes of mutations that can be attributed to two-hit events.

[27] K. C. Atwood and F. Mukai, *Am. Naturalist* **88**, 295 (1954).

Table 3. Survival and *ad-3* Mutation Induction in a Balanced Dikaryon Following X-Ray Treatment

X-Ray Dose (kr)	Percentage Survival	*ad-3* Mutations (per 10^6 survivors)
0	100.0	0.3
1	93.5	4.3
2	83.5	11.3
5	61.5	38.5
10	50.5	100.8
20	13.5	
40	2.4	

THE ACTION SPECTRA FOR UV-INDUCED CHROMOSOME ABERRATIONS OF MAMMALIAN CELLS PRETREATED WITH 5-BUdR AND 5-IUdR

E. H. Y. Chu

Introduction. – The action spectrum for ultraviolet-induced mammalian chromosome aberrations approximately parallels the uv absorption spectrum of nucleic acids.[28] Since DNA is a major component of the chromosome, our data are highly suggestive that DNA is the primary site of uv injury. Recent experiments indicate that incorporation of thymidine analogues into the cells significantly modifies the action spectrum, lending a strong support to the above conclusion.

Materials and Methods. – Chinese hamster cells grown directly in quartz dishes were irradiated with monochromatic uv light. For varying periods prior to irradiation, different concentrations of 5-bromodeoxyuridine (BUdR) or 5-iododeoxyuridine (IUdR) were included in the growth medium. Cells were washed after irradiation, allowed to continue growth for 24 hr (the last 18 hr in the presence of colchicine), fixed, and analyzed for chromatid aberrations.

Results. – A few hours' treatment with BUdR did not induce chromosome abnormalities. Prolonged exposures (24 and 48 hr) of cells to 10^{-5} *M* BUdR did not inhibit mitosis and induced only infrequent chromosome aberrations, although higher concentrations caused increasingly severe damages to the cell.

[28] E. H. Y. Chu, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 75–77.

Cells pretreated with BUdR for 24 hr showed an increased sensitivity to uv in terms of induced chromosome aberrations. The most significant modification of the uv action spectrum was observed in cells which had been exposed to BUdR for 48 hr (Table 4). Particularly noteworthy is the tremendous increment of the aberration rate at 2250 A and 3130 A. There seemed to be no effect at 3650 A. These results have been confirmed in another low-dose experiment in which a proportionate modification of the action spectrum was observed. Qualitatively, uv seemed to induce relatively higher frequencies of isochromatid deletions in BUdR-treated cells.

The IUdR-substituted cells also had a modified uv action spectrum. The IUdR- or BUdR-substituted cells responded differently to uv at various wavelengths.

Discussion. – 5-Bromouracil (BU) or BUdR has been shown to be incorporated, with concomitant thymine or thymidine replacement, into the DNA of bacteria,[29] bacteriophage,[30] and mammalian cells.[31] Hsu and Somers[32] showed that BUdR can induce chromosome breaks, particularly at the heterochromatic regions. The earliest BUdR-induced breakage occurred at 12 hr after treatment, after almost all cells had completed DNA synthesis. Our results confirmed this last finding,

[29] S. Zemenhof *et al.*, *Nature* **181**, 827 (1958).

[30] R. M. Litman and A. B. Pardee, *Nature* **178**, 529 (1956).

[31] B. Djordjevic and W. Szybalski, *J. Exptl. Med.* **112**, 509 (1960).

[32] T. C. Hsu and C. E. Somers, *Proc. Natl. Acad. Sci. U.S.* **47**, 396 (1961).

Table 4. Ultraviolet Action Spectra for Chromatid Aberrations of Thymidine Analogue-Substituted and -Unsubstituted Chinese Hamster Cells *in vitro*

Treatment	Cells Scored	Percent Normal	Terminal Deletions	Isochromatid Deletions	Exchanges	Aberrations per Cell	Ratio[a]
BUdR + 2250[b]	145	30	140	72	131	2.37	38.8
IUdR + 2250	199	75	45	11	12	0.34	4.0
2250	200	93	9	2	4	0.07	
BUdR + 2652	96	34	96	84	92	2.83	3.9
IUdR + 2652	193	58	81	10	74	0.85	1.1
2652	102	63	31	6	34	0.70	
BUdR + 3130	282	82	42	20	8	0.25	6.0
IUdR + 3130	137	83	16	10	4	0.27	7.5
3130	198	97	6	0	0	0.03	
BUdR + 3650	201	94	9	3	2	0.07	0
IUdR + 3650	200	95	9	1	0	0.05	0
3650	113	99	1	0	0	0.01	
BUdR	200	91	15	3	5	0.12	
IUdR	150	90	12	4	0	0.11	
Control	200	99	1	1	0	0.01	

[a] Corrected aberration frequency in analogue-substituted cells over that in analogue-unsubstituted cells.
[b] UV surface exposure of 100 ergs/mm^2 was given for each wavelength (A) series.

indicating perhaps that BUdR substitution results in errors of DNA duplication and thereby chromosome breakages.

The analogue-substituted cells exhibit a marked increase in radiosensitivity when exposed to uv or x rays.[29-31] Boyce and Setlow[33] presented evidence that *Escherichia coli* cells in which thymine had been partially substituted by BU were more uv sensitive, especially at wavelengths below 2400 A and above 2800 A. The present results also indicate a similar modification of uv-induced chromosome aberrations in BUdR-substituted cells. The effect is more pronounced when cells are given a longer period of preirradiation exposure to BUdR. The fact that a direct chemical alteration of the DNA molecules modifies the uv radiosensitivity is consistent with the view that the initial DNA damages by uv probably account for the majority of chromosome breakage.

[33]R. Boyce and R. B. Setlow, *Biochim. Biophys. Acta* (in press).

Drosophila Cytology and Genetics

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A COMPUTER PROGRAM FOR THE SEQUENTIAL SCORING OF *DROSOPHILA* DATA FOR THE PRESENCE OF X-RAY-INDUCED TRANSLOCATIONS

D. L. Lindsley, Jr.

Ordinarily in experiments designed to detect translocations, the progeny of sons of irradiated males are scored for the appearance of linkage between normally unlinked genes. The only euploid gametes that are produced by an individual heterozygous for a reciprocal translocation are those that receive both translocated elements and those that receive the normal homologues of the translocated elements. In *Drosophila* males, where there is no crossing over, this results in completely linked inheritance of genes that are on the translocated elements and of genes that are on their normal homologues; in females, on the other hand, crossing over may separate marker genes from the breakpoints, and the pseudolinkage associated with translocation heterozygosis becomes considerably obscured and difficult to detect. This is the reason that sons rather than daughters of irradiated males are chosen for translocation detection.

Depending on the type of female to which an irradiated male is crossed, the sons will inherit either an irradiated X or an irradiated Y chromosome, and consequently the sons may have a translocation involving the X or Y chromosome. It is known that more than 90% of translocations involving the X chromosome are either lethal or sterile in the male and about 50% of translocations involving the Y chromosome are sterile. Thus a large proportion of the sons of irradiated males that carry translocations of the sex chromosomes are unable to breed, and sex-linked translocations are preferentially eliminated from the scored sample.

We have been interested in X;autosome translocations, and so we have had to utilize the

daughters of irradiated males. It is possible to partially obviate the disastrous effect of crossing over on the recognition of translocations by using multiply inverted chromosomes, but absolute linkage of markers on the translocated elements or on their normal homologues cannot be achieved even with our most effective crossover suppressors. Consequently, the progenies must be counted. Since each progeny may contain 48 different phenotypes and tests but a single irradiated genome, to score a large number of treated genomes would involve a prodigious amount of counting if each progeny were counted to completion.

To minimize the amount of counting required to score translocations, we adopted the procedure of counting on alternate days and analyzing the data following each count. Each progeny is compared with control progenies at comparable stages of eclosion; those showing no difference from the control are discarded. Thus, following each day's count, the sample retained for additional counting is further reduced. The data from each culture are recorded on forms and immediately punched on cards. They are then added to the total from any previous counts of the culture, and a new deck of updated, subtotal cards is produced. This deck together with an IBM 7090 program deck is then sent to the Control Data Processing Facility at ORGDP, and the results are returned in time to decide which cultures to retain for the subsequent count.

The program pools classes in various ways. It determines the total number of flies counted in the culture, the number of exceptional males, the number of exceptional females, the number of flies that inherit a Y chromosome from their father, the number of males with the irradiated X chromosome (equals 0 if there is a sex-linked-recessive-lethal mutation), the number of flies that receive the irradiated X chromosome, the irradiated second chromosome, the irradiated third chromosome, or the irradiated fourth chromosome from their mother (this allows detection of abnormal transmission of any irradiated chromosome). Also recorded are the numbers of flies that agree with the cultures carrying a T(1;2) (i.e., those with both irradiated X and irradiated 2 or both unirradiated X and unirradiated 2 inherited from their mother), a T(1;3), a T(1;4), a T(2;3), a T(2;4), and a T(3;4). The proportion that each of these subtotals is of the total is compared with the corresponding proportion from a control count, and the probability that the two proportions are drawn from the same population is determined by using the binomial distribution. The decision to continue counting or to discard a culture is based on a combination of the following factors: (1) total flies counted, (2) presence of exceptional progeny, (3) evidence of a sex-linked-recessive lethal, and (4) the various probabilities.

This program is in use in several investigations currently in progress in the *Drosophila* Genetics Group.

MUTABILITY OF AN ASYNAPSED SECOND CHROMOSOME DUPLICATION IN *DROSOPHILA MELANOGASTER*

F. J. Ratty

This study was undertaken to determine the mutation rate of selected loci in Duplication (w) 51b7. The duplicated segment is located in the right arm of the second chromosome in region 52E or F and extends from 3C2 to 3D2. To determine the mutation rate in the asynapsed chromosome segment, larvae were irradiated in which the most mature cells were primary spermatocytes. These larvae were obtained from eggs collected during a 4-hr period and incubated for 112 hr; at this time the larvae were in the third instar just prior to pupation. The genotype of the male larvae was $y\ w\ fa^{no}/Y;\ Dp(1;2)w^{+}/vg^{U}\ Pin^{2}$. The duplication was made heterozygous for vg^{U} and Pin^{2} to detect losses due to induced male crossing-over. The 112-hr-old larvae were separated from the medium upon which the eggs were laid, irradiated with 1300 r, and returned to standard cornmeal medium. When the irradiated $y\ w\ fa^{no}/Y;\ Dp(1;2)w^{+}/vg^{U}\ Pin^{2}$ males emerged, they were isolated during a 12-hr period before they had an opportunity to mate. They were aged 24 hr and pair-mated with 48-hr-old $y\ w\ fa^{no}/y\ w\ fa^{no};\ +/+$ females. The males remained with the females for 24 hr so that they would copulate only once. If the males mated more than once, the sperm used would have been derived from cells that were spermatogonia at the time of irradiation.

The incidence of fertilized females obtained from the above mating was about 50%. Attempts to increase the fertility by varying both the age of the male at the time of mating and the length of

Table 5. Regular Progeny from the Cross Irradiated $y\ w\ fa^{no}/Y$; $Dp(1;2)w^{+}/vg^{U}\ Pin$ **Males by** $y\ w\ fa^{no}$ **Females**

Number of Males Mated	Number of Males Fertile	♀ (w^{+}–vg^{U}	Pin^{2})	♂ (w^{+}–vg^{U}	Pin^{2})	Total
1549	661	6196	6233	6260	5959	24,648

time that he remained with the female were not successful. Concern was expressed that the male infertility might be due to the killing of primary spermatocytes at the time of irradiation. Fertility tests were made on males that had remained infertile during a 5-day mating period. It was observed that the testes of these males contained motile sperm, and when similar males were subsequently mated, they proved to be uniformly fertile. Cytological observation of pupal testes at various times after irradiation did not show the presence of cellular debris caused by the death of radiation-injured cells. These observations suggest a delay in meiosis rather than a loss of cells that were killed by the radiation.

The progeny of the irradiated $y\ w\ fa^{no}/Y$; $Dp(1;2)w^{+}/vg^{U}\ Pin^{2}$ males can be classified into two groups of males and females. One class possesses the duplication w^{+}, while the other contains the two dominant markers $vg^{U}\ Pin^{2}$. A summary of the numbers and types of progeny in each class is presented in Table 5.

The mutants recovered following the irradiation of the duplication-bearing larvae can be divided into four categories. One class has a variegated eye color that is presumably caused by an association of the w^{+} locus with a large heterochromatic region. In addition to the heterochromatic involvements of the duplication, various parts or all of the segment may be deleted. The types of changes and their frequencies are indicated in Table 6. With the exception of the two mutants that carried the w^{+} locus but evidenced loss of N^{+} by showing the phenotype associated with

Table 6. Presumptive Changes of $Dp(1;2)w^{+}$ **Recovered from the Cross Shown in Table 5**

Variegated	Losses of w^{+}-N^{+}	w^{+}	N^{+}
2	7	0	2

Table 7. Recombination in the vg^{U} **to** $Dp(1;2)51b7$ **to** Pin **Region from** $Dp(1;2)51b7/vg^{U}\ Pin$ **Males**

	($vg\ w^{+}\ Pin^{+}$)	($vg^{+}\ w\ Pin$)	($vg\ w\ Pin^{+}$)	($vg^{+}\ w^{+}\ Pin$)	($vg^{+}\ w\ Pin^{+}$)	($vg\ w^{+}\ Pin$)
Males	3	1	3	3	*a*	1
Females	2	0	5	2	*a*	1
	Single crossovers region 1		Single crossovers region 2		Double crossovers	

$$\frac{+ \qquad w^{+} \qquad +}{vg \qquad w \qquad Pin}$$

24,648 Total F_1

[a]The seven losses of both the w^{+} and N^{+} loci recorded could have resulted from double crossing-over, nondisjunction, or breakage and loss. At the present time, it is not possible to distinguish between these alternatives.

fa^{no}, all of the mutants represented the simultaneous loss of w^+ and N^+. There were no mutants that indicated the loss of the w^+ locus from the duplication although this class of mutants could have been readily detected. It would appear that both loci are lost from the autosome more frequently than the individual genes.

Phenotypic changes can also be produced by induced crossing-over. The arrangement of the loci on the chromosome and the frequency of the various recombinant classes are indicated in Table 7.

A tentative conclusion derived from this study is that breaks which occur at points of nonhomology result more frequently in the loss of the entire duplication than the loss of individual loci contained therein. The studies are being continued by using a modification of the genotypes indicated above to rule out alternative hypotheses accounting for the simultaneous loss of the w^+ and N^+ loci from the duplication. Comparisons will also be made using the duplication in a homozygous rather than a heterozygous condition.

SOME EFFECTS OF OXYGEN, HELIUM, AND METABOLIC INHIBITION ON DOMINANT LETHAL PRODUCTION IN STAGE 7 AND STAGE 14 OOCYTES OF *DROSOPHILA MELANOGASTER*

R. R. Rinehart

Introduction. – Studies of x-ray-induced dominant lethality in two stages of *Drosophila melanogaster* primary oocytes, designated stage 7 and stage 14 by King, Rubinson, and Smith,[1] have shown that the stage 7 oocytes require approximately 10 times the x-ray dose needed to induce the same amount of lethality in the stage 14 oocytes. Dose fractionation studies of these meiotic cells have shown that there is a significant decrease in the dominant lethality induced in stage 7 oocytes when the doses are given 15 min or more apart. This indicates a very rapid repair of irradiation damage in these cells. No such repair has been noted in stage 14 oocytes, even when the x-ray doses were given 24 hr apart.[2] Two hypotheses are being tested in an effort to determine possible reasons for this differential radiosensitivity and for the lack of repair in the stage 14 oocytes. They are (1) some of the differences in the irradiation response of these two types of cells may be due to different amounts of cellular oxygen, a known enhancer of irradiation damage, and (2) a sufficient amount of energy or metabolic activity necessary for repair of irradiation damage may be lacking in the stage 14 oocytes. Thus meiotic movement, prior to the repair of broken chromosomes, might lead to chromosomal imbalance and lethality in the stage 14 oocytes, while restitution of many induced breaks in the stage 7 cells would not be detected as dominant lethals.

Methods. – *D. melanogaster* females contain approximately 24 ovarioles. In newly emerged females the most mature oocyte present in each ovariole is the stage 7 cell. Flies matured for 5 to 6 days usually contain one stage 14 oocyte per ovariole. Thus by irradiating either newly emerged or matured females and counting up to the first 24 eggs laid by each type of fly, a fairly homogeneous sample of stage 7 and stage 14 oocytes can be irradiated and collected. A ratio of the number of pupae developing to the total number of eggs laid gives the parameter of x-irradiation damage.

Results and Conclusions. – Table 8 shows that stage 7 and stage 14 oocytes respond almost identically to similar alterations in their environmental oxygen concentration. This is true whether (1) they are actively metabolizing or (2) enzymatic removal of oxygen has been greatly reduced due to a lowered temperature. Therefore, it is concluded that there is no difference in radiosensitivity between these cells due to a differential amount of cellular oxygen in either of these types of cells.

Table 9 shows the effect of pretreating the different types of flies under anoxic conditions for increasing lengths of time prior to x irradiation. Stage 7 oocytes become anoxic almost immediately. However, the stage 14 oocytes require approximately 5 min more pretreatment time to reach this anoxic state. After longer than 1 hr of pretreatment with helium, the stage 7 oocytes again become more sensitive to x rays. Since the amount of dominant lethality remains approximately the same in nonirradiated but helium pretreated egg samples, it is felt that the helium pretreatment is not simply

[1] R. C. King, A. C. Rubinson, and R. F. Smith, *Growth* **20**, 121 (1956).

[2] D. R. Parker, *Biological Contribution, Univ. Texas Publ.* **5914**, 113 (1959).

Table 8. Effect of Various Oxygen Tensions on Survival of Irradiated Stage 7 and Stage 14 Oocytes of *D. melanogaster*[a]

Oocyte Stage	Pretreatment Gas	Temperature (°C)	X-Ray Dose (r)	Survival (%)	
				X Ray	Control
7	Air	23–25	3000	45.3	91.3
14	Air	23–25	300	46.9	91.3
7	Air	3–5	3000	40.3	91.2
14	Air	3–5	300	39.6	93.0
7	60 min O_2	23–25	3000	42.5	92.4
14	60 min O_2	23–25	300	38.4	94.1
7	60 min O_2	3–5	3000	36.8	88.4
14	60 min O_2	3–5	300	35.5	92.5
7	60 min He	23–25	3000	75.4	91.7
14	60 min He	23–25	300	74.2	92.4
7	60 min He	3–5	3000	78.8	90.7
14	60 min He	3–5	300	77.8	92.2

[a]Irradiation was given in pretreatment gas and temperature.

Table 9. Effect of Increasing Periods of Anoxia Prior to X Irradiation in Stage 7 and Stage 14 Oocytes of *D. melanogaster*[a]

Oocyte Stage	Pretreatment Gas	Temperature (°C)	X-Ray Dose (r)	Survival (%)	
				X Ray	Control
7	Air	23–25	3000	45.3	90.8
14	Air	23–25	300	46.8	91.3
7	5 min He	23–25	3000	78.0	91.3
14	5 min He	23–25	300	69.0	92.9
7	10 min He	23–25	3000	75.4	88.4
14	10 min He	23–25	300	75.7	95.3
7	30 min He	23–25	3000	77.8	91.7
14	30 min He	23–25	300	75.2	93.1
7	60 min He	23–25	3000	74.8	90.3
14	60 min He	23–25	300	74.2	92.7
14	2 hr He	23–25	300	63.0	80.2
7	6 hr He	23–25	3000	66.2	92.6
7	14 hr He	23–25	3000	50.6	86.9

[a]Irradiation was given in pretreatment gas.

killing the eggs. The stage 14 oocytes also show an increase in dominant lethality after 1 hr anoxia; however, this lethality can be accounted for by cell death due to the gas, since the control egg samples show a similar amount of nondevelopment. Matured flies, containing stage 14 oocytes, are killed by more than 2 hr of anoxia.

Table 10 demonstrates the effect of pretreating the two cell types with helium and then irradiating under a normal air atmosphere. Stage 14 oocytes irradiated in air immediately after a 10-min pretreatment in helium show a greater amount of dominant lethality than cells irradiated in air with no prior anoxia. Increasing the helium pretreatment time to 60 min does not significantly increase the amount of damage observed. Stage 7 oocytes, however, show very little increase in damage after 10 min helium prior to the immediate irradiation in air. After 60 min anoxia the stage 7 cells show an increased x irradiation response, and after 6 hr of helium prior to being x irradiated, the amount of damage observed is approximately 38% more than if the irradiation were carried out in air with no preanoxia.

Since the amount of damage observed is less than the level of x-ray-induced dominant lethality with a similar x-ray dose but irradiated in 100% oxygen and at a low temperature to ensure a maximum amount of cellular oxygen, it is concluded that the observed damage must be attributed to factors other than just irradiation enhancement due to the oxygen effect.

At the present time two hypotheses seem the most likely to explain the observed differences in the irradiation and gas pretreatment response of these cells:

1. Residual amounts of energy may be depleted or metabolic activity may be reduced in the stage 7 oocytes by the anoxic treatment given prior to irradiation. This may lead to a lack of repair of

Table 10. Effect of Preirradiation Anoxia upon Survival of Stage 7 and Stage 14 Oocytes of *D. melanogaster*[a]

Oocyte Stage	Pretreatment Gas	Temperature (°C)	X-Ray Dose (r)	Survival (%)	
				X Ray	Control
14	Air	23–25	300	46.9	91.3
14	60 min O_2	3–5	300	35.5	92.5
14	10 min He 5 min Air	23–25	300	31.9	93.7
14	60 min He 5 min Air	23–25	300	29.2	90.5
14	60 min He 5 min O_2	23–25	300	28.3	87.8
7	Air	23–25	3000	45.3	91.3
7	60 min O_2	3–5	3000	36.8	88.0
7	10 min He 5 min Air	23–25	3000	42.7	90.0
7	60 min He 5 min Air	23–25	3000	30.0	93.1
7	60 min He 5 min O_2	23–25	3000	25.6	86.2
7	6 hr He 5 min Air	23–25	3000	6.7	89.9

[a]Irradiation was given in second pretreatment gas.

irradiation damage usually associated with these cells and to a greater dominant lethal rate in the stage 7 oocyte. This same effect is noted in the stage 14 oocytes; however, it is much less, it occurs with 10 min prehelium, and it cannot be shown to increase with a longer anoxic pretreatment period. This may mean that there is only a small amount of energy available for repair in these cells and that this is quickly depleted.

2. An alternate hypothesis is that the helium pretreatment sensitizes the cells to x irradiation.

Tests are in progress that should demonstrate more clearly whether either of these alternatives is more likely or if other explanations are indicated.

STUDIES ON COMPETITION BETWEEN CHROMOSOMES FOR GENETIC EXCHANGE

E. H. Grell

Competitive pairing is a term introduced by Dobzhansky[3] to account for reductions in crossing-over in three situations: (1) in regions homologous to duplications, (2) in regions near breakpoints of translocations when translocations are heterozygous, and (3) in the vicinity of heterozygous inversions.

A study of competitive pairing in a proximal section of the X chromosome of *Drosophila* was made using two 1,4 translocations, a multiply inverted X chromosome and normal sequence chromosomes. The crossing-over was always measured between *Bar* and $sc^{VI}y^{+}$ which is transposed to the right of the centromere.

In females with two normal X chromosomes this region is 9.9 ± 0.9 map units in length. In the homozygous translocations it is 13.7 ± 1.2 units. If the translocation is heterozygous the genetic length of the region drops to 2.1 ± 0.4. This decrease is a demonstration of competitive pairing in situation (2). Competitive pairing of the first kind is observed in an experiment in which females carried two normal chromosomes and the proximal part of X from *Bar* through the centromere as a duplication. Crossing-over within the duplication is only 0.4 ± 0.2 map unit. In an attempt to use a complexly rearranged chromosome to disrupt pairing between the two entire X chromosomes so the duplication could pair and cross over more often with the normal X, females were produced that carried FM4, a normal X, and the duplication. The presence of the rearrangement FM4 did not significantly increase crossing-over within the duplication. It is concluded that the rearranged X is able to interfere with crossing-over between the duplication and a normal X as much as a second normal X. An often-made assumption that two structurally very different homologues are asynapsed at exchange pairing does not appear to be warranted.

[3]Th. Dobzhansky, *Z. Induktive Abstammungs-Vererbungslehre* **68**, 134 (1935).

FACTORS AFFECTING DISTRIBUTIVE PAIRING OF NONHOMOLOGUES

Rhoda F. Grell

Meiotic associations between nonhomologues leading to nonrandom assortment may be induced in *Drosophila* females with extremely high frequencies. When the number of chromosomes available for nonhomologous pairing is greater than two, competition for partners occurs among them and marked preferences are shown.[4] Possible causes for the preferences include shared proximal homology, similarity in size of the nonhomologues, and the presence of sites so arranged as to favor pairing between particular nonhomologues. Earlier work indicated that proximal homology is probably a negligible factor.[4] Experiments have now been designed to study the role of chromosome size and of specific sites in the determination of preferences.

Materials and Methods. – A number of X-chromosome duplications that had been obtained and cytologically characterized by Cooper and Krivshenko[5] were utilized for the tests. All of the duplications carry the distal tip of the X chromosome and varying amounts of proximal X heterochromatin so that they form a series, which, upon comparison with a normal metaphase fourth chromosome, ranges in size from one barely discernible (1187) to one 3 times the length of chromosome 4. Each duplication was introduced into females of identical genotype so as to produce a number of stocks that differ only in the duplication they

[4]Rhoda F. Grell and E. H. Grell, *Proc. Natl. Acad. Sci. U.S.* **46**, 51 (1960).

[5]Personal communication.

possess. In addition to the duplication the females carried an extra fourth chromosome, a homozygous 3;4 translocation, and genetic markers suitable for following the segregation of the duplication, the extra fourth, and the X chromosomes. The pairing behavior of each duplication with the free fourth chromosome was measured by the frequency of duplication-4 nondisjunction.

Results and Discussion. – The data in Table 11 show that a strong correlation exists between pairing regularity and similarity in size of the duplication and chromosome 4. The lowest nondisjunction frequencies occur with duplications closest in size to chromosome 4 (class C). In this class, duplication-4 nondisjunction averages about 0.08% and does not differ significantly from the rate of primary nondisjunction of two normal X homologues in a normal genome. Thus, for this range (0.7–1.1) nonhomologues exhibit the same regularity in pairing as do homologues, while duplications of smaller (classes A and B) or larger (classes D and E) size show increased frequencies of nondisjunction.

The results indicate that size alone is not responsible for pairing efficiency since the progressive change in duplication size is not associated with correlated changes in nondisjunction frequency. Instead, the duplications form five discrete nonoverlapping classes (A–E) within which variations in size apparently cause no related change in segregation behavior. This suggests the presence of sites that contribute to or detract from pairing efficiency. One marked change in segregation occurs between the duplication in class E (size 2.5 to 3.0) which carries the nucleolar organizer region and those in class D (size 1.4 to 2.0) which do not. Classes A and B show the greatest shift in nondisjunction frequency, although, with respect to size, duplica-

Table 11. Frequencies of Nondisjunction Between X Duplications of Different Sizes and Chromosome 4

Class	Duplication Number	Duplication[a] Size	N.O.	Other Constrictions	Percent Duplication-4 Nondisjunction (X regulars)	Nondisjunction (% X)	Duplication-4 Nondisjunction (X exceptions)	Numbers
A	1187	~0.3	–	–	4.49 ± 0.19	0.45 ± 0.06	8/27	12,113
B	1162	0.5	–	–	0.39 ± 0.07	0.32 ± 0.06	1/13	8,000
C	816	0.7	–	–	0.12 ± 0.06	0.43 ± 0.11	0/7	3,276
	1204	0.9	–	+	0.08 ± 0.03	0.62 ± 0.10	0/23	7,391
	1193	1.0	–	?	0.13 ± 0.05	0.37 ± 0.08	0/10	5,371
	1144	1.1	–	–	0.02 ± 0.02	0.88 ± 0.10	1/37	8,476
	1339	1.1	–	?	0.08 ± 0.03	0.76 ± 0.10	1/27	7,126
D	1337	1.4	–	?	0.38 ± 0.08	0.75 ± 0.12	0/21	5,635
	1186	1.6	–	+	0.39 ± 0.08	0.70 ± 0.11	1/21	5,991
	1346	2.0	–	+	0.42 ± 0.07	0.97 ± 0.11	0/39	8,011
E	1488	2.5	+	+	2.03 ± 0.39	1.19 ± 0.30	2/8	1,344
	856	3.0	+	+	1.89 ± 0.24	1.42 ± 0.20	6/24	3,377

[a]As compared to chromosome 4.

tions 1187 and 1162 are less dissimilar than many within a single class.

The frequency of X nondisjunction rises gradually with the increasing size of the duplications. Among the exceptional X-progeny, duplication-4 segregation is fairly regular, particularly in classes B, C, and D, implying that these chromosomes are not involved in any major, direct way with nondisjunction of the X's.

STUDIES ON CHROMOSOMAL RNA IN *DROSOPHILA VIRILIS*

P. A. Roberts

RNA occurs in specific regions of the salivary chromosomes and may be identified by staining with the basic dye Azure B, which, through metachromasy, stains DNA blue and RNA purple. The maximum number of RNA bands is seen in the late third instar larva just prior to formation of the prepupa. Although as many as 175 RNA bands can be seen at this time, this represents the minimum number of active loci, since RNA distribution ranges from large amounts in puffs down to barely perceptible bands. At the beginning of the prepupa instar the contents of the glands are discharged and the glands begin to degenerate. However, RNA can still be found at numerous sites along the chromosomes as late as 6 hr after prepupa formation and it is not until the prepupa is 12 hr old that RNA has largely disappeared from the degenerating chromosomes. This raises the question of whether the accumulation of RNA at these many loci represents a breakdown of RNA transport or possibly a failure of inhibition of RNA synthesis.

Since there is now considerable evidence that somatic variegation is not the result of somatic mutation, several translocations were examined to see whether there is any disturbance in the formation of chromosomal RNA in euchromatic regions which have been placed next to heterochromatin. The translocations studied were $T(2;5)pe^{m39}$, $T(3;5)pe^{m4}$, and $T(3;5)pe^{m51}$, rearrangements which produce somatic variegation of *pe*, a gene normally located in heterochromatin. There was no observable difference in the euchromatic chromosomal RNA pattern between the rearranged chromosomes and controls of the Texmelucan strain.

AUTOSOMAL NONDISJUNCTION IN CLARET-NONDISJUNCTIONAL *DROSOPHILA* FEMALES

D. G. Davis

Introduction. – It is known that *Drosophila melanogaster* females homozygous for the mutant ca^{nd} produce a high incidence of nondisjunction and loss of chromosomes.[6] These phenomena produce exceptional sons (20%), exceptional daughters (4%), gynandromorphs (5%), haplo-4 (40%), and haplo-4 mosaic (5%) offspring in progenies from ca^{nd} females.[7] Regular offspring from such females were found, by test-crossing, to contain about 6% triplo-4 individuals.[8] The disparity in frequencies between exceptional males and females, the gynandromorphism, and the haplo-4 mosaicism are best explained by elimination or loss of chromosomes.

It was noted that haplo-4 frequencies differed strikingly among the exceptional male (63%), exceptional female (3%), and regular (38%) classes, suggesting possible nonrandom involvement of chromosomes 4 and X in loss. Nondisjunctional classes could not be so compared. Moreover, the above data were probably seriously biased by haplo-4 inviability. The mating systems described below were then devised to test simultaneously autosomal and X chromosomal nondisjunction and loss. Nondisjunction of the second or third chromosomes would ordinarily yield inviable zygotes. In fact, ca^{nd} females are highly sterile, while egg-laying is apparently normal, suggesting inviability from autosomal aneuploidy.

Methods. – Behavior of chromosomes 4 and X was studied by mating y/y; ca^{nd}/ca^{nd}; ci^{D}/spa^{Cat} females to males having a compound chromosome 4. This system permits recovery and recognition of regular and exceptional chromosome-4 classes without significant inviability. Behavior of chromosomes 2 and 3 is being studied by mating ca^{nd} females to males having either compound chromosomes 2 or 3.

Results and Discussion. – Table 12 shows percentage of recovery of chromosome-4 egg types

[6] E. B. Lewis and W. Gencarella, *Rec. Genet. Soc. America* **21**, 44 (1952).

[7] C. W. Hinton, unpublished data.

[8] D. G. Davis, unpublished data.

Table 12. Percentage Recovery of Chromosome-4 Egg Types

X Class (Egg Type)	Nullo-4 (%)	Diplo-4 (%)	Mono-4 (%)	Total Progeny
Regular males (X)	58.0	3.4	38.6	33.6
Regular females (X)	58.4	3.1	38.5	41.5
Regular gynandromorphs (X)	67.8	0.6	31.6	4.0
Exceptional males (O)	88.3	0.5	11.2	18.3
Exceptional females (XX)	27.5	27.0	45.5	2.3
Exceptional gynandromorphs (XX)	48.6	5.7	45.7	0.4
Total progeny	63.4	3.2	33.5	

in various X-chromosome genotypes and percentage of X types and percentage of four types in the total progeny ($N = 8307$).

Data currently being collected on chromosome-2 behavior indicate that a slight excess of nullo-2 over diplo-2 eggs are being recovered. However, the ratio appears considerably less than was observed for nullo-X:diplo-X (6:1) and nullo-4:diplo-4 (20:1). Since the mating system does not provide recovery of eggs derived from regular autosomal disjunction, and since frequencies of sperm derived from nondisjunction of compound autosomes in the male are unknown, it is not possible to derive frequencies.

Distribution of exceptional chromosome-4 egg types in relation to exceptional X-chromosome egg types indicates a correlation (1) between loss of X and loss of 4, and (2) between nondisjunction of X and nondisjunction of 4. The inference of coincident loss of X and 4 from earlier haplo-4 data is strengthened by present results. However, this is the first demonstration of coincident nondisjunction of X and 4 under the influence of ca^{nd}.

GENETIC AND CYTOLOGICAL EXTENSION OF THE NOTCH LOCUS

W. J. Welshons Elizabeth S. Von Halle
Bobbie J. Scandlyn

Introduction. – Previous investigations have demonstrated that the Notch locus in *Drosophila* is a complex one composed of pseudoallelic recessive visible mutants superimposed upon an array of pseudoallelic recessive lethals, alternatively referred to as dominant Notch (N) mutants. The linear order from left to right on the X chromosome of recessive visibles and lethals is as follows: fa–fa^{no}–N^{40}–N^{Nic}–spl–N^{103}–N^{j24}–N^{Co}–N^{g11}–nd. The order of N^{g11} and nd relative to each other is tentative, but both are certainly to the right of N^{Co}. The genetic length of the entire region fa to nd has been estimated as 0.11 unit.

Results. – In a previous publication[9] it was noted that no recessive lethals (N's) had yet been discovered in the region to the left of fa^{no}, but it was presumed that they would eventually be found there. Recently, recombination experiments performed with N^{e11} and spl yielded results which placed the N to the left of spl and in the vicinity of fa. Similar data obtained from N^{e11}/fa heterozygotes placed the lethal 0.03 unit to the left of fa, and thereby increased the genetic length of this complex locus to 0.14 unit.

Similarly designed experiments utilizing N^{39}, fa, and spl placed this recessive lethal in the region of fa^{no}. Since N^{39} was known to be cytologically deficient for salivary band 3C7, it was important to see if this deficiency would recombine with its fa^{no} pseudoallele. A total of 85,600 chromosomes derived from N^{39}/fa^{no} heterozygotes were tested,

[9] W. J. Welshons and E. S. Von Halle, *Genetics* **47**, 743 (1962).

but no recombinants were found. It seems likely, therefore, that the deficiency N^{39} includes the locus of fa^{no}.

Conclusions. – Obviously, the region left of fa^{no} is capable of producing recessive lethals as indicated by the localization of N^{e11}. Furthermore, it would appear from the N^{39} experiments that the single salivary band 3C7 cannot possibly contain all of the pseudoallelic loci comprising the functional Notch locus since mutants like N^{e11} and N^{Co} are appreciable distances to the left and right of deficiency N^{39}.

Cell Growth and Reproduction

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Insect Genetics

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PROTECTION OF BACTERIOPHAGE T4 FROM GAMMA RADIATION IN LIQUID AND FROZEN MEDIA

R. C. von Borstel M. J. Bond

Introduction. – Experiments have been performed in an attempt to achieve biologically observable events from transmutation of N^{14} by thermal neutrons in the bacteriophage T4 (ref 1). The rate of phage inactivation was high. Five decades of inactivation had occurred when the integrated thermal neutron flux had reached 1% of that necessary to provide an average of one transmutation event in N^{14} per phage genome. The inactivation was attributable to capture gamma radiation from neutron capture reactions in the phage environment.[2] The experiments reported here are studies on radiation protection using a Co^{60} gamma radiation source to determine if the inactivation can be reduced under conditions that would be usable when phage are exposed in the reactor.

Results. – The osmotic shock-resistant mutant stock o-11 of bacteriophage T4 at concentrations above 10^{10} phage/ml were prepared in M9 (simple salt) medium, 1:1 M9 and dilution (0.5% bacto-peptone and 0.5% NaCl) medium, and these same media with 1.2×10^{-1} and 1.2×10^{-2} *M* β-mercaptoethylamine (MEA) buffered to neutrality. These were then exposed to Co^{60} gamma radiation either in a liquid or a frozen state. Those in the liquid media were exposed in nitrogen atmospheres.

[1]R. C. von Borstel, E. G. Calef, and A. Colomb, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1962*, ORNL-3267, pp 40–41.

[2]R. C. von Borstel, E. G. Calef, and A. Colomb, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 63–66.

Those in the frozen state were kept in a nitrogen atmosphere before freezing, quick-frozen drop by drop on a block of lead immersed in liquid nitrogen, and then kept frozen by packing in frozen CO_2 before and during exposure. Thawing of the frozen drops was done by placing them in liquid media at room temperature. Temperature of the thawing medium was found to have no effect on phage survival between 5°C and 40°C. The quick-freezing and thawing procedure itself was found to cause no more than 15% inactivation of the o-11 stock of bacteriophage.

When these experiments were done, the gamma-dose rate of the source was approximately 30,500 r/hr.

The results from irradiation in liquid media (Fig. 4) show that the dilution medium is effective in protecting the phage against effects which are believed to be indirect. The shoulder on the curve for phage irradiated in M9 medium is real and has been observed in repeated experiments. The effect may be explained by assuming indirect inactivation of the phage by formation of radicals and peroxides that have finite lifetimes and diffusion distances. The dilution medium at least partially protects against the indirect effect. The other curves in Fig. 4 show that, under the conditions of our experiments, the MEA at two concentrations protects the phage from the effects of gamma radiation to a considerable extent.

The results from irradiation in frozen media (Fig. 5) show that freezing protects against the apparent indirect effect observed in phage suspended in liquid M9 medium. The rate of inactivation of phage in the 1:1 medium with 1.2×10^{-2} *M*

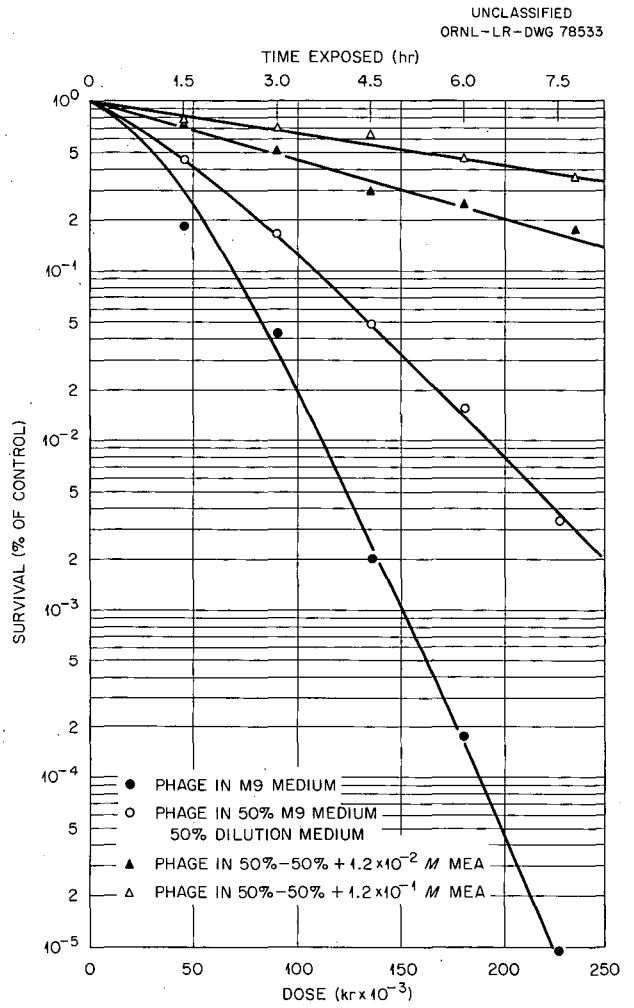

Fig. 4. Survival Curves for Bacteriophage Suspended in Liquid Media and Exposed to Co^{60} Gamma Radiation (Dose Rate, 30,500 r/hr).

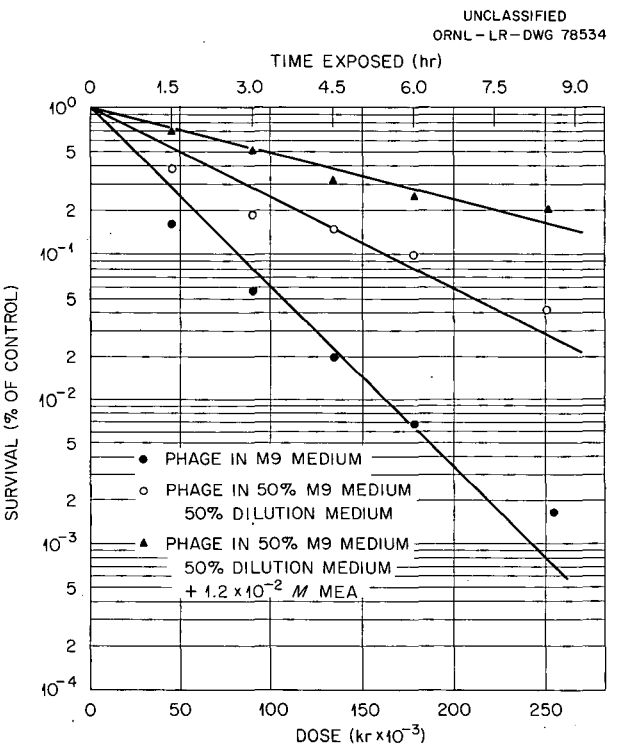

Fig. 5. Survival Curves for Bacteriophage Suspended in Frozen Media and Exposed to Co^{60} Gamma Radiation (Dose Rate, 30,500 r/hr).

MEA appears to be the same whether frozen or unfrozen. With MEA at a molarity of 1.2×10^{-1}, phage frozen in the suspension medium are not viable when thawed.

Discussion. – It appears that the effect of gamma radiation can be decreased by a factor of about 4 from the phage frozen in the M9 medium to the phage frozen in the 1:1 medium containing 1.2×10^{-2} *M* MEA. The next step in our experimental program will be to see if an equivalent decrease in phage inactivation can be observed from exposures within the reactor.

BIOCHEMISTRY OF YOLK

R. A. Wallace

Lipovitellin in Frog and Hen Egg Yolk. – During the past year we have come to realize that the yolks of many vertebrate species have a "granule" component. In the amphibian egg, such granules have been termed "platelets" and seem to be the sole source of the yolk proteins, whereas in the avian egg they constitute only about 23% of the total yolk solids. The granules from both the amphibian and avian egg contain only the proteins phosvitin (MW = 35,000) and lipovitellin (MW = 400,000) in a molecular ratio of 2:1. The lipovitellin from avian granules has been further resolved into α and β forms which differ from one another by their chromatographic behavior on hydroxyapatite columns, their protein phosphorus content, and their ease of dissociation (subunit MW = 200,000) in alkaline solvents. In all other respects studied thus far, the α and β forms seem to be similar, and there is evidence that their chromatographic and dissociative behavior is a direct function of their phosphorus contents (see Cook[3]).

Lipovitellin from the amphibian platelet has not been analyzed for possible components, however, and in collaboration with Cook (National Research Council, Canada) a comparison of amphibian and avian lipovitellin has been made, some aspects of which are given in Table 13. Apparently, the amphibian platelet completely lacks the less-phosphorylated β-lipovitellin present in the avian granule, since all the amphibian lipovitellin comes off the hydroxyapatite column as a single component at a phosphate buffer concentration of 1.3 *M*, and the phosphorus and dissociation analyses of the eluted protein have established its relation to α-lipovitellin.

Table 13. Comparison of Amphibian and Avian Lipovitellin

	Frog	Chicken	
		α	β
Eluted from hydroxyapatite (*M* PO_4 buffer)	1.3	1.1	0.6
Protein P (%)	0.8	0.6	0.3
50% dissociation (pH)	11.0	10.5	7.8

This finding is of interest in view of the fact that the amphibian granule is actually a perfect crystalline (hexagonal $\{10\bar{1}0\}$ face = 81 A) inclusion, whereas the avian granule apparently is not.[4,5] Since both α- and β-lipovitellin seem to have an identical *protein* composition, perhaps it is the β form which is supplied from the serum to the oocyte and is there phosphorylated further (see below) to provide α-lipovitellin. In the rapidly growing avian oocyte (volume increases 350 times in 6 days), phosphorylation may not be as complete as in the more slowly developing amphibian egg (volume increases 60 times in 7 months) and optimum conditions for crystal growth are not maintained. This possibility is under investigation.

The Formation of Yolk Crystals in the Amphibian Oocyte. – Ward[6] recently demonstrated that regular hexagonal crystals of yolk first appear within the

[3]W. H. Cook, *Proceedings of the 1st International Congress on Food Science and Technology* (in press).

[4]S. Karasaki, *J. Cell Biol.* (in press).

[5]R. A. Wallace, *Biochim. Biophys. Acta* (in press).

[6]R. T. Ward, *J. Cell Biol.* **14**, 309 (1962).

mitochondria of young oocytes (*Rana pipiens*) and suggested that the growth of these crystals probably resulted from mitochondrial protein synthesis. In view of the isolation of phosvitin from the serum of laying hens[7] and the large body of evidence indicating that the yolk proteins are synthesized externally from the oocyte in a variety of species (see Urist and Schjeide[8]), it would seem that an alternate explanation for the mitochondrial involvement in crystal formation is needed.

Nass[9] has noticed that the removal of only about 25% of the phosphorus from the amphibian yolk proteins (those present in the insoluble crystal) by a protein phosphatase results in their solubilization. Presumably, then, a rephosphorylation would render them insoluble and a mitochondrial protein phosphorylating system may be involved in the crystal growth. Rabinowitz and Lipmann[10] have described just such a system ("protein kinase") which they have purified from yeast and brain extracts. The reaction:

$$\text{partially phosphorylated protein} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \text{fully phosphorylated protein} + \text{ADP}$$

is freely reversible and the enzyme involved is thus far known to be specific for the proteins (and peptides of) casein and avian phosvitin only, is mitochondrial in origin, and will not promote exchange if the protein is less than 50% phosphorylated (maximum exchange obtained with 75% phosphorylated phosvitin).

A similar system has now been found in the ovary of *R. pipiens*, as measured by its ability to promote incorporation of phosphorus from terminally labeled ATP^{32} into phosvitin. The enzyme can be readily solubilized only after acetone-butanol extraction of the tissue, and the extract has a higher specific activity than those previously described for yeast and brain. Purification and characterization of the ovarian protein kinase is now in progress. Surprisingly, it has thus far been noticed that the ovarian enzyme behaves in a manner more similar to the yeast than the brain protein kinase (inhibited by phosphate, inactivated at pH 4.5).

[7] C.-C. Mok, W. G. Martin, and R. H. Common, *Can. J. Biochem. Physiol.* **39**, 109 (1961).

[8] M. R. Urist and A. O. Schjeide, *J. Gen. Physiol.* **44**, 743 (1961).

[9] S. Nass, *Biol. Bull.* **122**, 232 (1962).

[10] M. Rabinowitz and F. Lipmann, *J. Biol. Chem.* **235**, 1043 (1960).

PROGRESSIVE APPEARANCE OF LENS SPECIFIC ANTIGENS IN REGENERATIVE TISSUE TRANSFORMATION OF THE IRIS INTO THE LENS

Chinami Takata J. F. Albright
Tuneo Yamada

Introduction. – As a part of the programs of analysis of the tissue transformation occurring in lens regeneration in the adult newt,[11] acquirement of lens specificity by the regenerating cell population was studied by immunoelectrophoresis and gel diffusion technique, using *Triturus viridescens* and *T. pyrrhogaster.*

Methods. – The rabbit antiserum was prepared by injecting the *viridescens* lens homogenate in 0.15 *M* NaCl. The serum from normal healthy rabbits was used as the control. As antigens, 0.15 *M* NaCl extracts were prepared from the following tissues: normal lens, normal iris, the regenerating lens of various stages, liver, and blood serum of *T. viridescens*; adult lens, regenerating lens of various stages of *T. pyrrhogaster.* After electrophoresis of antigen extracts in agar was completed, the antilens serum was poured into the rectangular trough, and precipitin bands were allowed to develop for 2 to 4 days. The diffusion test was performed on the agar plate at room temperature.

Results and Discussion. – As shown in Table 14, the normal *T. viridescens* lens extract formed eight precipitin bands on immunoelectrophoresis, when tested with *T. viridescens* antilens serum. No evidence was obtained for the presence of any of these antigens in extracts of normal iris, liver, and blood serum of *T. viridescens*. Antigen extracts from the regenerating lens of 20 and 25 days after lens removal formed two and four bands, respectively, with antilens serum. These results suggest stepwise appearance of lens specific antigens, during tissue transformation, in the regenerating cell population from the iris which normally does not contain any of these

[11] T. Yamada and S. Karasaki, *Develop. Biol.* **7**, 595 (1963).

Table 14. Antibody-Antigen Reactions in Gel

Antigens	Immunoelectrophoresis (No. of bands)	Gel Diffusion Test (No. of bands)
T. viridescens		
Normal lens	8 (a–h)	5
Normal iris	0	—
Regenerating lens (12 days)	0	—
Regenerating lens (20 days)	2 (a,b)	2
Regenerating lens (25 days)	4 (a,b,e,h)	2
Liver	—	0
Blood serum	—	0
T. pyrrhogaster		
Normal lens	4 (a,b,e,h)	—
Regenerating lens (15 days)	—	0
Regenerating lens (18 days)	—	0
Regenerating lens (20 days)	0	1
Regenerating lens (25 days)	4 (a,b,e,h)	1
Regenerating lens (30 days)	4 (a,b,e,h)	1

antigens in a detectable amount. Studies with the extracts from *T. pyrrhogaster* showed that the normal lens has four antigens common to the *viridescens* lens, and that all of them are already present in the regenerating lens of 25 and 30 days. Preliminary electrophoretic analyses indicated that these four antigens correspond to the four antigens observed in the 25-day *viridescens* regenerating lens (Table 14). Although the extract of the 20-day *pyrrhogaster* regenerate failed to show reaction with antilens serum, it showed a recognizable band on the diffusion test. So far this is the youngest regeneration stage which indicated the presence of any lens antigen (Table 14).

It should be pointed out that the stage of regeneration at which the lens-specific antigens have been first detected is morphologically characterized by appearance of lens fibers in the regenerated lens. This is more than 10 days after the enhancement of protein synthesis and almost 20 days after the enhancement of RNA synthesis as demonstrated by autoradiography.[11] It is preceded by the first morphological sign of lens specificity, increase in cytoplasmic volume and in acidophily, for more than 5 days.

THE PATTERN OF MACROMOLECULAR SYNTHESIS IN REGENERATIVE TISSUE TRANSFORMATION

Tuneo Yamada Chinami Takata
Marion E. Roesel

As reported earlier, when the iris tissue transforms into the lens after surgical removal of the lens in the adult newt (*Triturus viridescens*), the first observable change in the iris cells is enhancement of nuclear synthesis of RNA.[12,13]

[12] T. Yamada, S. Karasaki, and D. M. Prescott, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1962*, ORNL-3267, p 42.

[13] T. Yamada and S. Karasaki, *Develop. Biol.* **7**, 595 (1963).

Further data concerning the pattern of macromolecular synthesis in the same system are summarized briefly as follows:

Intracellular Localization of Synthesized RNA. – When the regenerating cell population and the control iris are exposed to H^3-uridine or H^3-cytidine before the 7th day of lens removal, fixed 12 hr later, and studied on autoradiographs, there is no essential difference in the intracellular distribution of the autoradiographic grains (in spite of the large difference in the number of grains per nuclear section or per unit nuclear area); most of the grains (about 90%) are over the interphase nuclei. Few grains can be detected over the cytoplasm and mitotic chromosomes. When the above experiment is repeated at a later stage of regeneration, the autoradiograph of the regenerating cell population indicates a large fraction of the grains over the cytoplasm. With the progress of regeneration, the cytoplasmic fraction of the grains increases from about 15% to 65% at the expense of the nuclear fraction. The pattern of intracellular distribution of grains, as indicated above, is dependent upon the time interval in which the tissues are exposed to isotopes. When the interval is shortened from 12 hr to 3 hr or 30 min, while the other conditions are kept unaltered, the grains are found restricted over the nucleus, even in the cells of later stages of transformation. The simplest interpretation of these two sets of observations is that a transfer of RNA synthesized in the nucleus occurs more rapidly in the regenerating cell population of the early phase than in that of the later phase. It should be noted that this suggested shift in the distribution pattern of labeled RNA coincides temporally with an increase of the ribosomal population, as indicated by an electron microscopic observation.[14]

Specific Inhibition of Lens Formation with Actinomycin D. – Administration of actinomycin D inhibits transformation of the iris into the lens. A complete suppression of lens formation is possible without affecting tissue integrity of the normal lens and iris. Further studies of the effect revealed two phases of transformation process which can be distinguished by susceptibility toward the antibiotic. Within a range of doses, a treatment beginning before 12 days of lens removal leads to suppression of lens formation, whereas a comparable treatment beginning after 13 days of lens removal allows formation of an amount of lens fibers. Morphology of the lens obtained in the second series of experiments varies a great deal according to the stage of treatment – the later the treatment the larger the amount of lens fibers, and the more normal the lens structure. Since actinomycin D inhibits specifically RNA polymerase activity by binding to the guanine residue of the DNA molecule, suppression of lens formation with the antibiotic means dependency of the transformation process on the DNA primed RNA synthesis. Observed phase-specific susceptibility may be interpreted by assuming a phase depending on information based on guanine-rich DNA and the other phase depending on another type of DNA, although other possibilities, like difference in permeability of the tissue toward the antibiotic, are not excluded.

The Pattern of Protein Synthesis. – An autoradiographic study of the problem is being made using H^3-leucine as the isotopes. Two days after lens removal, hardly any significant difference in the grain count per unit area is observed between the regenerating part of the iris and the corresponding part of the control iris, which in its turn shows a count much lower than that of the normal retina and cornea. Seven days after lens removal the number of grains per unit area over the regenerating iris is about four times as much as that over the control. A further increase of the grain count is noted over the regenerating cell population from 16–20 days of lens removal. The grains are observable all over the cells without being concentrated either to the nucleus or to the cytoplasm. The enhancement of protein synthesis in the regenerating cell population occurs much later than the enhancement of nuclear synthesis of RNA, but precedes the appearance of lens-specific antigens, as detected with immunoelectrophoresis, by more than 10 days.[15]

Results presented indicate that during the process of tissue transformation at least three phases can be distinguished according to the extent of RNA and protein synthesis; intracellular localization of RNA, susceptibility toward actinomycin, and occurrence of lens-specific antigens. Consideration of different types of

[14] S. Karasaki, this report.

[15] C. Takata, J. F. Albright, and T. Yamada, this report.

information leads us to suggest that, before the lens-specific antigens become detectable, some decisive events are occurring that directly control the differentiation type of the cell population.

AN ELECTRON MICROSCOPE STUDY OF LENS REGENERATION IN THE NEWT

Shuichi Karasaki

Introduction. – This study deals primarily with an analysis of the regenerative transformation of iris cells into lens cells after the removal of the lens in the newt (*Triturus viridescens*) eye.[16,17] The successive changes in fine structure during this process were studied by electron microscopy.

Results and Discussion. – The pigment epithelium of the normal iris consists of two layers of flattened cells and is separated from an endothelium and from other tissues by the investing "basement membrane." The cytoplasm of these epithelial cells is characterized by a remarkable abundance of pigment granules (melanin granules) and an extensive development of smooth-surfaced endoplasmic reticulum. Ribosomes (100–200 A in diameter) occur in very small numbers and do not show special affinity for the surface of the endoplasmic reticulum. The nucleus is frequently indented and contains a very small nucleolus consisting of fine fibrous components. Four to six days after lens removal, the first visible changes are detected in the nuclei of the mediodorsal iris cells. The indentation of the nuclear surface begins to disappear. Next, the nucleoli become prominent and acquire a granular cortex around the fibrous core mentioned earlier. This new component may consist mainly of granules that are similar to ribosomes. During this period pigment granules gradually disappear from the cytoplasm. They seem to be extruded into the intercellular spaces and then taken up into leukocytes. The endoplasmic reticulum diminishes and, finally, almost completely disappears. In these depigmenting cells, lysosomes can frequently be seen. After complete depigmentation (8–20 days) the density of cytoplasmic matrix becomes relatively low and the free ribosomes are scattered throughout the cytoplasm. Nucleoli again become less prominent and frequently show only the fibrous core. Later (15–40 days) the differentiating lens cells contain large numbers of free ribosomes in the cytoplasm, although a small number of ribosomes is associated with the endoplasmic reticulum, which appears at this stage in a very restricted amount. The cytoplasmic matrix is occupied by fibrous elements (about 50 A wide) of low density, probably corresponding to the main component of structural protein of the lens, which has been observed in various other vertebrates.[18]

FUNCTION SPECIFIC CHANGES IN THE SULFUR METABOLISM OF PIGMENT CELLS

Günter Cleffmann

Introduction. – The coloration pattern of agouti mice is caused by the rhythmic activity of the hair bulb pigment cells, which produce and deliver yellow melanin in a certain period of hair development and black melanin in the other periods. This pattern is based on a single gene. Sulfhydryl compounds are potent agents in experimentally inducing a change in the rhythm,[19] and probably are the normally occurring triggers for the change in pigment production. The sulfur metabolism of the pigment cells in these distinctly definable, functional stages has been studied by autoradiography in order to obtain information about its role in causing the alteration.

Methods. – The rhythm in pigment production is closely related to the development of the hair, and hair development is rather synchronous in young animals. Therefore, animals 3 to 7 days old were used. $S^{35}O_4$ (5 μc per g of body weight) and GSH-S^{35} (1 to 2 μc per g of body weight) were injected, and the animals killed at different times afterward. Grains were counted per unit area over the pigment cells and other tissues of the hair follicle in autoradiographs of sections through skin.

[16] T. Yamada, S. Karasaki, and D. M. Prescott, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1962*, ORNL-3267, p 42.

[17] T. Yamada, C. Takata, and M. E. Roesel, this report.

[18] R. A. Resnik, T. Wanko, and M. A. Gavin, *J. Biophys. Biochem. Cytol.* **7**, 403 (1960).

[19] G. Cleffmann, *Arch. Entwicklungsmech. Organ.* **154**, 239 (1963).

Results and Discussion. – According to preliminary results, there is a rapid increase in the grain count over the pigment cells shortly after injection of $S^{35}O_4$ or GSH-S^{35}, reaching a maximum at 6 hr, followed by a rapid decrease to about half the maximum value during the next 5 hr. Thereafter, the count decreases rather slowly. This pattern is the same for pigment cells producing yellow and for those producing black pigment, both of which can be found in the same section. In the case of GSH-S^{35} the absolute grain number per unit area over yellow pigment cells is twice as high as that over black pigment cells throughout the period. For $S^{35}O_4$, however, no significant difference between the two types of cells could be found. This indicates that the turnover rate for organic sulfur compounds in hair bulb pigment cells is considerably higher in the yellow phase than in the black phase.

It has been shown[19,20] that the rhythmic activity of the agouti hair bulb pigment cells is initiated by some properties of the surrounding hair bulb tissue rather than being dependent on an inherent rhythm in the pigment cells. Therefore, a test was made to determine whether the papilla and the hair-forming tissue supply sulfur compounds in different amounts for black and yellow pigment cells. Grain counts over these tissues revealed no differences between hair follicles containing yellow and those containing black pigment cells.

Comparative studies of the other alleles at the agouti locus, on the one hand, and for other amino acids and melanin precursors, on the other, are expected to give detailed information on whether (1) the rhythm in metabolism is specific for GSH, and (2) it is dependent on the agouti gene.

ELECTRON MICROSCOPE OBSERVATIONS ON THE UTILIZATION OF YOLK PLATELETS DURING AMPHIBIAN EMBRYOGENESIS

Shuichi Karasaki

Introduction. – Since the yolk platelet must bear an important relation to the progressive differentiation of embryonic cells, the fine-structure changes of the yolk platelet during the utilization process were examined in the developing ectoderm of five different species of amphibia.

Results and Discussion. – Previous electron microscope analysis of yolk platelets[21] has revealed two basic components: an inner crystalline main body and an outer superficial layer. The first change in the yolk platelets is a decrease in thickness and a subsequent disappearance of the superficial layer. This process occurs in cells undergoing primary differentiation. The surface of the crystalline structure then begins to decompose into separate units (40–60 A in size). The subsequent disintegration of the main body is coupled with the formation of laminar or vesicular membranes of about 70 A in thickness, fine particles of about 150 A in diameter, and fibrils of about 50 A in diameter. The membranous components have the triple-layered structure of the unit membrane and show the fundamental ultrastructure of cytomembranes.

The phenomenon of the transformation from crystalline structure with a simple hexagonal lattice into laminated "unit membrane" may be explained in part by the high polymorphism of a lipid-water system as suggested by Stoeckinius.[22] During the decomposition of yolk platelets, mitochondria, pigment granules, and lipoid bodies are found in close association with the yolk platelets. In species of *Rana* (as opposed to other amphibian genera), crystalline structures typical of yolk platelets are frequently located within the mitochondria throughout embryonic development. This "yolk-mitochondria" relation may be interpreted as the abortive formation of yolk crystals within mitochondria during early embryogenesis. This is directly opposed to the view of Lanzavecchia and Le Coultre,[23] who assume that the above phenomenon represents a direct transformation of yolk platelets into mitochondria.

[20] W. K. Silvers, *J. Exptl. Zool.* **137**, 181 (1958).

[21] S. Karasaki, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 62.

[22] W. Stoeckinius, *J. Cell Biol.* **12**, 221 (1962).

[23] G. Lanzavecchia and A. Le Coultre, *Arch. Ital. Anat. Embriol.* **63**, 445 (1958).

FURTHER STUDIES ON METABOLISM OF NUCLEOLI IN AMPHIBIAN OOCYTES

O. L. Miller, Jr.

Previous studies[24,25] have shown that peripheral nucleoli in medium-sized oocytes of both *Triturus viridescens* and *Rana clamitans* are composed of a finely fibrous core surrounded by a granular cortex. In addition, autoradiographic studies on isolated nucleolar components in *T. viridescens* showed that essentially all H^3-cytidine incorporated into nucleolar RNA is in the cortex portion, although the core component also contains an appreciable amount of RNA. RNA and protein labeling experiments have since been performed with *R. clamitans* oocytes.

In thick sections of *R. clamitans* oocytes, core and cortex components of the nucleoli can be distinguished after toluidine blue staining. After incorporation of precursors in both RNA and protein, a large majority of the autoradiograph grains appears over the cortex component. The distinction is particularly clear-cut after RNA labeling. In the latter case, there is some indication that the initial point of incorporation occurs at the interface between the fibrous core and the granular cortex.

The autoradiographic results, however, do not distinguish between nucleolar RNA and protein syntheses *in situ* versus nucleolar accumulation of material synthesized elsewhere in the nucleus. In this vein, the feasibility of isolating the nucleolar components in large enough quantities for microanalyses of RNA and protein fractions is being investigated.

RELATION BETWEEN THE AMOUNT OF DNA PER CELL AND THE DURATION OF DNA SYNTHESIS IN SEVERAL STRAINS OF *TETRAHYMENA PYRIFORMIS*

I. L. Cameron G. E. Stone

Introduction. – Studies on many mammalian diploid somatic cell types have revealed a DNA synthesis period (S period) of relatively constant duration.[26] This period is found in cells growing *in vivo* and *in vitro* and is independent of the cell generation time. Because the amount of DNA per mammalian diploid somatic cell is apparently similar regardless of the species (~6.2 picograms), we were led to ask whether the duration of the S period might be directly proportional to the absolute amount of DNA per cell. Different strains of *Tetrahymena pyriformis* that were known to contain different amounts of DNA in their macronucleus[27] were used to investigate this relation under constant culture conditions.

Methods. – Spectrophotometric measurements were made on 15 Feulgen-stained cells of each of three strains of *T. pyriformis* (strains HSM, 6000, and 6002). Only those cells that were in G_1 (the pre-DNA synthesis period) were measured. Cells in similar stages of division were isolated with a braking pipette and given a pulse exposure to H^3-thymidine at known parts of their growth-duplication cycle. Autoradiographs of these cells were made and analyzed to determine the placement and duration of the S period in the cell cycle.

Results and Discussion. – The results of the spectrophotometric measurements are shown in Table 15. Strains EU 6000 and EU 6002 have about 50% more DNA per cell than strain HSM. If the duration of S were directly proportional to the absolute amount of DNA in the macronucleus, the S period for strain HSM should be shorter than for either strain EU 6000 or EU 6002. Because these latter two strains have similar amounts of DNA, they might be expected to have S periods of similar length.

Preliminary results reveal the following: Under identical culture conditions the S period is about 75 min in all three strains. The generation time is about 123 min in HSM, 129 min in EU 6000, and 159 min in EU 6002. It appears from these results that the duration of the S period is not a function of the amount of DNA in the nucleus nor is it closely related to the generation time.

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[24] O. L. Miller, Jr., *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 57.

[25] O. L. Miller, Jr., in *Electron Microscopy: Fifth International Congress for Electron Microscopy Held in Philadelphia, Pa., Aug. 29 to Sept. 5, 1962*, vol 2, ed. by S. S. Breese, Jr., Academic Press, New York, 1962.

[26] I. L. Cameron and R. C. Greulich, *J. Cell Biol.* (in press).

[27] C. Wells, *J. Cell Comp. Physiol.* **55**, 207, 1960.

Table 15. Results of Spectrophotometric Measurements on Feulgen-Stained *T. pyriformis*

Strain of *T. pyriformis*	Number of Cells	Amount of DNA (relative units) $\bar{X}$	95% Confidence Limit of $\bar{X}$
HSM	15	0.809	±0.0806
EU 6000	15	1.318	±0.0505
EU 6002	15	1.268	±0.1506

RNA AND PROTEIN METABOLISM IN THE MATURATION OF THE NUCLEATED CHICKEN ERYTHROCYTE

I. L. Cameron D. M. Prescott

Introduction. – The autoradiographic studies of Pinheiro *et al.*[28] have shown that extrusion of the nucleus from rat normoblasts to form immature erythrocytes is coincident with the complete cessation of RNA synthesis, although protein synthesis continues for a substantial but restricted period during the remainder of erythrocyte maturation. Mature erythrocytes, therefore, do not synthesize either RNA or protein. In avians the nucleus is not extruded during erythrocyte formation and all mature erythrocytes are nucleated. This situation led us to ask whether the changes in RNA and protein synthesis were uniquely associated with the extrusion of the nucleus or whether similar changes occurred during maturation of the nucleated erythrocyte in avians.

Methods. – Newly hatched chickens were injected with H^3-leucine or with H^3-uridine. Autoradiographs were made from the blood smears of these animals. The slides were then stained in eosin and toluidine blue or 0.1% aqueous methylene blue. The erythrocyte series is listed in Fig. 6 in its chronological order of development.

Results and Discussion. – The number of silver grains over individual cell types was counted, recorded, and corrected for background. At 45 min after H^3-uridine administration, in those cells which were labeled, most of the silver grains appeared over the nucleus. At later sampling times, the grains were more evident over the cytoplasm. Comparison of the other blood cell types indicates that the thrombocytes (nucleated) and granulocytes are not synthesizing RNA or protein. The lymphocytes and monocytes are engaged in both RNA and protein metabolism.

Figure 6 contains a plot of the average grain count in each stage of the erythrocyte series 45 min after injection of the particular isotope. The average grain count per cell in the H^3-uridine–treated birds dropped rapidly between the mid- and

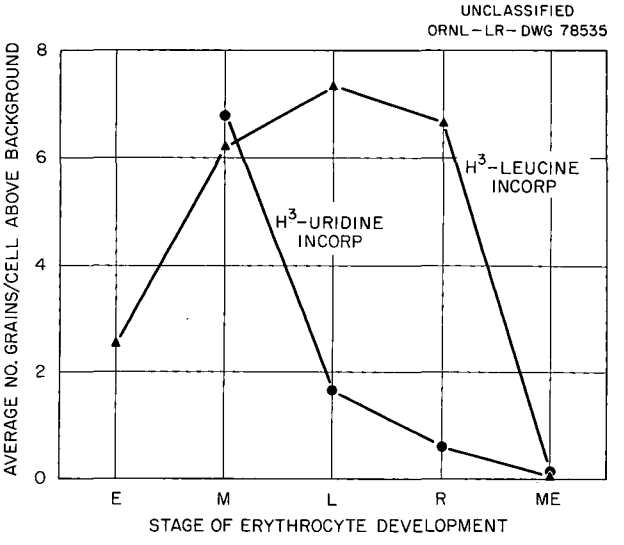

Fig. 6. A Plot of the Average Grain Count in Each Stage of the Erythrocyte Series 45 min After Injection of Either H^3-Uridine or H^3-Leucine. Erythrocyte series: *E*, early polychromatic; *M*, mid-polychromatic; *L*, late polychromatic; *R*, reticulocyte; *ME*, mature erythrocyte.

[28] F. Pinheiro, C. P. Leblond, and B. Droy, submitted to *Exptl. Cell Res.*

late-polychromatic stages, and continued to decrease until no incorporation was found in the mature erythrocyte. Protein synthesis, as measured by H^3-leucine incorporation, increased from early through late polychromatic stages and was still high in the reticulocyte stage. The decline in protein synthesis thus lagged behind the decrease in RNA synthesis, but H^3-leucine incorporation fell to zero in the mature erythrocyte. Thus, with the maturation of both enucleated erythrocytes (rat) and nucleated erythrocytes (chicken), RNA synthesis stops; but in both cases, protein synthesis continues thereafter for a limited time. Although RNA synthesis stops in the rat erythrocyte about the time of nuclear extrusion, enucleation is not a prerequisite for the loss of RNA synthesis during maturation of the chicken erythrocyte.

These findings are consistent with the thesis that the information released by the nucleus of the developing erythrocyte becomes progressively restricted in spectrum, and the final task before shutdown is the release of information for a relatively massive synthesis of one protein, hemoglobin. The nucleus of the mature erythrocyte is therefore unique, since it is not engaged in any syntheses associated with self-replication and its entire genome is permanently turned off.

DETECTION OF PRECURSOR POOLS IN SINGLE CELLS

O. L. Miller, Jr. G. E. Stone
D. M. Prescott

Introduction. – Knowledge concerning the monomer pools for nucleic acids, proteins, and other cellular compounds during the cell duplication cycle has been obtained primarily through biochemical analyses. The synchronized systems necessary for such studies are in many respects inadequate and difficult to maintain. Therefore, the development of a technique to detect pools of specific precursors in single cells, cell parts, or small groups of cells has been undertaken. Specifically, H^3-thymidine uptake into the acid-soluble pools of *Tetrahymena pyriformis* has been studied by a simple autoradiographic method.

Method. – Small groups of *T. pyriformis* were synchronized by selecting dividers from a log phase culture during a short time interval. Brief pulses of H^3-thymidine were applied to these groups at various times during the cell cycle and followed by rapid, thorough washing with cold medium. Samples were obtained at intervals following the pulses by quickly drying single cells on slides. Control slides were extracted with 1:3 acetic-alcohol and 70% alcohol. A film of Kodak NTB3 emulsion, diluted 1:1 with H_2O, was obtained by dipping with a wire loop. Although the film gels at room temperature, it was cooled further over an ice bath. The gelled film was applied to cooled slides and stored at 10°C in light-tight boxes containing Drierite.

Results. – After development, experimental slides pulse-labeled during the S period showed macronuclear labeling and a dense halo of grains over the cytoplasm and immediately surrounding the cells. A few cells that were labeled near the beginning of the S period exhibited halos without macronuclear labeling. Control slides (extracted with 3:1) from the S period showed incorporation into macronuclear DNA but no halos. Cells labeled in early or mid-G_1 period exhibited no macronuclear or halo label in experimental or control slides in samples taken immediately after the pulse and after the S period. The halos are presumed to result from diffusion of nucleotides from dying cells at the last instant of drying.

These preliminary results suggest, in agreement with Stern's experiments, that the thymidine pool utilized in DNA synthesis in *T. pyriformis* probably is present only during or perhaps slightly previous to the time of DNA synthesis. Investigations of methods to keep precursor pools localized within the cell after death and experiments with other precursors and cell types are continuing. Confirmation of the validity of the technique by correlation with biochemically analyzed systems is planned.

Mammalian Genetics and Development

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EFFECT OF AGE AND PARITY ON MUTATION INDUCTION IN FEMALE MICE

W. L. Russell Elizabeth M. Kelly

Data presented in earlier reports on mutation frequencies in mouse oocytes were from females irradiated as young adult virgins (approximately 2 to 4 months old). A surprising result has developed following the use of somewhat older females (mostly from 6 to 9 months of age at irradiation). There was nothing unusual in the first postirradiation litters from these females, but the second litters showed a considerably higher mutation rate. The dose rate used in this experiment was 0.8 r/min, and the total dose was 400 r (Cs^{137} gamma rays). The mutation frequency in first postirradiation litters was 11 mutations in 40,944 offspring, while that in second litters was 12 mutations in 9094 offspring, a highly significant increase ($P = 0.00024$). The first and second litters of young females show no significant difference, and the combined mutation frequency in these agrees closely with the frequency for first postirradiation litters of the older females.

Some information has also been obtained on older females at the lower dose rate of 0.009 r/min. This is presented in Table 16 along with the results described above and other earlier data. In this table the results for first postirradiation litters of older females are combined with those from first and later litters of younger females, since no

Table 16. Effect of Age and Parity on Mutation Induction in Oocytes of Mice Exposed to 400 r

Radiation	Approximate Dose Rate (r/min)	All Litters of Young Females and First Litters of Older Females			Second[a] Litters of Older Females		
		Number of Offspring	Number of Mutations at 7 Loci	Mean Number of Mutations per Locus per Gamete	Number of Offspring	Number of Mutations at 7 Loci	Mean Number of Mutations per Locus per Gamete
				$\times 10^{-5}$			$\times 10^{-5}$
Gamma (Cs^{137})	0.009	44,811	2	0.64	7,321	2	3.90
Gamma (Cs^{137})	0.8	61,771	18	4.16	9,094	12	18.85
X rays (250 kvp)	90	11,124	15	19.26			

[a]Includes some later litters.

significant difference has been found between these groups.

As yet, no explanation has been found for this high mutation frequency in second litters of older females. No cytological differences have been seen in the oocytes involved. In the first experiment with older females, the animals had had a few litters before irradiation, and the possibility existed that it was this factor rather than age *per se* which was responsible for their differing from the young females. It will be remembered that the young females were virgin at the time of irradiation. However, new data now being accumulated tend toward ruling out this possibility and favor the view that age is the important factor. The fact that the mutation frequency in second litters of older females irradiated at 0.8 r/min is similar to that for first litters of females irradiated at 90 r/min (Table 16) suggested the possibility that the oocytes involved in the production of these second litters may lack the capacity for repair of premutational damage and, therefore, that they respond to the 0.8-r/min dose rate as strongly as to 90 r/min irradiation. However, the data for second litters of older females exposed to 0.009 r/min irradiation show a significantly ($P = 0.018$) reduced mutation frequency (Table 16), and, thereby, indicate that the oocytes in question do have at least some capacity for repair of premutational damage. In this connection it will be interesting to watch for the results in second litters in a current experiment using 90 r/min irradiation of older females.

Although this finding of an unexpected age-dependent variation in mutational sensitivity, or in capacity for repair of premutational damage, *within* a particular cytological stage (dictyate) could complicate the measurement of dose-rate effects, it does not seem to invalidate any of our past conclusions about the reality of dose-rate effects in females. These conclusions were based on oocyte stages or states that show no such age dependence. Furthermore, the stage that is now shown to be age dependent also shows a dose-rate effect within age groups.

EVIDENCE FROM SIX X-AUTOSOME TRANSLOCATIONS BEARING ON THE SINGLE-ACTIVE X HYPOTHESIS

Liane B. Russell Jean W. Bangham
Clyde L. Saylors

The finding that two X chromosomes were necessary for the expression of variegated-type position effects from X-autosome translocations in the mouse [XO being nonvariegated like XY (ref 1),

[1]Liane B. Russell and Jean W. Bangham, *Genetics* **45**, 1008 (1960).

and XXY being variegated like XX (ref 2)], as well as the results of various cytological investigations, led us to suggest[3] that the genetic activity of *one* X was required for gene balance. Any additional X's present were able to express the properties of heterochromatin, one of these being the production of V-type position effect. Shortly afterward, Lyon[4] stated a somewhat similar theory which has been named the single-active X hypothesis. This differs somewhat in the explanation of the V-type position effect.

According to the single-active X hypothesis, position effect in the mouse results from the circumstance that the X-translocated autosome shares the inactivity of that X in those cells (approximately one-half of all cells) in which the standard X is active, allowing, in this manner, the recessives on the standard autosome to express themselves. This hypothesis, in its simplest form, would imply that for any given autosomal locus translocated to the X, variegation characteristics should be independent of the position of the break points. Whether or not variegation is observed at all, and if so, the amount and pattern of variegation that is seen, should be determined only by the relation of gene action to differentiation for the character under consideration. In work reported here, we have, by using six X-autosome translocations, obtained evidence that variegation is, on the contrary, dependent on position of break points.

Material. – The first two cases of variegated-type position effect in the mouse were reported by us in 1959 (ref 5), and these involved an X;8 and an X;1 translocation. A third case, also T(X;1), was reported in 1960 (ref 1). Since the last full publication,[6] we have discovered three new cases of V-type position effect, all of them associated with X;1 translocations. Two of these were radiation-induced in spermatozoa; the third occurred spontaneously either in C3H or 101 mice. All translocations are visible in mitotic metaphase preparations, some more easily than others.

The three new translocations are analogous to our three earlier ones in that translocation heterozygotes carrying appropriate recessive markers on the standard autosome are variegated for the marker and partially sterile if female, but wild type and completely sterile if male. Unbalanced types apparently do not survive. In some of these features our six X-autosome translocations thus differ from the T(X;1) reported by Cattanach.[2]

Method. – In an attempt to determine if there is any relation between position of rearrangement points and variegation, we have, for all six of our X-autosome translocations, carried out a number of crosses designed to localize breaks, and other crosses designed to test which loci are variegating. Because of the nonvariegation of males, crossing-over determinations must include fertility test of progeny or else be based on female progeny only. This, added to the small litter sizes produced by translocation heterozygotes and the occasional poor viability, makes such determinations tedious. Detailed evidence cannot be presented here.

In crosses designed to test for variegation, heterozygous translocation females with the wild-type allele were mated to normal males carrying either the single marker in question, or linked markers to test for spreading effect. Markers so far tested in linkage group 1 are *c*, *tp*, and *p*; we have recently obtained the cooperation of R. A. Popp of this Division to test the hemoglobin locus, *Hb*. Markers tested in LG8 are *m*, *b*, and *wi*. If, in any instance, variegated daughters failed to be produced, the wild-type daughters were tested against a marker known to yield variegation to prove that the translocation was still present.

Results and Discussion. – According to present indications the position of rearrangement points in linkage group 1 may be assigned tentatively as shown in Fig. 7.

Three types of findings speak against the independence of variegation from location of break point that is implied by the single-active X hypothesis in its simplest form. First, the amount

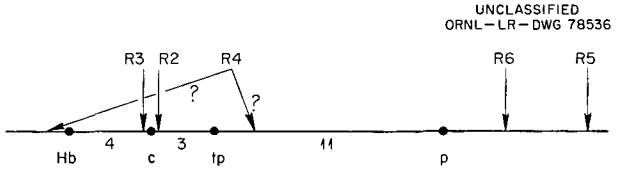

Fig. 7. Tentative Position of Rearrangement Points in Linkage Group 1.

[2]B. M. Cattanach, *Z. Vererbungslehere* **92**, 165 (1961).

[3]Liane B. Russell, *Science* **133**, 1795 (1961).

[4]M. F. Lyon, *Nature* **190**, 372 (1961).

[5]Liane B. Russell and Jean W. Bangham, *Genetics* **44**, 532 (1959).

[6]Liane B. Russell and Jean W. Bangham, *Genetics* **46**, 509 (1961).

of variegation for a given locus can be different for different translocations. Thus, *p*-locus variegation is barely perceptible in the case of R3, moderate in R2 and R5, and strong in R6. The indication that amount of variegation is inversely related to distance from R may be fortuitous in view of the finding that, in the case of the *c* locus, R5, which gives 21% crossing over with *c*, variegates as heavily as does R3, which gives only 0.3% crossing over. However, the essential finding is that different *p*-locus variegations differ markedly, a result not predicted on the single-active X in its simplest form.

Second, some loci entirely fail to variegate in some of the translocations. This is true of the *m* locus in T(X;8) and of the *c* locus in the case of R2. Although it is not known for *m* whether gene action is such that variegation can be expressed, there is ample evidence from all the other X;1 translocations that the *c* locus can variegate strongly. Further, R2 is less than 0.2 crossover unit distant from the *c* locus. We have concluded that the R2 rearrangement separates *c* and *p* loci and has placed c^+ next to a portion of X chromosome that does *not* have inactivating functions.

Third, we have detected a spreading effect in the cases of R3, R5, and R6, in that most mutant patches appear to be the color of the marker nearest to the break, some the color of both markers, but none, to date, the color of the distant marker only. A spreading effect has also been reported by Cattanach.[2]

We conclude that (1) not the entire X has inactivating powers and (2) where the X does inactivate neighboring portions of autosome, this inactivation may not involve the entire translocated piece.

MEIOTIC PROPHASE IN THE FEMALE GUINEA PIG

E. F. Oakberg Evelyn Clark
Patricia A. Alday

Previously, we had observed a species difference in nuclear morphology of the earliest oocyte stages in adult guinea pigs and mice.[7] Oocytes of the mouse had one large acidophilic and several small basophilic nucleoli and "diffuse" chromosomes characteristic of the dictyate stage, giving a nucleus with an interphase-like appearance. Nuclei of oocytes in the earliest follicle stages of guinea pigs, however, had several basophilic nucleoli and were much condensed by fixation, resembling synizesis stages. The chromosomes, however, were less distinct and not as strongly basophilic as is typical of early meiotic prophase. A study of meiotic prophase in fetal, neonatal, and adult females was undertaken in order to determine the stage of oocyte "arrest" in the guinea pig.

Pair matings were observed daily, and the male was allowed to remain with the female 24 hr after birth of a litter. If a *postpartum* estrus occurred, time of mating would be known. Day of birth of a litter was taken as *day* 0 of the subsequent pregnancy, and females were killed at 5, 6, 7, 8, and 9 weeks gestation. Immature animals were killed from the day of birth to 35 days of age; adults were 8 to 12 months old. All ovaries were fixed in Bouins, embedded in paraffin, and stained in Heidenhain's iron hematoxylin and eosin.

Great variation was observed in the meiotic prophase stages present at any given fetal age. At 5 weeks gestation, most of the primordial germ cells still were undergoing mitosis, but a few were in zygotene. At 6 weeks, all stages from leptotene to pachytene were present; a few dictyate-like nuclei were observed at 7 weeks. Leptotene still was common at 8 weeks, and in the 9-week fetus, some early zygotene stages still were present, but later stages, especially pachytene, were most common. The range of nuclear stages present made determination of developmental sequences difficult, but the pachytenes of the 9-week fetus appeared to enter a diffuse, interphase-like stage characteristic of the newborn female. Additional data will be required before the occurrence of a diffuse stage between pachytene and diplotene can be considered established. Under our conditions of breeding, birth occurred on the 65th day of gestation. Diplotene was observed *in utero* in some oocytes, but the majority underwent diplotene after birth. All oocytes had reached the "arrested" stage by 35 days *postpartum*. As the oocyte matures in the adult, chromosomes of the "condensed" stage disperse into the growing nucleus but remain acidophilic and do not change in structure until just before ovulation. We have

[7] E. F. Oakberg and Evelyn Clark, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 57–58.

not yet obtained females at appropriate intervals for demonstration of diakinesis and meiotic divisions.

A species difference in the state of the oocyte chromosomes results in a contracted, dense nucleus in the "arrested" stage of the guinea pig and an interphase-like nucleus in the mouse. The stage of nuclear arrest in both species, however, is postdiplotene.

CHEMICAL MUTATION STUDIES IN MICE

B. M. Cattanach

Introduction. – The chemical, triethylene melamine (TEM), has been shown to be very effective in breaking mouse chromosomes; dominant lethals and translocations have been recovered in very high frequencies from treated postmeiotic germ cells.[8,9] A program of testing for TEM-induced spermatogonial mutations is now under way.

Methods. – Adult hybrid 101 × C3H male mice were treated with doses of 4 mg of TEM per kg of body weight (administered in two intraperitoneal injections of 2 mg/kg 24 hr apart), 1 mg/kg, or 0.5 mg/kg. Mutations in the offspring were scored by the specific loci method – the method widely used in radiation experiments.[10] The fertility of the males was noted in the days following treatment for comparison with that following irradiation.

Results. – The TEM reduced male mouse fertility in the first 2 weeks following treatment by the induction of dominant lethals, and, with the higher doses, it also caused a period of temporary sterility due to aspermia 2 to 3 weeks later. With the 4-mg/kg dose, 100% dominant lethality was induced up until the temporary aspermic period. With the 1-mg/kg dose, dominant lethality was high only in the first 3 weeks, after which near-normal fertility was recovered until the onset of the second temporary sterile (aspermic) period. The timing of the fertile period indicated that it is the spermatocytic stages that are resistant to the mutagenic action of TEM – a markedly different result from that found with x rays. An early sterile period was also obtained with the 0.5-mg/kg dose, but it is not yet known whether the second temporary sterile period occurs.

The median times for recovery from the second sterile period were 89 days (4 mg/kg) and 65 days (1 mg/kg). Therefore, 4 mg/kg of TEM causes considerably more and 1 mg/kg of TEM causes slightly less spermatogonial killing than does 600-r x rays (median recovery time, 72 days).

The numbers of F_1 animals scored are as yet far too small to allow an estimation of the induced-mutation frequencies, but two specific loci mutants, as well as a probable dominant mutant, have been recovered.

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[8] B. M. Cattanach and R. G. Edwards, *Proc. Roy. Soc. Edinburgh* B**67**, 54 (1958).

[9] B. M. Cattanach, *Z. Vererbungslehre* **90**, 1 (1959).

[10] W. L. Russell, *Cold Spring Harbor Symp. Quant. Biol.* **16**, 327 (1951).

Radiation Microbiology

Alexander Hollaender	
Mutagen Mechanisms – Bacterial Metabolism	**Bacterial Genetics**
G. E. Stapleton; Annie S. Angel; H. L. Scott[a]	H. I. Adler; Alice A. Hardigree; R. Curtiss III
[a]ORINS Fellow.	

INFLUENCE OF CULTURE CONDITIONS ON RADIOSENSITIVITY OF *ESCHERICHIA COLI*

G. E. Stapleton

Previous reports[1–3] have described some of the characteristics of development of resistance of a number of strains of *Escherichia coli*. Much of our present thinking involves the possibility that the resistance developed in cell populations reflects an active postirradiation repair or recovery process. It was considered likely that the response of radiosensitive and resistant populations of the same strain to postirradiation conditions, such as the plating medium and temperature, would influence the viability of such populations in a differential manner. The dependence of viability of the B and B/r strains of this species on postirradiation conditions has been amply demonstrated.[4,5]

E. coli B/r (CSH), the strain most used in the present investigations, when grown in nutrient broth or peptone (sensitive), responds to the presence and absence of amino acids or other supplements in the postirradiation plating medium similarly as did B/r (ORNL) in previous studies.[3] Resistant populations, from glucose-containing cultures, respond also to the addition of complex nutritional supplementation to the postirradiation medium. Figure 8 shows the relative response of sensitive and resistant populations of the B/r (CSH) strain to plating on M9 (mineral-salts-glucose) medium and on nutrient agar. It is clear that the presence of components in the complex medium is required for maximum expression of radioresistance of these cell populations. Results of preliminary studies indicate that individual amino acids, notably methionine, are the major requirements supplied in the complex media.

It is of interest that the major effect of the complex media on survival of glucose-grown cells is reflected in the shoulder of the curve. Almost identical survival curves were obtained for (1) early stationary phase cultures of this strain on glucose-containing media[3] when plated on complex media, (2) for late stationary phase cultures when plated on M9 media, and (3) in earlier studies[4] for cells that arrive at intermediate pH levels. It is tempting to postulate that all three situations result in limiting amounts of active repair systems in

[1]G. E. Stapleton and M. S. Engel, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 87–90.

[2]G. E. Stapleton, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1962*, ORNL-3267, p 62.

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

[4]G. E. Stapleton and M. S. Engel, *J. Bacteriol.* **80**, 544 (1960).

[5]H. L. Cromroy and H. I. Adler, *J. Gen. Microbiol.* **28**, 431 (1962).

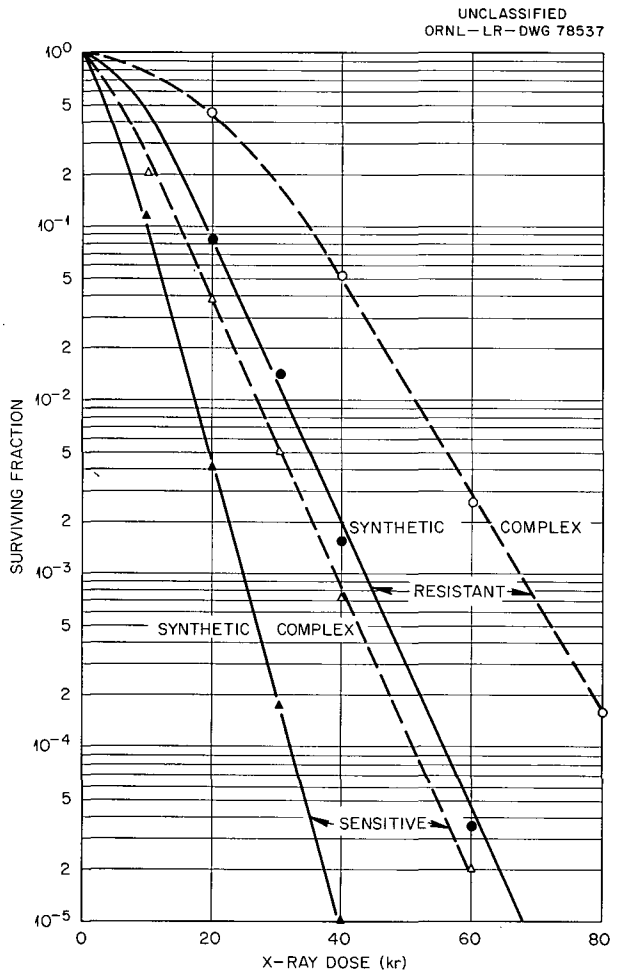

Fig. 8. Effect of Postirradiation Media on Survival of *E. coli* B/r (CSH).

this strain of *E. coli*. These suggestions are the basis of future experiments concerned with development of radioresistance in this and other species of bacteria.

CHEMICAL PROTECTION AGAINST RADIATION DAMAGE BY SULFHYDRYL COMPOUNDS

G. E. Stapleton H. L. Scott

Earlier evidence suggests at least two separable phenomena associated with the protection afforded by sulfhydryl compounds.[5] When β-mercaptoethylamine (MEA) is introduced into a bacterial suspension in equilibrium with air, and the containing vessel closed off, this compound will react with and remove oxygen from the suspension. However, when suspensions are bubbled with purified nitrogen and then reacted with MEA in closed vessels, a large additional protection is found. The present investigations are directed toward elucidation on the latter process. By use of various strains of *E. coli* it is possible to show that the reported variations in protective capacity of MEA among bacteria may be due to variability in that part of protection resulting from oxygen removal, the residual protection observed in anoxic suspensions being constant for the several strains investigated.

We are attempting to separate the two mechanisms by choice of organisms, by investigation of the differential rates of reaction, and by the differences in concentration required for the two processes. To date, we can report that the relative rates of the two processes are different by a large factor, the slower one being the oxygen-removal process. Both processes require the reduced form of the compound.

SPACE-RELATED PROJECTS

G. E. Stapleton

This group is cooperating with the NASA-ORNL Cooperative Program to the extent of determining the relative biological effectiveness (RBE) for particular radiations, principally protons, ranging in energy from a few Mev to 160 Mev. The RBE for inactivation and mutation of *Escherichia coli* is being investigated. The efficiency of protective agents, such as MEA, is being studied over the broadest range of linear energy transfer (LET) available. To date, 160-Mev protons have been used, and at present 22-Mev protons are being used. In cooperation with the staff of the Lawrence Radiation Laboratories of the University of California, the investigations will be extended to lower-energy protons, to alpha particles, and finally to heavy ions from the Berkeley heavy-ion, linear accelerator.

GENETIC ANALYSIS OF RADIATION SENSITIVITY IN *ESCHERICHIA COLI*

H. I. Adler Alice A. Hardigree

In earlier reports[6,7] we established that a group of genes in the Histidine-Proline region of the *E. coli* K12 linkage map influenced the ability of cells to survive exposure to either ionizing or ultraviolet (2537 A) radiation. More detailed analysis of this data suggests that the small segment of the map between the genes controlling lactose utilization and sensitivity to bacteriophage T6 is particularly "rich" in such genes. We suggested that, for a clear understanding of the genetic and biochemical basis of control of radiation sensitivity, it would be necessary to isolate single gene radiation mutants of *E. coli* K12. We have been unsuccessful in isolating such mutants from *E. coli* K12 but did obtain a bacterium from Paul Howard-Flanders of the Department of Radiology, Yale University, New Haven, Connecticut, which has proven to be a valuable mutant. This bacterium *E. coli* K12 AB1899 was isolated by Howard-Flanders after treatment of the parental culture (AB1157) with nitrous acid. His preliminary data indicated that AB1899 might be an x-ray-sensitive F^- (recipient) strain. We have been able to establish that the basis for the apparent x-ray sensitivity is the failure of AB1899 to become resistant when grown prior to irradiation in a glucose-containing complex medium. Many *E. coli* strains do become resistant when grown in this manner.[8] Figure 9 demonstrates the failure of AB1899 to become resistant under these conditions. The parental culture, AB1157, shows an effect typical of many *E. coli* strains. AB1899 apparently contains an inactive allele of a gene(s) that normally is concerned with the development of glucose-induced radiation resistance. Its failure to develop resistance is not due to an inability to utilize the sugar since

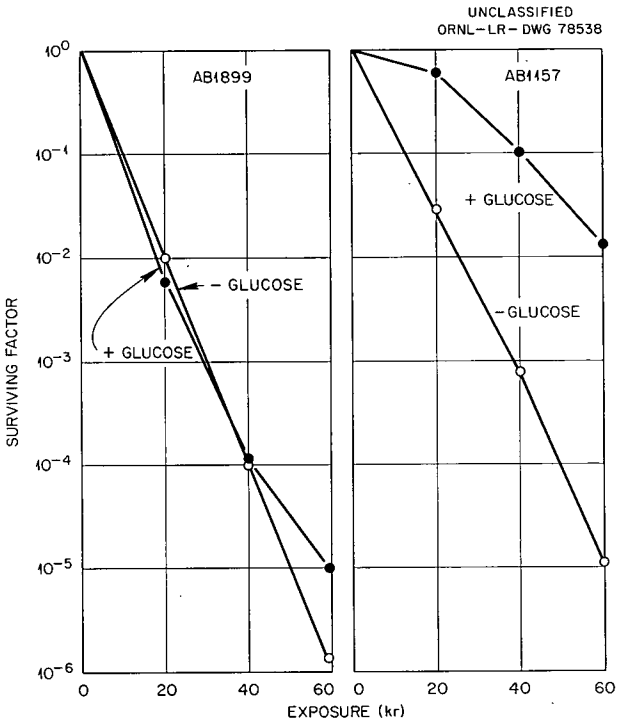

Fig. 9. Effect of Growth in Glucose on the X-Ray Sensitivity of *E. coli* K12 AB1899 and AB1157.

AB1899 grows rapidly on media in which glucose is the sole carbon source and produces considerable quantities of acetic acid.

We have now surveyed several HFr (donor) strains of *E. coli* K12 in order to find one that does develop resistance when grown in glucose and is suitable for matings with AB1899. *E. coli* HFrH is satisfactory for this purpose, and preliminary genetic mapping indicates that a gene(s) controlling the ability of *E. coli* to become resistant to ionizing radiation when grown in a glucose-containing medium is located in the Lactose-T_6 region of the linkage map. More accurate genetic mapping experiments are in progress. Biochemical experiments to determine the unusual features of glucose metabolism in *E. coli* AB1899 have been initiated. The organism produces a large amount of extracellular slime when grown on glucose-containing solid media. This slime is water-soluble and is probably a polysaccharide, but its exact nature has not yet been established.

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[6] H. I. Adler and J. C. Copeland, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 80.

[7] H. I. Adler and J. C. Copeland, *Genetics* **47**, 701 (1962).

[8] G. E. Stapleton, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 79.

EARLY STAGES IN THE DEVELOPMENT OF COLONIES OF VARIOUS *ESCHERICHIA COLI* STRAINS

H. I. Adler Alice A. Hardigree

Macrocolony formation on solid media is frequently the criterion used as an end point in radiation experiments with bacteria. It is assayed simply by routine bacteriological techniques but does not provide information on the first cell divisions accomplished by an irradiated population. We have employed a simple technique that allows us to make qualitative and quantitative observations on the timing and morphological changes associated with the first divisions of an irradiated population of bacterial cells.

Stationary phase bacteria are spread over the surface of a thin layer of nutrient agar on a microscope slide. A No. 1 coverslip is placed over the midsection of the slide. Thick blocks of agar are put on each side of the coverslip to retard desiccation. The bacteria are observed with an oil immersion 97× bright phase-contrast objective at 37°C; and it can be demonstrated that in unirradiated control populations, cell division proceeds at an optimum rate (one division per 20–25 min) for at least 4 hr.

Comparison of early stages in colony development of unirradiated *Escherichia coli* B_{s-1} (Hill) (radiation sensitive), B (Hill) (intermediate), and B/r (ORNL) (radiation resistant) reveal that the sensitive and resistant mutants both divide regularly without the formation of long forms ("snakes"). The *E. coli* B (Hill) culture divides irregularly, and many cells form long snakes which break down into individual cells as colony development progresses. Irradiation with either low doses of uv light (2537 A) or x rays (250 kvp) induces some snake formation in B_{s-1} (Hill), enhances snake formation in B (Hill), but fails to produce snakes in B/r (ORNL).

Because the "B" culture used and other B cultures tested form snakes during the early stages of colony development even in unirradiated cells, it has proved difficult to quantitate the results obtained; and attention has been focused on the radiation-sensitive mutant *E. coli* B_{s-1} (Hill) and the radiation-resistant mutant *E. coli* B/r (ORNL). Data accumulated so far indicate that, in an *E. coli* B_{s-1} (Hill) culture plated after exposure to 1 kr of x rays, 82% form microcolonies (at least four divisions), 10% fail to divide even once, and 8% undergo limited division.

A macrocolony count on the same population of irradiated cells indicated 68% survival. By accumulating more data, at several dose levels, it should be possible to establish whether the difference between the microcolony estimates of survival (82%) and the macrocolony estimates (68%) are significantly different. It will also be interesting to accumulate more data on that class of cells which undergoes only a limited number of divisions after exposure to radiation.

Mammalian Recovery

C. C Congdon

Recovery *in vitro*

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

Hemopoietic and Lymphatic Stem Cells

Joan W. Goodman
Wanda R. Shelton
Adele S. Shaw

Secondary Disease

Immo Hoppe[a]
D. C. Swartzendruber[b]
C. C Congdon
A. L. Kretchmar[c]
Vu-Thi-Suu[a]
W. H. McArthur[d]
M. G. Hanna, Jr.[e]
Mary K. Hill
Alberta P. Henley

[a]Visiting investigator from abroad.
[b]Research Associate.
[c]Medical Division, ORINS. In collaboration with Mammalian Recovery Group.
[d]Consultant.
[e]ORINS Fellow.

STUDIES OF Fe^{59} INCORPORATION BY SPLEEN OF LETHALLY IRRADIATED MICE INJECTED WITH ISOLOGOUS BONE MARROW

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

Introduction. – It was previously reported that Fe^{59} uptake by the spleen of lethally irradiated mice is a function of the number of isologous bone marrow cells injected.[1] Uptake reflects erythropoietic activity in the spleen which is dependent on quantitative transplantation of marrow cells. We have continued development of this system as a method of evaluating *in vitro* treatment of radiation injury of cells. Coupled with this system is development of short-term culture conditions that permit physical and chemical manipulation of irradiated cells.

Results and Discussion. – The 24-hr uptake of Fe^{59} by the spleen has been replaced by a 6-hr uptake; otherwise, the methods are essentially identical to those previously used.[1] Use of the 6-hr assay not only shortens the procedure, but extends the range over which uptake is linear with marrow dose. This extension is probably a reflection of release of newly formed iron-containing erythrocytes from the spleen that is less after 6 hr than after 24 hr. Figure 10 shows that a fall in iron uptake occurred after 0.8×10^6 cells rather than the expected plateau. Spleen weight shows the same decrease at high bone marrow doses. The explanation for this effect is not clear, but perhaps the decrease represents a physiological response that signals a shutdown in cell proliferation in the hyperactive spleen. In this connection, the spleen of an irradiated mouse injected with 0.8×10^6 cells takes up as much as four times the iron as that of the normal mouse.

[1]L. H. Smith, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 85–86.

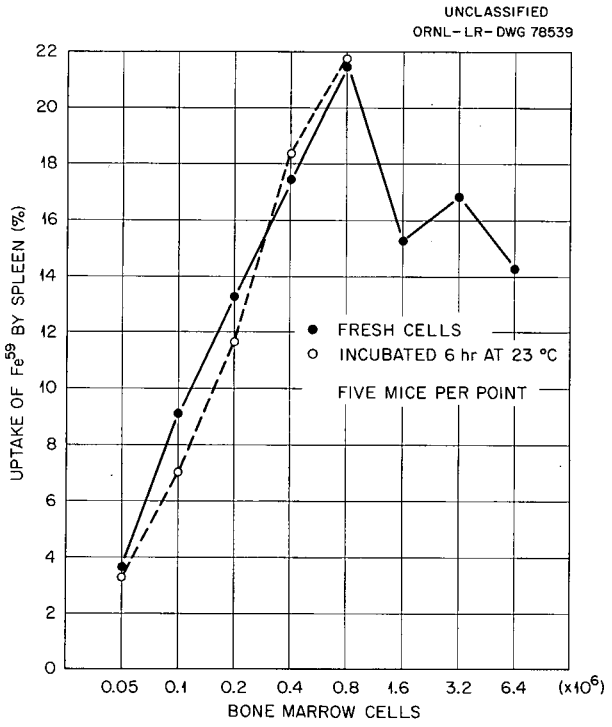

Fig. 10. Percentage Uptake of Fe^{59} by Spleens of Lethally X-Irradiated (900 r) Mice Injected with Isologous Bone Marrow Cells.

To study treatment of radiation injury to marrow cells *in vitro*, culture conditions must be optimal. Initially we used Tyrode's solution for a 6-hr incubation period, noting both iron uptake of spleen induced by these cells and cell counts in the incubated cultures. Up to 50% variation was observed not only in erythropoietic activity of cells, but also in the number of cells remaining after a 6-hr incubation at 23°C. Variation has been reduced by use of 10% calf serum in Tyrode's solution without calcium. Reduction in cell number (10–15%) still is observed, but reduction in erythropoietic activity is minimized (Fig. 10).

ORIGIN OF PERITONEAL FLUID CELLS

Joan W. Goodman

Introduction. – Free cells of the peritoneal fluid (PFC) have been studied extensively from the cytologic and immunologic points of view. Recently they have been shown to have hemopoietic activity.[2] The origin of these free cells has been discussed in the literature for over 60 years. The possible sources include circulating blood leukocytes and submesothelial connective tissue cells. The relative importance of these two potential sources is being assessed by typing of PFC taken from homologous radiation–bone marrow chimeras. In such mice, circulating leukocytes are known to be of donor type, whereas submesothelial connective tissue cells are presumably host type.

Methods. – Chimeras that had received, at least 3 months before this study, a single lethal dose of x rays followed within 24 hr by injection of bone marrow from nonisologous mice, were used along with control normal mice of appropriate strains. All chimeras tested retained their foreign marrow transplants. The PFC were harvested in heparinized saline, adjusted to a concentration of 5–10 million cells/ml, and typed by reaction with donor-specific and host-specific isoimmune sera in the presence of rabbit complement.[3]

Results and Discussion. – Table 17 shows that all chimeras tested had peritoneal cells of donor type, and there was no evidence that any PFC had been derived from host cells. However, it should be pointed out that a fraction (from 5 to 20%) of the cells could not be tested by this method because they were nonspecifically killed (i.e., were dead after incubation in nonimmune serum plus complement). Thus, the most that can be said is that 95% (and the least, 80%) of chimeric PFC are donor-bone-marrow-derived.

These results indicate that the submesothelial connective tissue cells play at best a secondary role in supplying free cells to the peritoneal fluid. It is suggested that stem cells, circulating normally in the peripheral blood, wander through the peritoneal cavity and nearby connective tissue. These stem cells, in response to specific stimuli, differentiate into macrophages or other cell types normally found among PFC.

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[2] Joan W. Goodman, "Transplantation of Peritoneal Fluid Cells," *Transplantation* (in press).

[3] P. A. Gorer and P. O'Gorman, *Transplant. Bull.* **3**, 142 (1956).

Table 17. Peritoneal Fluid Cell (PFC) Type in Bone Marrow Chimeras

No. of Mice[a]	Days After Transplantation	Host	Bone Marrow Donor	PFC Type
		Bone Marrow Chimeras		
9	314–383	$1C3F_1$	$B6D2F_1$	$B6D2F_1$
22	299–400	$1C3F_1$	LAF_1	LAF_1
8	309	LAF_1	$1C3F_1$	$1C3F_1$
		Normal Control Mice		
16		$1C3F_1$		$1C3F_1$
6		$B6D2F_1$		$B6D2F_1$
11		LAF_1		LAF_1

[a]All mice reported here retained bone marrow transplants and had donor-type PFC.

SHORT-TERM CULTURE OF MOUSE LEUKOCYTES

Immo Hoppe

Introduction. – The existence of dividing cells in preparations of fresh or tissue cultured peripheral leukocytes has been amply demonstrated. In addition, fresh leukocytes from laboratory animals show hemopoietic stem cell function and immunologic competency.[4,5] A clear relation between specific function and cell type has yet to be established. Tissue culture of peripheral leukocytes conceivably offers an approach to elucidation of this relation, particularly in regard to a current method whereby stimulation of mitosis of human leukocytes after short-term culture can be demonstrated.[6] The present approach deals with a study of the morphologic characterization and functional capacity of mouse peripheral leukocytes in short-term (to 4 days) culture.

Methods and Results. – The initial step is to obtain successful cultures of mouse leukocytes; that is, to show mitotic stimulation of cells in short-term culture as has been demonstrated for human leukocytes. Nichols and Levan[7] published a technique for culturing leukocytes of different mammals, including mice. Following their procedure, however, we were not able to obtain more than a few mitotic figures in the preparations. These results were independent of mouse strain, as well as age and sex of the animals. Varying such factors as the leukocyte isolation technique, heparin preparation, antibiotics, pH, phytohemagglutinin (PHA) and leukocyte concentration, and time of application and concentration of colchicine, afforded no better results. The quality of PHA was critical, since none of several commercial preparations were effective in human leukocyte cultures, whereas an effective preparation was obtained from local cranberry beans. The PHA from cranberry beans, nonetheless, was ineffective for mouse leukocytes.

A decrease in leukocyte counts to about 20% of initial value was observed by *day* 3 of culture. Granulocytes were almost completely eliminated by the separation procedure. During culture, lymphocytes and monocytes showed increasing necrobiotic alterations. By *day* 3, erythrophagocytosis and infrequent phagocytosis of nuclei were observed, in addition to the appearance of cells

[4]Joan W. Goodman and C. C Congdon, *Arch. Pathol.* **72**, 18 (1961).

[5]Joan W. Goodman and G. S. Hodgson, *Blood* **19**, 702 (1962).

[6]P. C. Nowell, *Cancer Res.* **20**, 462 (1960).

[7]W. W. Nichols and A. Levan, in *Conference on Blood–Bone Marrow Tissue Culture and Cell Separation*, Bethesda, Md., Oct. 20–21, 1961.

that had plasmocytic features and showed various degrees of vacuolization.

Cultured cells retained some functional capacity. Isologous leukocytes cultured up to 4 days caused rejection of homologous marrow transplants in lethally irradiated mice.

Conclusion. – A working tissue culture of mouse peripheral leukocytes has not yet been obtained. Under present culture conditions used, cell death occurs quickly, although after 4 days there remain viable cells that retain immunologic competency.

ELECTRON MICROSCOPE OBSERVATIONS OF LYMPHATIC TISSUE

D. C. Swartzendruber

Mouse splenic white pulp undergoes characteristic alterations following the intravenous injection of 100×10^6 rat bone marrow cells. These changes have been investigated by Congdon,[8] and

[8]C. C Congdon, *J. Natl. Cancer Inst.* **28**, 305 (1962).

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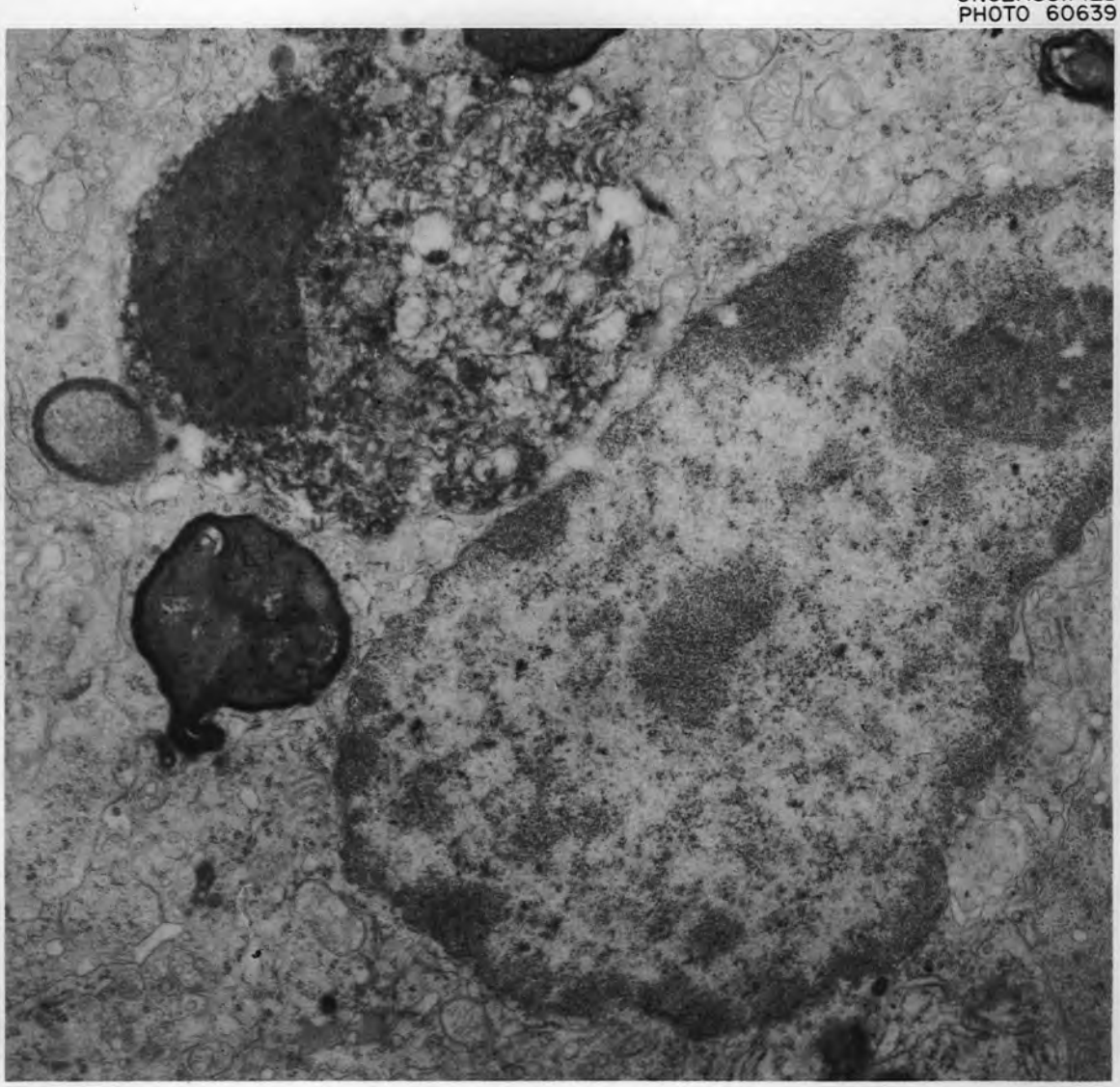

Fig. 11. Reticular Macrophage or Tingible Body. Note large vesicular nucleus to the right with a phagocytosed plasma cell and other inclusions to the left. 25,000X.

the role of germinal centers in the immune response has been reemphasized. The electron microscope is being used to extend these observations since high-resolution microscopy affords additional and more precise morphologic criteria than conventional microscopy for distinguishing among cellular types of lymphatic tissue.

In the first 3 days following antigen injection, large, presumably plasmablast, cells are numerous in the splenic white pulp. These cells are characterized by an abundance of free ribosomes and a small amount of ergastoplasm. They resemble precisely the cells described by Binet and Mathé[9] in the homograft reaction. Germinal centers are re-formed by *day* 4 or 5, and a characteristic feature is the appearance of reticular macrophages or tingible bodies of Flemming. Tingible bodies are thought to contain phagocytosed nuclear debris of lymphocytes. Electron micrographs of these macrophages provide evidence that plasmocytic cells are also phagocytosed (Fig. 11).

The cytoplasm of reticular macrophages appears to contain numerous mitochondria and profiles of smooth or agranular endoplasmic reticulum (ER) as well as rough ER. Also scattered free ribosomes are present. The above features probably account for the empty or clear cytoplasm of tingible bodies as seen in Zenker-formol-fixed, hematoxylin and eosin-stained sections.

Studies are continuing in attempts to describe reticular macrophages and their relation in the germinal centers to the immune response.

ANTIGEN LOCALIZATION IN THE SPLEENS OF LAF_1 MICE

M. G. Hanna, Jr.

Introduction. – Considerable attention has been given antigen localization in lymphatic tissues. Soluble antigens labeled with I^{131} have been used in these studies based on the assumption that these antigens would more closely represent the "antigenic stimulant." In general, the autoradiographic results have demonstrated that the soluble antigens are evenly distributed within the spleens and lymph nodes of stimulated animals. Due to the rather high-energy beta particles emitted from I^{131}, its resolution in autoradiographs is very poor. The tendency has also been to inject high concentrations of the labeled soluble antigens. With these two considerations, precise localization could not be obtained; consequently, detailed topographical studies of the lymphatic tissue have not been adequately performed.

In this study, a low concentration of bovine serum albumin (BSA) labeled with I^{125} was used to obtain better autoradiographic resolution and more precise localization. Iodine-125 is a nuclide which decays by electron capture, emitting principally photons with energies in the range of 27.3 to 35.5 kev. Accompanying the photon radiation are low-energy conversions and Auger electrons which have the same general energy range as tritium beta particles.

Methods and Results. – LAF_1/Cum male mice (15 to 16 weeks of age) were injected intravenously with 0.1 ml of BSA that was labeled with I^{125}. Each animal received 23 μc I^{125} and 7.5 mg BSA in each 0.1-ml injection. Four animals were autopsied hourly for 23 hr. Autoradiographs were prepared with Kodak NTB-2 liquid emulsion of the spleen, liver, thymus, and mesenteric lymph node. After 10 days' exposure the autoradiographs were stained with hematoxylin and eosin. At present, the only quantitative data obtained has been on the antigen localization in the spleen. Total grain counts were made on four randomly selected high-powered fields in both the germinal center and the surrounding lymphocyte mass. This procedure was followed in four white pulp areas of each spleen section studied. In the red pulp, 16 randomly selected areas were counted.

The data thus far obtained (Table 18) demonstrate a positive gradient for the soluble antigen in the germinal center. It is accepted that adjusted ratios may affect the magnitude of the gradient; however, it would not affect the overall trend exhibited in the 24-hr interval. The activity of the red pulp appears to be more closely aligned to the germinal center than to the lymphocyte mass.

Discussion. – The more refined technique utilized in this study reveals an unequal distribution of soluble antigen in the spleen. This fact has also been observed in unstained slides where the grains were demonstrated with a dark-field condenser. Further experiments are planned to test whether this response persists in later post-injection intervals and to determine its significance.

[9] J. L. Binet and G. Mathé, *Nature* **193**, 992 (1962).

Table 18. Antigen Localization in the Spleen

Hours After Injection	Average Grain Count		A/B	Average Grain Count in Red Pulp Area
	Germinal Center Area (A)	Lymphocyte Mass (B)		
1	121.3	119.0	0.98	165.7
2	47.8	41.8	0.87	58.5
3	60.2	46.1	0.77	64.5
4	54.4	38.0	0.69	55.2
5	39.5	29.3	0.74	46.8
6	29.1	20.1	0.69	33.0
16	44.4	29.0	0.65	46.0
17	22.3	16.7	0.75	22.4
18	36.9	28.7	0.77	36.8
19	44.0	30.7	0.70	53.2
20	31.3	22.1	0.71	35.0
21	48.2	30.6	0.63	50.2
22	36.5	25.3	0.69	42.6
23	37.6	20.2	0.54	32.4
24	30.6	20.0	0.65	35.3

EARLY HISTOLOGIC EFFECTS OF ANTIGENIC STIMULATION IN THE MOUSE SPLEEN

C. C Congdon

Introduction. – A unique series of cellular changes takes place in the white pulp of the mouse spleen during the first few days after intravenous injection of antigenic materials.[10] Since the changes are well-established at 24 hr, an investigation was made of the histologic material taken at hourly intervals for the first 24 hr following primary antigen administration.

Results. – The findings with 100×10^6 rat bone marrow cells consisted of marginal zone cell changes at 1 to 2 hr, increased debris in tingible bodies and beginning germinal center dissolution at 3 hr, beginning loss of lymphocytes near central artery at 6 hr, presence of new pyroninophilic cells at 8 hr, loss of most germinal centers at 10 hr, and a fully developed picture of white pulp blurring at 15 hr.

Especially interesting were the changes in tingible bodies during the 24-hr period of observation. The tingible body is a macrophage containing cells and cell debris. It is located within the germinal center. The germinal center is a collection of dividing cells lying in a mass of lymphocytes that constitute the white pulp of the spleen.

The amount of cell debris in the tingible bodies was greatly increased at 3 to 4 hr after the marrow injection. It remained at this increased level until approximately 8 hr after injection, at which time there was an abrupt cessation of nearly all tingible body activity.

A second intravenous injection of 100×10^6 rat bone marrow cells given 6 weeks after the first showed marked changes in marginal zone cells, a great increase in debris in the tingible bodies, and dissolution of the light-staining side of the germinal centers during the 8-hr period after injection in which the spleen white pulp was examined.

Discussion. – These findings, which can be considered the prompt effects of antigenic stimulation, point to the structures that initially come in contact with the antigen. Apparently the blood circulation within the spleen red pulp is such that foreign materials are directed against the surface or marginal zone of the masses of white pulp substance causing immediate changes in the

[10] C. C Congdon and Joan W. Goodman, p 181 in *International Symposium on Tissue Transplantation*, Santiago, Chile, 1962.

cellular composition of this surface. Next, the macrophages within the germinal centers close to the marginal zone began to show a great increase in activity and continued to do so for about 8 hr. This is the amount of time required to initiate an intense cell proliferation process, which we speculate either comes from the dark-staining cells of the germinal center or from a transformation of the surrounding lymphocyte mass. The proliferative phase of cellular activity in the white pulp replaces most of the white pulp structure by 15 hr after antigen injection. One also assumes that during the proliferative phase new substances are first produced which destroy the foreign cells or interact with the antigenic materials. Lymphatic tissue changes in foreign bone marrow chimeras can be interpreted as variations on the types of changes seen in normal mice rejecting homologous or heterologous marrow.[10]

SERINE METABOLISM IN MICE CARRYING HOMOLOGOUS BONE MARROW GRAFTS

A. L. Kretchmar[11]

Introduction. – As indicated in previous reports, the secondary disease process in lethally irradiated mice is believed to be a metabolic disorder superimposed on, or resulting from, the graft-against-host immune reaction. Investigations carried out so far indicate marked liver enlargement with increased free aspartic acid as relatively unique features of the foreign bone marrow chimera prior to the development of clinical secondary disease. Suu has shown, in addition, that kidney lysozyme content is considerably elevated during the first 3 weeks after marrow transplantation (this report). The present work demonstrates another difference between isologous and homologous bone marrow chimeras – the homologous chimera has a low free serine content of the liver, plasma, and muscle.

Results and Discussion. – The concentration of free serine of liver is low in irradiated mice with homologous bone marrow grafts as compared to unirradiated animals or to irradiated mice given isologous bone marrow cells. At 7 days after injection of homologous marrow, the concentration is within the range of levels in control animals; at 12 days, it is reduced by about one-third; and at 21 and 35 days it is reduced by one-half. Levels in plasma are low at 12, 21, and 35 days. The concentration of serine in muscle is not affected until after *day* 21, when it is decreased. Data obtained from an analogue computer allowed us to construct a speculative model of serine metabolism that relates the serine pools in liver, plasma, and muscle. This model suggests that a marked increase in net loss of serine (either greatly accelerated utilization, blocked synthesis, or a combination) can explain the experimental results. This imbalance in serine metabolism would begin on about the 4th to 6th day after injection of marrow. This result is consistent with other findings, indicating that the disturbance in host metabolism brought about by a homologous graft begins very early after injection of the foreign cells.

[11]ORINS Medical Division.

INCREASE IN LYSOZYME ACTIVITY IN KIDNEYS OF IRRADIATION CHIMERAS

Vu-Thi-Suu

Introduction. – The wide distribution of lysozymes in plants and animals (tears, saliva, blood plasma, tissues, and secretions) and their lytic action against certain gram-positive and gram-negative bacteria[12] suggest a role for these enzymes in defense of plants and animals against bacterial invasion. The observations[13–15] that lysozyme activity in serum and kidney of rabbits increases during sensitization to horse serum, after injection of heat-killed *Salmonella typhosa* or Newcastle disease virus, during anaphylaxis, and, in serum of children, after injection of adrenocorticotropin indicate that mobilization of lysozyme activity may be a rather general part of an animal's reaction to bacterial, immunological, and

[12]P. Jollès, pp 434–45, part A, in *The Enzymes*, vol 4, ed. by P. D. Boyer, H. Lardy, and K. Myrbäck, Academic Press, New York, 1960.

[13]T. Hamamoto, *Ann. Paediat. Japon.* **5**, 80 (1958).

[14]H. Nagai *et al.*, *Ann. Paediat. Japon.* **6**, 622 (1960).

[15]J. C. Ribble, *Proc. Soc. Exptl. Biol. Med.* **107**, 597 (1961).

noxious insult. In this connection, the observations of Perri[16,17] on the increased lysozyme activity in kidney of rats with certain tumors, and those given in this report showing increased lysozyme activity in kidney of homologous irradiation chimeras, are of interest.

Methods and Results. – Male mice (LAF_1/Cum), 12 to 20 weeks of age, were used as recipients or donors of isologous bone marrow. For the donors of homologous bone marrow, male mice ($1C3F_1$/Cum) of the same age were used.

The lysozyme activity in kidney of irradiation chimeras was measured with a Zeiss spectrophotometer at 25°C (wavelength: 450 mμ). Absorbance was read at 30-sec intervals, and the course of the reaction was recorded for 2½ min. The procedure of Shugar[18] standardized against Worthington lysozyme was used.

This assay has shown that a marked increase in lysozyme activity is associated with the presence of grafted homologous bone marrow (Fig. 12). This increase begins about 3 days after injection of the marrow cells, reaches its peak at 20–40 days, and returns to normal at 100–200 days after treatment.

[16]G. C. Perri and R. Anigstein, *Cancer Res.* **20**, 1054 (1960).

[17]G. C. Perri *et al.*, *Nature* **193**, 649 (1962).

[18]D. Shugar, *Bull. Soc. Chim. Biol.* **33**, 710 (1951).

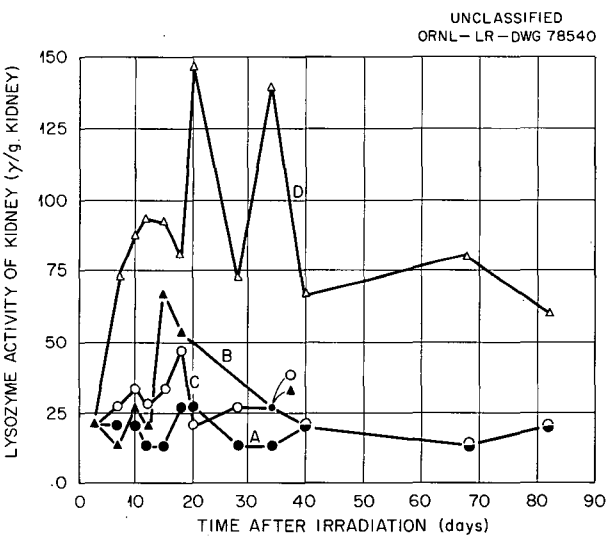

Fig. 12. Lysozyme Activity in the Kidneys of Mice. Normal control, *A*; animals given 950 r total-body irradiation, *B*; irradiation and 40 × 10^6 isologous bone marrow cells, *C*; 40 × 10^6 homologous cells, *D*. Each point is the average of data from 1 to 3 mice.

STUDY OF NITROGEN BALANCE IN IRRADIATED MICE GIVEN FOREIGN BONE MARROW

W. H. McArthur

Introduction. – Irradiated mice can recover from lethal exposure if given bone marrow cells intravenously. Animals treated with isologous bone marrow usually have an uneventful recovery period and show delayed effects of irradiation and premature aging. In contrast, irradiated animals given foreign bone marrow will start to regain their initial loss of body weight, then die when a secondary weight loss, beginning on or about the 12th day after treatment, reaches a level that is unable to sustain life. This weight loss occurs despite a nearly normal intake of food.

It has been suggested that animals treated with foreign bone marrow die of a "metabolic starvation." Metabolic changes may not be specific for a "homologous disease syndrome," but may instead be the result of debility caused by reaction between grafted foreign cells and host in some rather nonspecific way. However, if metabolic changes are specific, then these experiments may serve as models for study of immune diseases in general, since it is rather generally felt that the basic underlying mechanism of homologous disease is immunological interaction between grafted cells and host.

The metabolism of homologous animals was compared to normal mice and irradiated mice given isologous bone marrow cells. This investigation attempts to measure a rather general metabolic parameter – that of determination of nitrogen balance during recovery in treated animals, and determination of any influence of the homologous disease development.

It is believed that work of this kind will aid in designing more specifically mechanistic studies and, in particular, will help to pinpoint the beginning stages of homologous disease.

Methods. – Twenty-four animals were used, including six normal, six x-irradiated, six isologous, and six homologous marrow-treated mice. These data cover the 30-day-period experiments. Sixty- and ninety-day-period experiments are in progress.

LAF_1/Cum male mice were x-irradiated recipients; homologous bone marrow donors were $1C3F_1$/Cum. Recipients received 900 r and were injected with 40×10^6 isologous or homologous bone marrow cells.

Urine and feces were collected every 48 hr from specially designed metabolic cages. Nitrogen balance experiments were run on collections in which the feces were separated from the urine. Other experiments were made on collections in which feces and urine were combined. Nitrogen determinations were made by means of the automatic nitrogen analyzer. Data presented here will cover experiments on feces that were collected separately from urine.

Results and Discussion. – Nitrogen balance in normal and irradiated animals given bone marrow is summarized in Fig. 13. The x-irradiated animals not given bone marrow show progressively negative nitrogen balance until death. Normal animals maintained a positive nitrogen balance during these experiments. Irradiated isologous and homologous marrow-treated animals were able to maintain a positive nitrogen balance.

Dry weight of feces in normal animals varies from 1.90 to 2.32 g, while the nitrogen ranges from 103 to 140 mg of nitrogen excreted per day. Among the x-irradiated groups, only one animal not treated with bone marrow showed abnormal weight changes and nitrogen balance. Weight in x-irradiated controls varied from 1.23 to 1.57 g, while nitrogen range varied from 81 to 96 mg. Weight in isologous animals varied from 1.05 to 2.41 g, while nitrogen range varied from 121 to 157 mg. Weight in homologous animals varied from 1.56 to 2.42 g with nitrogen range varying from 94 to 144 mg.

Conclusion. – There was no difference between nitrogen excreted in isologous and homologous animals. These data agree with conclusions reached in earlier experiments.

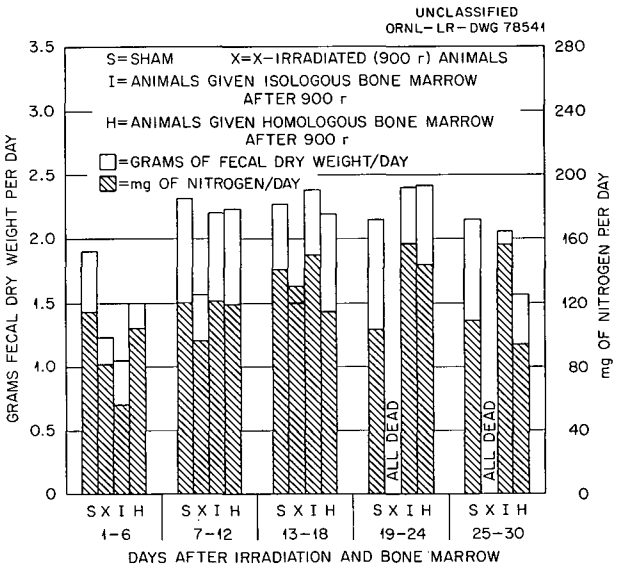

Fig. 13. Nitrogen Balance in Normal and Irradiated Mice Given Bone Marrow.

Radiation Immunology

T. Makinodan
J. F. Albright
E. H. Perkins
E. E. Capalbo[a]
T. Sado[a]
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CHANGES IN POOL SIZE OF POTENTIALLY COMPETENT ANTIBODY-FORMING CELLS FROM SPLEENS OF PRIMED AND NONPRIMED DONORS

E. H. Perkins

Introduction. – Data which must be considered in assessing the differentiation status of antibody-producing cells are presented. We have shown previously that spleen cells (N_0) proliferate when cultured in lethally irradiated, isologous recipients and that there is a direct log relation between N_0 and the amount of antibody produced.[1] When antisera are collected at the end of the logarithmic growth phase of the infused spleen cells and $\log_2$ titer is plotted against $\log_2$ cell number, the resulting regression line has a slope of $\sim$1 (ref 2). Therefore, the relative pool size of potentially competent (PC) antibody-forming cells can be estimated by injecting the test antigen at varying intervals after culture of N_0.

Results. – No significant change in relative pool size of PC cells from primed donors was detected during a 13-day period of culture. Furthermore, the stability of the relative pool size was independent of N_0, within a range of 0.5×10^6 to 10×10^6, primed cells during the 13-day period of culture. The relative pool size of PC cells from nonprimed donors decreased as the interval between initiation of culture and antigen stimulation increased and reached a minumum 5 days after culture. This characteristic decrease in pool size of PC cells from nonprimed donors could be reduced by (1) decreasing the irradiation dose given to the recipient, (2) injecting bone-marrow cells along with N_0, and (3) using these two procedures simultaneously. Injecting a non-cross-reacting antigen 1 day after culture of N_0 and prior to injection of the test antigen resulted in a further decrease in pool size. These latter treatments had no effect on the pool size of N_0 from primed donors.

Discussion. – These results suggest that there exist at least two sequential compartments of PC cells. The primary compartment is made up of pluripotent cells capable of differentiating along

[1]E. H. Perkins, M. A. Robinson, and T. Makinodan, *J. Immunol.* **86**, 533 (1961).

[2]T. Makinodan, M. A. Kastenbaum, and W. J. Peterson, *J. Immunol.* **88**, 31 (1962).

several paths, depending upon the stimulant. The secondary compartment consists of cells whose path of differentiation is fixed.

COMPETITIVE EFFECTS OF NON-CROSS-REACTING ANTIGENS IN PRIMARY ANTIBODY RESPONSE

T. Makinodan W. J. Peterson

The interval between the time of antigen injection and the time of appearance of antibody-synthesizing cells can be defined as the latent phase. During this phase the most pronounced histological change is taking place in the germinal center of lymphoidal organs.[3] It follows then that interference of this germinal center reaction should confound the sequential events. Studies were therefore carried out to assess the effect of sheep RBC antigen (Ag_1) injection on antibody response to rat RBC antigen (Ag_2).

Materials and Results. – Twelve-week-old (C3H × 101)F_1 mice were used in this study. At varying intervals after intraperitoneal injection of an optimal dose of Ag_1, an optimal dose of Ag_2 was injected intraperitoneally. Sera were collected 6 and 8 days after Ag_1 injection and were titrated for antibody content in the usual manner. In view of the relation, antibody response = constant $(N_0)^{k_1}(Ag)^{k_2}$, where $k_1 = 1.0$ and $k_2 = 0.4$ (ref 4), we have expressed our data in terms of activity relative to that when Ag is given alone (see Fig 14). It can be seen that the maximum suppressive effect of Ag_1 on antibody response to Ag_2, and vice versa, can be detected when the time interval between injection of Ag_1 and Ag_2 is 48 hr.

Conclusions. – These results emphasize the following: (1) at the height of the germinal center reaction, the immune system of an animal cannot efficiently cope with another non-cross-reacting antigen, and (2) transformation of potentially competent cells into competent antibody-synthesizing cells can be most easily confounded when a non-cross-reacting antigen is introduced at the height of the germinal center reaction.

[3]C. C Congdon and T. Makinodan, *Am. J. Pathol.* **39**, 697 (1961).

[4]T. Makinodan and J. F. Albright, *Third International Symposium on Immunopathology*, in press.

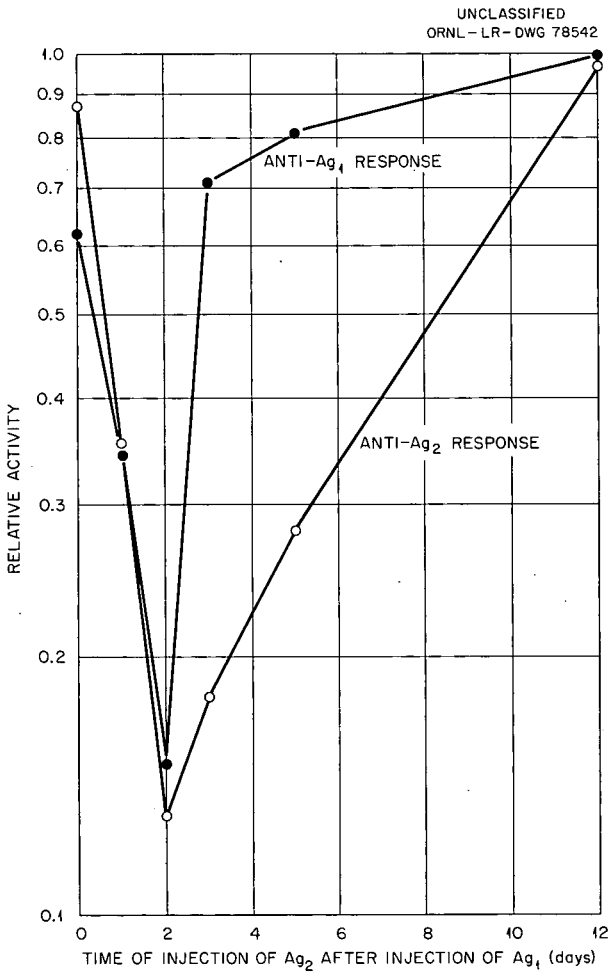

Fig. 14. Effect of Non-Cross-Reacting Antigenic Stimulation on Specific Primary Antibody Response.

PROLIFERATIVE CAPACITY OF ANTIBODY-PRODUCING CELLS

Toshihiko Sado Martha R. Leonard

Introduction. – It seems to be established that somatic cellular division is characteristic of the immune response. However, little is known about the proliferation of cells that are actually synthesizing antibodies. To obtain more detailed information about antibody-producing cells, we have estimated their proliferative capacity at various stages of antibody response by combined tritiated thymidine (H^3T) labeling and immunofluorescent techniques.

Method and Results. – Rabbit spleen cells preimmunized against bovine serum albumin (BSA) were cultured *in vivo* in diffusion chambers in sublethally irradiated mice and stimulated with the secondary dose of the same antigen. At frequent intervals after the antigenic stimulation, animals were injected intraperitoneally with a single dose of H^3-thymidine (5 μc/mouse). One hour later, chambers were removed from the injected mice, and cells were collected and stained by the fluorescent antibody (anti-BSA) technique. Fluorescence-positive, or antibody-containing, cells were located and then processed for autoradiography (for details, see ref 5).

Antibody-containing cells were classified into two types on the basis of size and appearance: a large or *immature* cell type and a small or *mature* type.

Figure 15 shows the uptake of H^3-thymidine by large and small antibody-containing cells. It is clear that, during the log phase (*day* 5) of antibody production, immature cells are very active in DNA synthesis. Very few such cells are capable of dividing on *days* 9 and 11. During the log phase, some of the mature cells also incorporate H^3-thymidine. From *day* 7, there are no mature cells synthesizing DNA. This indicates that these cells are differentiated, or terminal, cells.

Roughly speaking, H^3T index, or percentage of labeled cells, could be expressed as

$$H^3T\ (\%) = \frac{\text{duration of DNA synthesis}}{\text{mean generation time}} .$$

If we assume the duration of DNA synthesis as 5 hr, independent of cell types, we can estimate the mean generation time of immature and mature antibody-containing cells at various times after antigenic stimulation as indicated in Table 19.

Table 19. Mean Generation Time of Antibody-Producing Cells

Days After Antigenic Stimulation	Mean Generation Time (hr)	
	Large Ab Cells	Small Ab Cells
5	8.6	85.5
6	35.0	595.2
7	98.4	
9	462.0	
11		

[5]P. Urso and T. Makinodan, *Journal of Immunology*, in press.

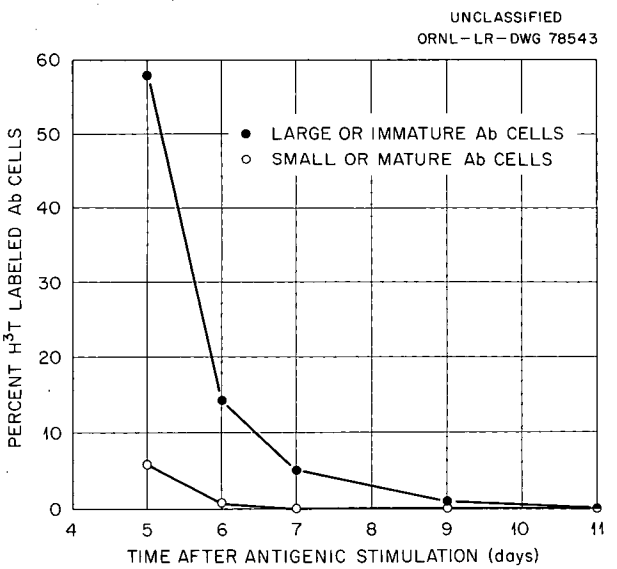

Fig. 15. Uptake of H^3-Thymidine by Antibody-Containing Cells.

Conclusions. – The data indicate that during the log phase the mean generation time of immature antibody-producing cells is less than 9 hr. It is interesting to note that doubling time of antibody titer during this period is about 7 hr.[4] During the stationary and decay phases, proliferative capacity of these cells declines, and the mean generation time becomes much longer. These results strongly suggest the view that antibody response is a reflection of proliferation of antibody-synthesizing cells. It is also shown that small or mature antibody-containing cells recovered from *day* 7 on are terminal, nondividing cells.

ESTIMATION OF THE PROPORTION OF POTENTIAL ANTIBODY-PRODUCING CELLS WITHIN THE POPULATION OF CELLS COMPRISING THE MOUSE SPLEEN

J. F. Albright
T. Makinodan
W. J. Peterson
Martha R. Leonard

Introduction. – Previous results have permitted us to estimate that only about 2×10^7 spleen

cells are required to produce a secondary antibody response equivalent to that of the normal intact mouse and that, during antibody (Ab) production, one cell initially stimulated can generate, through somatic division, about 10^3 antibody-synthesizing cells.[2,4] These estimates suggest that the number of cells which are susceptible to stimulation by a given test antigen may constitute a surprisingly small proportion of the total spleen-cell population. The results of direct tests of the number of potential antibody-producing cells (PC cells) in the spleens of both nonimmunized and previously immunized animals are presented.

Results. – One phase of the antibody-production curve is the temporary stationary level of titer, which occurs at the end of the phase of exponential increase in titer. This phase occurs by *days* 8 to 9 after antigen stimulation of any number of spleen cells which is sufficient to yield detectable antibody when cultured *in vivo*. We have, therefore, determined the minimum number of spleen cells which, in 50% of replicate samples, will produce detectable antibody by *day* 8 or 9 after antigen stimulation. Figure 16 is a probability plot of the data obtained from testing the response of preimmunized spleen cells against rat RBC. It can be seen that there is one unit (presumably one cell) of PC cells in about 10^5 spleen cells with respect to this test antigen. There is one unit of PC cells with respect to rat RBC in about 10^7 spleen cells of the nonimmunized mouse. Similar results have been obtained in preliminary estimates of the number of PC cells capable of responding to sheep RBC.

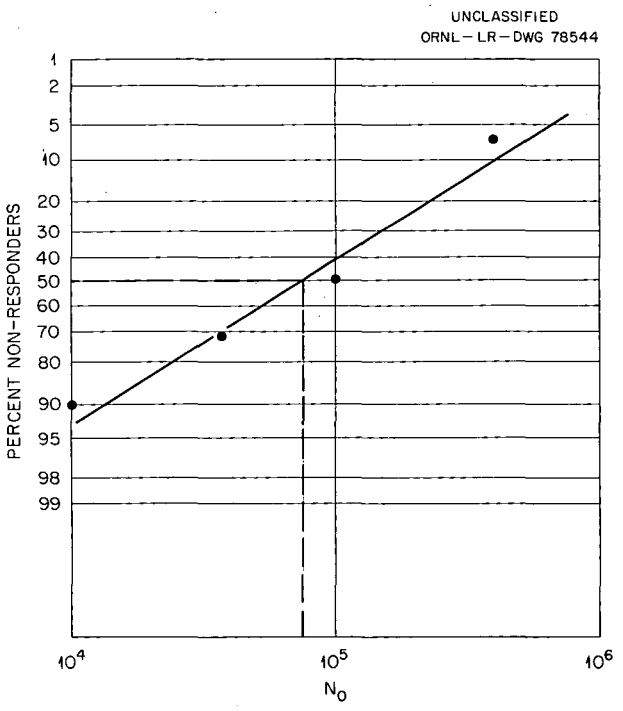

Fig. 16. "All-or-None" Assay for Fraction of PC Cells in Total Spleen Cells (N_0).

Discussion. – In the spleen of a preimmunized animal there is one PC cell in about 10^5 cells for a given antigen, as shown above. Upon exposure to antigen each PC cell which is stimulated will generate about 10^3 antibody-synthesizing cells by the end of the exponential phase. At this time the proportion of antibody-synthesizing cells to total spleen cells will be 10^{-2}. Actually, this high proportion can probably never be realized under normal conditions for physiological reasons (homeostasis), and this probably affords us some insight into the reasons why, in the intact animal, only about 10% of the PC cells are involved in maximum antibody response (in this case the proportion of Ab cells to total cells would be about 10^{-3} at the end of the exponential phase). If more than one immunogen were introduced, the proportion of Ab to total cells would be even more exaggerated, and it is possible that, because of the physiological limitations, one might obtain results which would suggest competition of immunogens.

STORAGE OF POTENTIAL ANTIBODY-PRODUCING CELLS AT LOW TEMPERATURE

J. F. Albright Peter Mazur[6]
W. J. Peterson

Introduction. – To continue our studies on purification, kinetics, and mechanisms of antibody (Ab) producing systems, it is necessary to have a method for accumulating and storing competent and potentially competent Ab-producing cells. Our quantitative techniques for evaluating Ab production permit accurate and systematic assessment of methods for preserving Ab-producing cells. Results are presented which show that under certain conditions Ab cells may be stored

[6]Cell Physiology Group.

with virtually no loss in their Ab-producing capacity.

Results. – Pools of spleen cells were prepared from preimmunized mice. The Ab-producing potential of fresh aliquots of cells from the pools was determined in *in vivo* cultures. Other aliquots were suspended in various concentrations of either glycerol (GLY) or dimethyl sulfoxide (DMSO) and frozen. The most suitable procedure for freezing was found to be controlled reduction of temperature at the rate of 1°C/min from 4°C to −50°C. The cells were then cooled rapidly to −196°C by immersion in liquid nitrogen. They were stored for 1 hr at −196°C and then thawed rapidly. The concentration of the suspension of cells during freezing was 2×10^8/ml. After thawing, the cells were diluted tenfold with Tyrode's medium to obtain a suitable concentration of cells for the assay and to reduce the concentration of the preservative to a level which is not toxic for the test mice. Figure 17 shows the amount of Ab produced by a fixed number of cells that were frozen and stored in various concentrations of DMSO or GLY. Antibody titer is expressed as percentage of that produced by control, unfrozen cells.

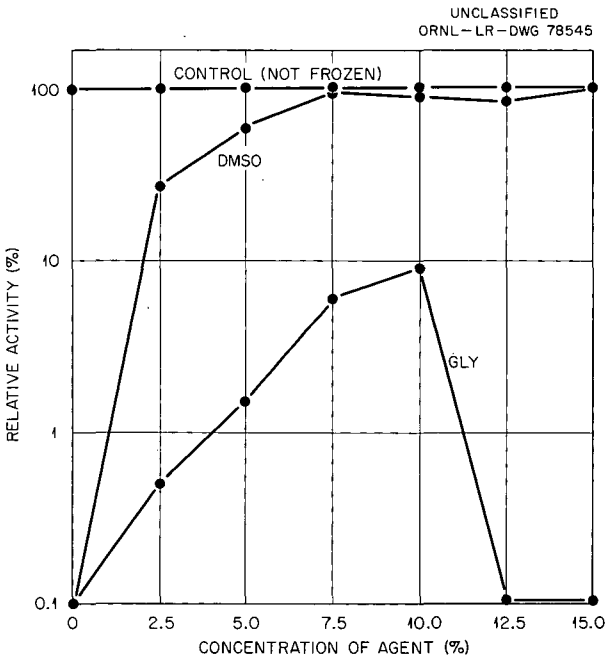

Fig. 17. Antibody-Producing Capacity of Mouse Spleen Cells Frozen and Thawed in Various Concentrations of Glycerol (GLY) and Dimethyl Sulfoxide (DMSO).

Discussion. – It is clear that DMSO is a more satisfactory preservative for Ab-producing cells than is GLY. At concentrations in the range 7 to 15%, DMSO permits the Ab-producing cells to be frozen and thawed with little or no loss in activity. It is probable that little additional loss in activity of the cells will occur on long-term storage at −196°C; experiments to establish this point are in progress.

HUMORAL HOMOGRAFT-REJECTING FACTOR

E. E. Capalbo

Introduction. – Two types of mechanisms, cellular and humoral, have been suggested to explain the rejection of homografts by competent cells. Using the diffusion chamber technique as a cell culture method and tritiated thymidine (H^3T) as a cell marker, we found that a profound inhibition of proliferation of H^3T-labeled target cells occurs during the first 24 hr of culture with presensitized cells.[7] We also reported that in terms of relative antibody-forming activity, 94% of target cells were destroyed when cultured with presensitized cells.[8] This information indicates: (1) the diffusion chamber technique is a suitable method to study homograft rejection, and (2) target cells are most probably destroyed when cultured in contact with presensitized competent cells. A series of experiments, reported here, were carried out using double-compartment diffusion chambers to investigate the presence of a humoral homograft-rejecting factor. As criteria for rejection, the decreases in secondary antibody response and in the percentage of H^3T-labeled cells were utilized.

Results. – For this work two parental strains of mice differing at the H-2 locus were used as a source of spleen cells. Mice of C3H strain were used as donors of killer (P1) cells, and mice of C57BL strain as source of target (P2) cells. Spleen cells were inoculated into double-compartment diffusion chambers which were implanted into the peritoneal cavity of x-irradiated (500 r) C3H

[7] E. E. Capalbo, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1962*, ORNL-3267, p 81.

[8] E. E. Capalbo, F. Celada, and T. Makinodan, *Federation Proc.* **21**, 41 (1962).

mice. In one group of experiments, 24 million P1 spleen cells, presensitized with P2 antigens, were cultured in one compartment of the diffusion chambers. The second compartment contained 24 million P2 spleen cells preimmunized against rat red blood cells (RBC). A control group consisted of diffusion chambers carrying 24 million preimmunized P2 spleen cells plus 3 million rat RBC in one compartment and Tyrode's solution in the second. Antirat RBC titer was determined in chamber fluid samples collected on the fifth day of culture. In terms of relative antibody-forming activity, 84% of the target cells were destroyed. In a second group of experiments, 24 million P1 cells and 24 million P2 cells were cultured in double-compartment diffusion chambers and labeled with H^3T by injecting the recipients. A control group consisted of diffusion chambers carrying only P2 spleen cells. Figure 18 shows that the proliferation of H^3T-labeled target P2 cells in the experimental group is greatly inhibited. The doubling time of the control group was about 24 hr. Killer (P1) cells, on the other hand, proliferated at an accelerated rate with a doubling time of ~12 hr.

Discussion. – In these experiments, killer and target cells were separated from each other by a porous membrane that is impermeable to cells. The results presented here suggest that rejection of the homologous test cells can be effected by means of a humoral factor, which diffuses through the membrane and has the ability to act across, at least, short distances (~3 mm). The nature of the factor is unknown.

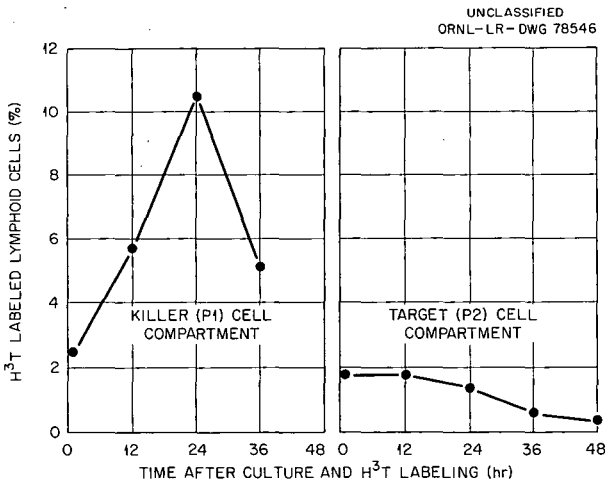

Fig. 18. Proliferation of Killer and Target Cells Cultured in Double-Compartment Diffusion Chambers as Indicated by the Percentage of H^3T-Labeled Cells.

QUANTITATIVE STUDIES OF SPLENIC ENLARGEMENT IN CHICK EMBRYOS IN RESPONSE TO HOMOLOGOUS GRAFTS

Frank Seto

Introduction. – Data from earlier experiments in this laboratory,[9] in which the splenomegaly response following the grafting of heterologous and homologous spleens was measured, indicate that, contrary to popular belief, the host embryo is able to react immunologically to foreign tissues. Since it is difficult to obtain quantitative data with the chorioallantoic membrane (CAM) grafting technic, information of this nature is generally lacking in earlier studies. An alternative procedure has been adopted to study the splenomegaly phenomenon quantitatively.

Methods and Results. – Spleens from adult chicken donors were teased in Tyrode's solution, and cell suspensions of known concentrations were prepared. Using the technic of Beveridge and Burnet,[10] 0.05 ml of spleen cell suspension was injected intravenously into incubated, fertile eggs. At specific time intervals thereafter, the embryos were killed, and the body, liver, and spleen weights were estimated. In most of the experiments both adult donors and host embryos were derived from the same commercial, non-inbred, white leghorn stock from the University of Tennessee. Experiments were conducted to determine the splenic enlargement as a function of (1) the time after cell injection, (2) the number of splenic cells injected, (3) the age of the host embryos, and (4) irradiation of host embryos and donor cells. The results of the first three parts of the study are summarized in Fig. 19 (*a–c*). Relative splenic enlargement was estimated by using the difference between the experimental and control spleen weights and plotting the values with the control level arbitrarily set at 1. It can be seen

[9] J. F. Albright, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 98–100.

[10] W. I. B. Beveridge and F. M. Burnet, *Medical Research Council Report No. 256*, Her Majesty's Stationery Office, London, 1946.

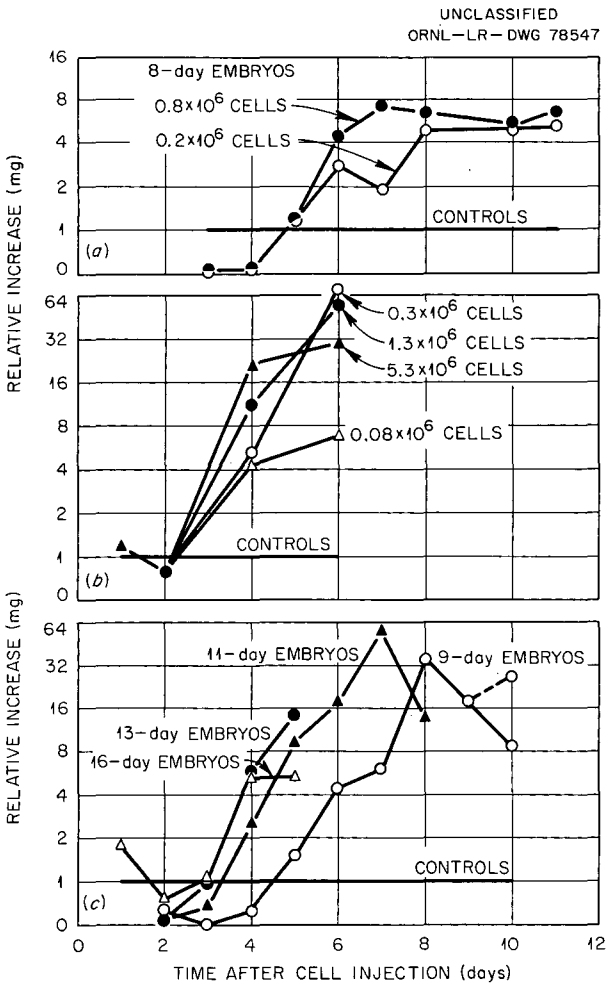

Fig. 19. Relative Enlargement of Chick Embryo Spleen Following Intravenous Injections of Adult Chicken Splenic Cells. *a*, Temporal change in spleen enlargement of 8-day embryos; *b*, relative splenic enlargement following injections of different donor cell concentrations; *c*, splenic responses of embryos of different incubation ages – illustrating differences in induction periods.

in Fig. 19*a* that the temporal change in the splenic enlargement resembles the hemagglutinin immune response curve – with an initial latent period, followed by a sharp rise of a few days duration, a plateau, and subsequent decline. Figure 19*b* illustrates the effect of injecting different donor cell concentrations. For moderate and low cell dosages, the enlargement is roughly proportional to the numbers of injected spleen cells. When host embryos of different ages are used (Fig. 19*c*), the latent period is of longer duration in the younger hosts. In experiments in which host embryos were x-irradiated at different doses and injected with fixed numbers of cells, the splenic enlargement of irradiated embryos was consistently lower than the unirradiated controls and was dose-dependent. On the other hand, the correlation between dose of irradiation delivered to donor spleen cells and decrease in splenomegaly was not consistent; that is, at certain doses, enlargement greater than that with unirradiated cells was obtained.

Discussion. – The experimental data are more readily explained if one assumes that it is the host system which is responding immunologically to the donor cells (antigens). The longer induction period in the younger hosts may reflect a lesser development of immunological competence. The depressed response at high dosages and the proportional enlargement at lower dosages can be expected in view of our present knowledge of antigen dosage effects.[11] Since irradiation is known to reduce the immunological capacity, the reduction in splenomegaly after the irradiation of the host embryos undeniably indicates an active role of the embryo in splenomegaly. These data strengthen the viewpoint that the embryo is immunologically competent.

EVALUATION OF THE RELATION BETWEEN ANTIGEN DOSE AND THE AMOUNT OF HEMAGGLUTINATING ANTIBODY PRODUCED BY MOUSE SPLEEN CELLS CULTURED *IN VIVO*

J. F. Albright Rachel R. Carter

Introduction. – The factors which influence antibody production can be readily visualized in the following expression:

$$\mathrm{Ab} = f(\mathrm{Ag}, N_0, V) ,$$

where Ab is the amount of antibody produced (e.g., titer), Ag is the amount of a given antigen, N_0 is the number of competent antibody-producing cells, and V is the volume in which the reaction is conducted. Each of the independent variables may be analyzed when the other two are kept constant. The relation between Ab and N_0 has

[11] J. F. Albright and T. Makinodan, unpublished data.

been examined in detail.[12] The present report is concerned with our progress in the analysis of the relation between Ab and Ag.

Methods and Results. – Spleens were removed from preimmunized, 14-week-old, $C31F_1$ mice, 5 to 6 weeks after injection with either 2.5×10^8 sheep RBC or 1.6×10^8 rat RBC. Fixed numbers of cells were transferred into isologous, irradiated culture mice and immediately exposed to secondary antigen. The secondary antigen dosage was varied from 2×10^5 to 2×10^9 RBC. The production of antibody was followed by the collection of serum from groups of mice at daily intervals. The amount of antibody produced (stationary phase of the response) by a fixed number of spleen cells is directly related to the dosage of stimulating antigen. Figure 20 is a plot of log Ab/PC (PC is the number of potentially competent antibody-producing cells as determined by our "all-or-none assay")[13] as a function of antigen dosage expressed as log of the number of stimulating sheep or rat RBC.

Discussion. – The data shown in Fig. 20 may be fitted in the expression $Ab = (\text{constant})\,(PC)^{k_1}\,(Ag)^{k_2}$, where V is constant. Within certain experimentally determined limits, the values of k_1 and k_2 are constant. In previous studies, the value of k_1 has been found to be 1.0. From Fig. 20, the value of k_2 is found to be ~0.4 for the rat RBC antigen and ~0.5 for sheep RBC. These values suggest an approximate proportionality between Ab and the square root of the amount of antigen.

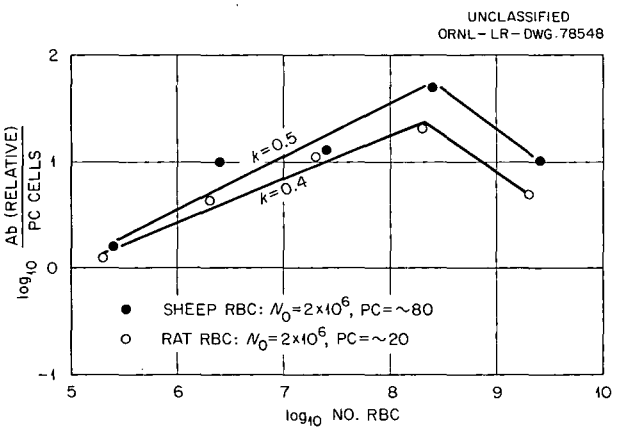

Fig. 20. Relation Between Antibody Production and Amount of Antigen (RBC) Used to Stimulate Secondary Hemagglutinin Response.

[12] T. Makinodan and J. F. Albright, *J. Cellular Comp. Physiol.* **60** (suppl), 129 (1962).

[13] J. F. Albright, T. Makinodan, M. R. Leonard, and W. J. Peterson, this report.

Pathology and Physiology

Section Chief – A. C. Upton

Pathological Effects of Neutrons and High-Energy Radiation in Mammals

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DELAYED SOMATIC EFFECTS OF RADIOMIMETIC AGENTS AND THE INFLUENCE OF AET ON THEIR PATHOGENESIS

J. W. Conklin
T. P. McDonald
K. W. Christenberry
W. D. Gude

Preliminary observations on the life-shortening effects of midlethal doses of the radiomimetic agents, methyl bis(β-chloroethyl)amine (HN2) and triethylene melamine (TEM), were previously reported.[1] A follow-up study employed fractionated doses of these same agents and a third radiomimetic chemical, 1,4-dimethanesulfonoxybutane (Myleran), in an attempt to define more clearly the effect of dosage upon survival and delayed somatic effects in mice. Since it was also noted that the effectiveness of HN2 for 30-day lethality was reduced about 40 percent by preinjection with AET,[2] the AET-pretreated HN2 survivors were also observed throughout life for delayed somatic effects.

Results. – Ten-week-old RF female mice receiving fractionated doses of HN2, TEM, Myleran, and x rays or an intraperitoneal injection of 360 mg per kg of AET 20 min before a single injection of HN2 were observed throughout life. The effects of

[1]J. W. Conklin *et al.*, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 84–85.

[2]M. Asano, T. P. McDonald, and T. T. Odell, Jr., *Intern. J. Radiation Biol.* **4**, 591 (1962).

Table 20. Effects of HN2, AET + HN2, TEM, Myleran, and X Rays on the Longevity and Incidence of Neoplasms in RF Female Mice

Treatment		Number of Treatments[a]	Interval Between Treatments (days)	Number of Mice	Mean Survival Time (months)	Incidence of Neoplasms (%)			
						Leukemia			
Agent	Total Dose					Myeloid	Thymic Lymphoma	Lung	Ovary
HN2	3.7–4.5 mg/kg i.v.	1		158	18.4	9	5	59	13
AET + HN2	360 mg/kg, AET i.p. + 4.0–10.0 mg/kg HN2 i.v.	1		160	19.5	3	10	70	28
HN2	9.6 mg/kg i.v.	4	14	115	14.9	2	19	62	23
TEM	3.0–4.0 mg/kg i.p.	1		146	16.6	6	16	52	58
TEM	6.0 mg/kg i.p.	4	14	113	12.7	4	29	46	45
X rays	500–600 r	1		242	13.0	7	26	8	37
X rays	1200 r	4	14	110	11.6	26	33	13	39
Myleran	48 mg/kg i.v.	4	14	110	16.2	5	38	11	44
Controls		1		114	20.9	3	5	20	7
Controls		4	14	112	20.5	4	10	17	21

[a]Data for groups given single treatment reported earlier.[1]

the various treatments on survival and neoplasia (Table 20) and on lens opacification (Fig. 21) are reported.

Discussion. – The radiomimetic agents resembled x rays in shortening the life span and inducing neoplasms and lens opacities when administered in fractionated doses, as observed earlier in acute dose experiments.[1] In regard to oncogenesis, the compounds exhibited varying degrees of effectiveness, and no consistently greater effect was noted in the fractionated groups for all neoplasms. Interpretation of the results in quantitative terms will require additional data on dose response relations and on organ distribution. AET appeared ineffective in inhibiting the induction of neoplasms and lens opacities in HN2-treated mice surviving 30 days, but it exerted some measure of protection against life-shortening in such animals.

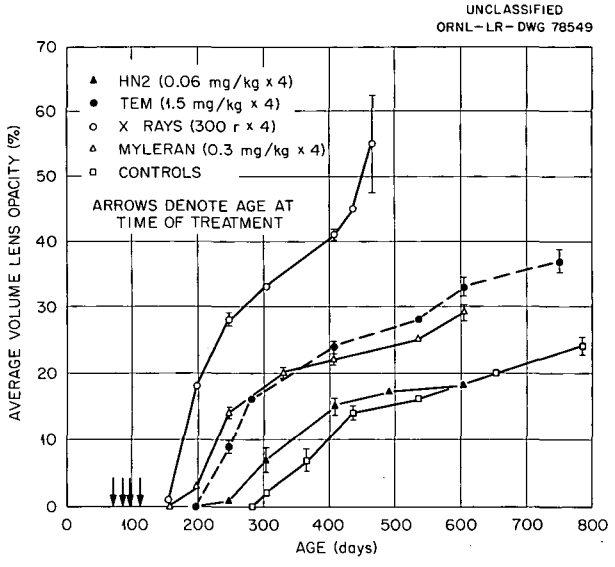

Fig. 21. Lens Opacities in Mice Treated with Fractionated Doses of X Rays, TEM, HN2, and Myleran.

RADIATION MYELOID LEUKEMOGENESIS IN THE RF MOUSE

V. K. Jenkins N. G. Anderson[3]

Results obtained from serial passage of cells and cell-free filtrates from radiogenic myeloid leukemias of the RF mouse suggested a progressive increase in the potency of a subcellular leukemogenic agent.[4] The following experiments were carried out to study further the nature of the etiologic factor(s).

Methods and Results. – Spleen cells from RF mice with radiogenic myeloid leukemia were serially passaged in irradiated and nonirradiated adult mice of the same strain. The incidence of takes increased in successive transplant generations, averaging about 70% after the 20th passage. Spleen cell suspensions from mice with passaged leukemia centrifuged for 1 hr yielded supernatant fluids which transmitted the leukemia to ~50% of adult and ~60% of newborns within 60 days after inoculation. Spontaneous myeloid leukemias have not been observed in control mice younger than 10 months of age. Cell-free filtrates from passaged leukemias induced the disease in ~3% of 310 unirradiated adult males and in ~8% of 129 unirradiated, newborn male recipients within 60 days. In 146 unirradiated, newborn female recipients, cell-free filtrates induced thymic lymphomas as well as myeloid leukemia (~4% of each type within 120 days). On fractionation by zonal centrifugation in sucrose gradients, the leukemogenic activity in homogenized myeloid leukemic spleen cells sedimented in a manner appropriate for a virus particle of the type noted with the electron microscope in this leukemia.[4] Leukemogenic activity was also recovered from the blood plasma of leukemic donors.

Discussion. – Implication of a viral etiology is further strengthened by recovery of leukemogenic activity from subcellular particles of leukemic spleen cells, which are comparable in size and density to virus particles observed with the electron microscope in this disease.[4] The precise character of the leukemogenic agent, however, as well as the role of radiation and host factors, remains to be established.

[3]Cell Physiology Group.

[4]D. F. Parsons *et al.*, *Cancer Res.* **22**, 728 (1962).

LIVER DAMAGE FOLLOWING INJECTION OF THE RADIOMIMETIC COMPOUND 4M20

M. L. Davis W. D. Gude
G. E. Cosgrove M. Asano

In a previous study comparing hematologic and pathologic effects of various radiomimetic drugs with those of x rays, Asano *et al.*[5] noted acute liver changes following the injection of 4-(*p*-dimethylaminostyryl) quinoline (4M20). Further study of the hepatotoxic effect of 4M20 was undertaken.

Methods and Results. – Young adult mice of the RF and $(101 \times C3H)F_1$ strains were given doses of 4 mg or 2 mg of 4M20 intravenously. One group received an injection of dilute HCl at pH 1.5 (the pH of the 4M20 solution) to serve as injection-acidity controls, and another group was set up as non-injected controls. At intervals from 1 to 17 days after injection, selected animals in each group were tested for liver function by the BSP test (using a dose of Bromsulphalein equivalent to 100 mg per kg of body weight). Forty minutes after injection of the dye they were killed and bled, and liver tissues were sectioned for fat, hematoxylin, and cosin staining. Fat deposition and liver cell vacuolation were graded on a scale of 0 to 4 – zero denoting no abnormality and 4, severe changes. BSP retention increased rapidly to a maximum of 100% in $(101 \times C3H)F_1$ mice on *day* 3 and in RF mice on *day* 6 after a 4-mg dose of 4M20 (Table 21). Retention rapidly decreased over subsequent sacrifice days until most animals gave normal test levels at day 10 to 13. Fat in liver cells and vacuolation of liver cells were prominent by 24 hr after injection and increased in severity through the period of increasing BSP retention, then subsiding to control levels as the BSP retention subsided. A 2-mg dose of 4M20 caused a maximum BSP retention of 18% in RF mice, but caused no increase in retention in $1C3F_1$ mice despite some fatty degeneration of liver cells. Necrosis of liver cells around the central veins occurred in both strains of mice in the early days after a 4-mg dose of 4M20 but not after a 2-mg dose.

Discussion. – Function tests and morphologic change indicate that 4M20 is highly toxic to the liver, especially at the higher dose level used.

[5]M. Asano *et al.*, *AMA Arch. Pathol.* (in press).

Table 21. BSP Retention, Fat Deposition, and Liver Cell Vacuolation in (101 × C3H)F_1 Mice Receiving 4M20

Material and Dose Injected	Day Tested	BSP Retention (%)	Histologic Stains of Liver	
			Fat Deposition	Liver Cell Vacuolation
4M20 (4 mg i.v.)	1	9	3	3
	2	72	3	4
	3	100	4	4
	7	57	2	3
	9	12	2	2
	13	5	1	0
4M20 (2 mg i.v.)	1	4	2	1
	4	4	0	0
	7	4	2	1
	11	3	0	0
Noninjected controls	1–17	5	0	0

The changes are much more marked than those seen in anoxia, severe illness, or following irradiation. Residual damage is indicated by giantism and pleomorphism of hepatic cells, which persist indefinitely. The long-term effects of 4M20 are under further investigation for possible carcinogenic aspects.

AN APPROACH TO THE CHARACTERIZATION OF STEM CELLS IN MOUSE BONE MARROW

Gustavo Cudkowicz G. D. Gosslee[7]
L. H. Smith[6] W. L. Hughes[8]

Introduction. – The competence of bone marrow to promote recovery of hemopoietic function in irradiated recipients varies on serial transplantation.[9] The purpose of this study was to investigate the nature and cellular basis of such variations. In order to obtain a quantitative measure of the proliferative capacity of the transplanted marrow cells, their ability to form hemopoietic colonies and to promote the uptake of I^{131}-labeled iododeoxyuridine (I^{131}-UdR) in the spleen of an irradiated isogenic recipient was determined. The proliferative capacity was also related to the cellular constitution of the donor marrow as judged by differential cell counts on marrow smears.

Results. – Experiments were carried out in (BALB/c × A/He)F_1 mice subjected to 850-r whole-body 300-kvp x irradiation followed immediately by intravenous injection of 10^7 or 2×10^7 nucleated syngeneic bone marrow cells. These mice were killed at 5-day intervals up to 40 days after transplantation, and their marrow was retransplanted into syngeneic recipients exposed to 700-r whole-body 300-kvp x radiation.

When bone marrow was retransplanted within 10 days, its ability to colonize the recipient spleen and to give rise to DNA-synthesizing cells (as judged by uptake of I^{131}-UdR) was drastically reduced (Figs. 22 and 23), although its content of myeloblasts and erythroblasts was relatively high.

[6]Mammalian Recovery Group.

[7]Biometrics Group.

[8]Brookhaven National Laboratory, Medical Research Center, Upton, N. Y.

[9]D. W. H. Barnes *et al.*, *Ann. N. Y. Acad. Sci.* **99**, 374 (1962).

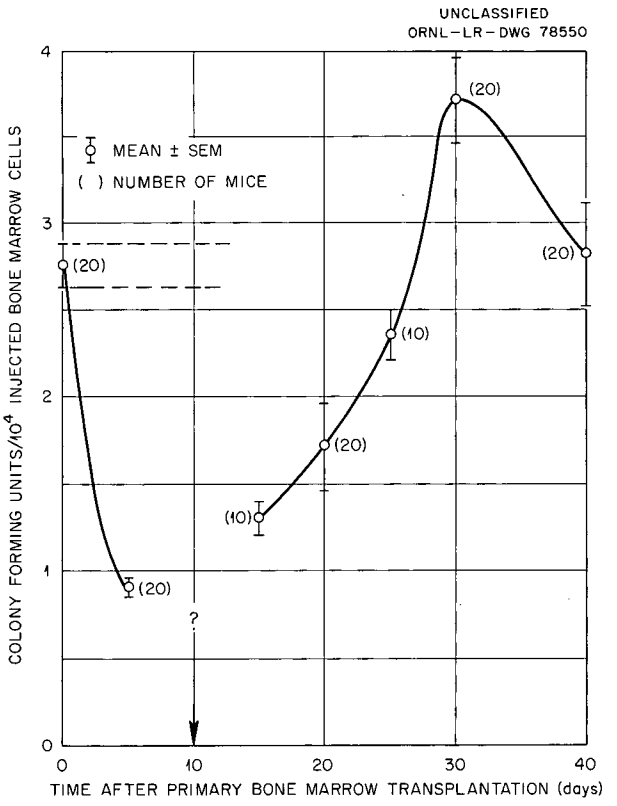

Fig. 22. Colony-Forming Ability of Retransplanted Marrow in Relation to Time After Initial Transplantation.

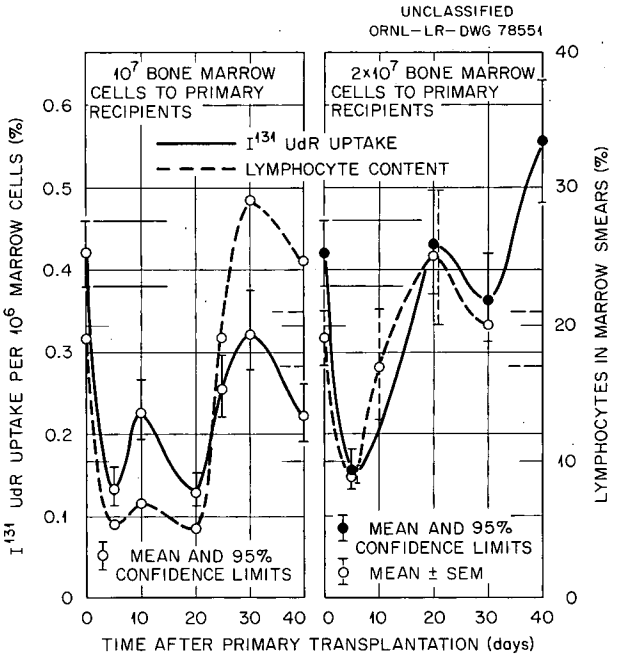

Fig. 23. Ability of Marrow to Promote Uptake of I^{131} UdR in Relation to Time of Retransplantation and Lymphocyte Content.

The normal repopulating capacity of the marrow returned only after 20 or more days following transplantation, depending on the number of marrow cells given to the primary recipients, and was correlated with restoration of its content of lymphocyte-like cells (Fig. 23). This observation suggested that the "lymphocyte" might be the stem cell responsible for splenic repopulation. To test this hypothesis, autoradiographs of flash-labeled marrow smears were examined to determine whether marrow lymphocytes incorporated tritiated thymidine more actively than normal soon after transplantation into a lethally irradiated recipient. Cells having the morphology of the small lymphocyte were seldom labeled in normal marrow, but a few days after transplantation such cells were seen to label relatively intensely and in increased numbers.

Discussion. – These results indicate that the capacity of the marrow to repopulate hemopoietic sites in irradiated recipients is not dependent on its content of erythroblasts and myeloblasts but rather on its content of more primitive precursors. Furthermore, the results suggest that the stem cell in question may be related morphologically to the small lymphocyte. It was also demonstrated that I^{131}-UdR may be used to advantage in the quantitative assessment of growth of hemopoietic elements.

A NONCODOMINANT HISTOCOMPATIBILITY FACTOR IN THE C57BL MOUSE

Gustavo Cudkowicz
J. H. Stimpfling[10]
G. M. Shearer
Lou C. Satterfield

Introduction. – Bone marrow cells of C57BL phenotype transplanted in sufficient numbers to provide optimal growth in syngeneic x-irradiated recipients failed to grow under comparable conditions in F_1 hybrids when the parent mice differed by a histocompatibility allele at the strong histocompatibility-2 (H-2) locus. However, the

[10]Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

same cells proliferated well in F_1 hybrids when the parent possessed the same allele at the H-2 locus, and different alleles at the weaker H-1 or H-3 loci.[11] The present study was undertaken to explore whether a noncodominant histocompatibility factor would segregate in the backcross progeny from matings of (C3H × C57BL/10)F_1 and of (A × C57BL/10)F_1 hybrids to either parental strain and whether such a factor would be linked to the H-2, agouti (*A*), or hemoglobin (*Hb*) loci.

Results. – Growth of parental strain marrow cells was assessed by the I^{131}-deoxyuridine (I^{131}-UdR) method[12] 5 days after infusion of the cells into x-irradiated (700 r) recipients. The H-2 and Hb phenotype of the backcross progeny was determined by hemagglutination and by hemoglobin[13] characteristics. Forty-five [(C3H × C57BL/10)F_1 × C57BL/10] mice were tested. In 21 of them the C57BL/10 marrow cells proliferated as well as in syngeneic recipients. In the remaining 24 backcross mice, the marrow cells failed to grow, as in F_1 hybrid recipients. Twenty-five [(A × C57BL/10)F_1 × C57BL/10] mice were also tested, and in 12 of them the C57BL/10 cells proliferated. In the remaining 13 mice, the same marrow cells did not grow. The results of both experiments were pooled and are presented in Fig. 24. No linkage could be detected of the trait under study (resistance to marrow grafts) with sex or with the markers A and Hb. On the other hand, the trait seems to be linked with H-2. Marrow cells of C3H and A strain donors grow equally well in syngeneic recipients, F_1 hybrid recipients, and in progeny of F_1 hybrids mated to C3H and A mice, respectively.

[11] Gustavo Cudkowicz *et al.*, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 108.

[12] G. Cudkowicz *et al.*, this report.

[13] R. A. Popp *et al.*, *Proc. Soc. Exptl. Biol. Med.* **101**, 754 (1959).

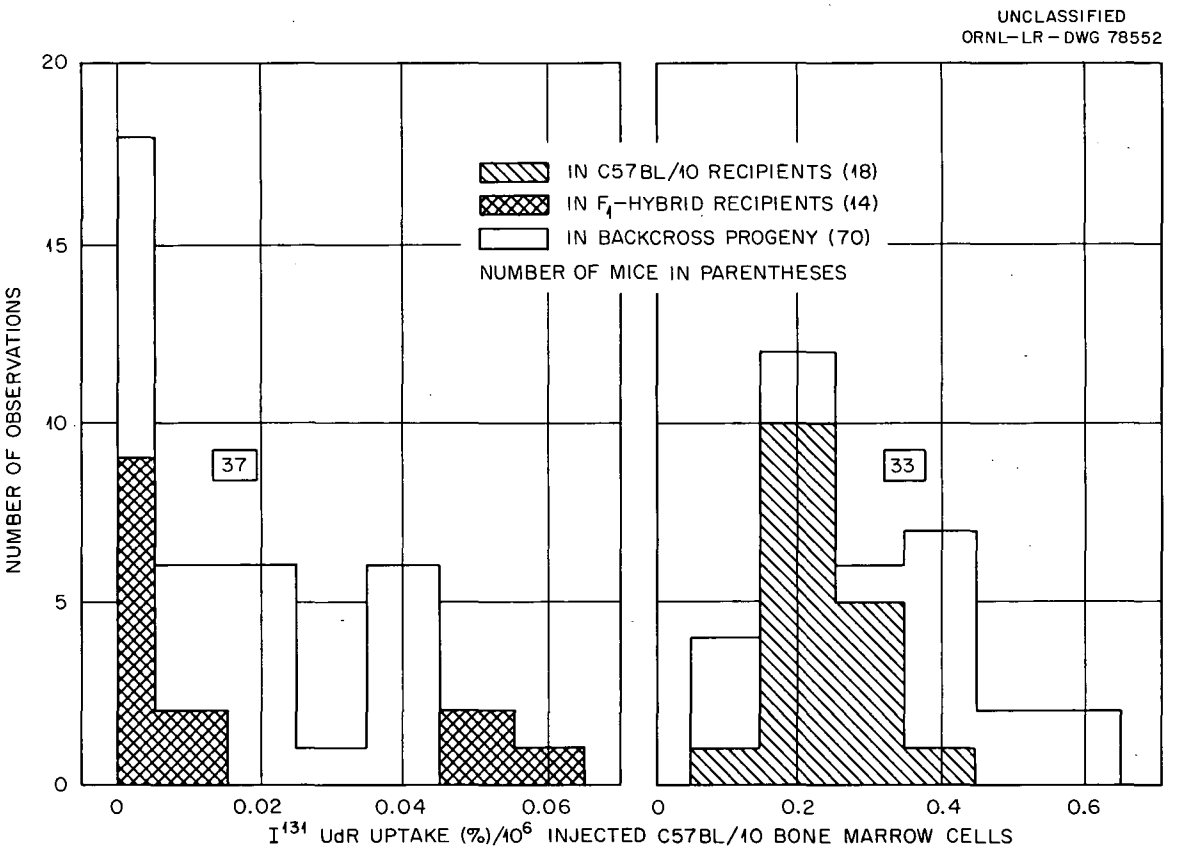

Fig. 24. Growth of C57BL/10 Marrow Cells Transplanted into Syngeneic, F_1 Hybrid, and Backcross Progeny Mice, as Determined by I^{131}UdR Incorporation in Recipient Spleens.

Discussion. – The results presented suggest that the C57BL/10 mouse is homozygous for a recessive gene that is linked with H-2 and influences the growth of transplanted marrow cells. The recessive allele is not expressed in F_1 hybrid mice and in approximately half of the backcross progeny mice. C3H and A mice do not share the recessive allele with C57BL/10 mice. It is not yet established whether the allele determines a recessive specificity or interacts with the expression of other histocompatibility factors.

STUDIES ON RETICULOENDOTHELIAL FUNCTION IN CHRONICALLY IRRADIATED MICE

H. E. Walburg, Jr. Edna I. Mynatt

Phagocytosis and killing of bacteria by mouse Kupffer's cells were studied in irradiated and unirradiated mice using organ bacterial counts.

Methods and Results. – X radiation was administered in doses of 50 r every 48 hr to CF_1 female mice 8 weeks of age at the start of the experiment. Forty-eight hours after having received various accumulated doses, groups of mice were injected with a suspension of *Pseudomonas aeruginosa.* Organs were subsequently removed aseptically, homogenized, and diluted, and the number of bacteria determined by plate count. Twenty-four hours after injection of the bacterial suspension, the hepatic bacterial levels in unirradiated mice had declined to 0.1% of the number injected. In the livers of irradiated mice, no greater numbers of bacteria were present 0.5, 4, 8, and 12 hr after injection of bacteria, but 24 hr after injection, elevated liver bacterial counts (greater than the control mean plus three standard errors) were found in some individuals in all irradiated groups. The proportion of such individuals increased as the accumulated dose rose from 500 to 1500 r. Spleen and sternal marrow always contained less than 10% as many bacteria as were found in the liver. The blood bacteria levels decreased rapidly for the first half hour after injection in irradiated mice, and no bacteria were detected between 4 and 12 hr, but bacteria reappeared in the peripheral blood between 12 and 24 hr after injection. To determine if the hepatic bacterial increase resulted from organisms outside or inside the liver, all organs were examined for bacteria. The visceral organs other than the liver contained only a few bacteria, but the tails of injected mice contained a large number of *P. aeruginosa*, exceeding the liver levels sometimes by a factor of 1000. The peripheral blood had an intense leukopenia, ranging from 30% of the control mean at 450 r to 10% of the control mean at 2050 r.

Discussion. – The information thus far obtained indicates that the higher hepatic bacterial levels of chronically irradiated mice do not necessarily indicate a failure of the phagocytic or bactericidal capabilities of the Kupffer's cell. Rather, it appears that the hepatic reticuloendothelial system is continuing to function in the presence of a focal infection caused by a defect in the subcutaneous inflammatory reaction. This defect is possibly due to the lack of leukocytes in the peripheral circulatory system. Nelson and Becker,[14] using a similar experimental system, concluded that bacteria survived and multiplied within the spleens and livers of mice receiving single doses of 500 to 700 r x radiation. The number of bacteria at the injection site was not determined. Miller, Hammond, and Anderle[15] have shown that a small focus of infection at the inoculation site results from leakage of the inoculum into the perivenous tissues of the tail.

PLATELET LABELING WITH $Na_2S^{35}O_4$

T. T. Odell, Jr. T. P. McDonald

Introduction. – Earlier work demonstrated that rat platelets contain a sulfated mucopolysaccharide (MPS) which becomes labeled on injection of $Na_2S^{35}O_4$. Experiments were undertaken to determine whether platelets are labeled with S^{35}-sulfate in any additional way, especially during the first few hours after a single injection of the isotope.

Methods. – Rats were given a single intravenous injection of $Na_2S^{35}O_4$, and the platelets were collected 1, 3½, 8, 12, 16, 20, 24, and 32 hr later. The platelet MPS was extracted by hydrolysis, separated from protein by treatment with chloroform–amyl alcohol reagent, and dialyzed against water

[14] E. L. Nelson and J. R. Becker, *J. Infect. Dis.* **104**, 20 (1959).

[15] C. P. Miller, C. W. Hammond, and S. K. Anderle, *J. Exptl. Med.* **111**, 773 (1960).

to remove free sulfate. The MPS remaining in the dialysis bags was electrophoresed on paper, and the paper strips counted for radioactivity in a gas-flow paper strip counter.

Results and Discussion. – The radioactivity of whole platelets declined between 1 and 12 hr and increased thereafter. The radioactivity of the material remaining in the dialysis bag (MPS-S^{35}) was negligible at 1 and 3½ hr and rose thereafter, approximately doubling between 8 and 20 hr. Very little radioactivity was apparent on the paper electrophoresis strips until the 20-hr period. The radioactivity on the strips migrated to the same position as chondroitin sulfate thus indicating its acid MPS nature, in agreement with earlier studies. In addition, it was stainable with Alcian Blue 8GN, a dye specific for acid mucopolysaccharides.

The results, therefore, indicate two modes of platelet labeling: (1) a labeling of (circulating) platelets soon after injection of $Na_2S^{35}O_4$ and its disappearance in about 12 hr; and (2) a labeling of platelet MPS, first seen in a significant amount about 8 hr after injection of sulfate and increasing thereafter. The latter label is presumably acquired by the platelet while it is still a part of the cytoplasm of the megakaryocyte in the bone marrow.

IDENTIFICATION OF THE HEMOGLOBIN TYPES OF INBRED STRAINS OF MICE

R. A. Popp

Alleles at *Hb*, which control the electrophoretic pattern of mouse hemoglobin and also the primary structure of the beta chain,[16] have been reported for many strains of mice.[17] Alleles at *Sol*, which govern the solubility property of hemoglobin and also the primary structure of the alpha chain,[18] had been identified for only a few strains of mice. Therefore, an investigation of the alleles at *Sol* for many strains of mice available at this laboratory was undertaken, and the results are presented here.

[16]R. A. Popp, *J. Heredity* **53**, 142 (1962).

[17]R. A. Popp and W. St. Amand, *J. Heredity* **51**, 141 (1960).

[18]R. A. Popp, *J. Heredity* **53**, 149 (1962).

Methods and Results. – Techniques for identifying alleles at *Sol* and *Hb* with the use of K_2HPO_4-KH_2PO_4 buffer have been described earlier.[19] C57BL and SEC mice, whose hemoglobin genotypes are $Hb^1/Hb^1;Sol^1/Sol^1$ and $Hb^1/Hb^1;Sol^2/Sol^2$, respectively, were mated with strains of mice whose *Sol* types had not been determined. The hemoglobin types of F_1, F_2, and BC_1 progeny were assessed by comparison with hemoglobins of known types. The strains of mice examined and their hemotypes are shown in Table 22. The crossover frequency between *c* and *Hb* was determined when such alleles were phenotypically expressed. The average crossover frequency for all groups was 0.0636 ± 0.0070; the values ranged from 0.02 to 0.15 in individual groups.

[19]R. A. Popp, *J. Heredity* **53**, 77 (1962).

Table 22. Hemotype of 18 Strains of Mice[a]

Hemotype	Strain
$Hb^1/Hb^1;Sol^1/Sol^1$	C57BL/Cum
	C57BL/6 Cum
	C57BL/6 ccLs
	C57L/Rl
	FUS/Ls
$Hb^1/Hb^1;Sol^2/Sol^2$	SEC/Rl
$Hb^1/Hb^1;Sol^3/Sol^3$	NB/Rl
$Hb^2/Hb^2;Sol^1/Sol^1$	A/Cum
	AKR/Up
	RFM/Up
	DBA/2 Cum
	CFW/Rl
	CFCW/Rl
	RUS/Rl
	101/Cum
	129/Rl
$Hb^2/Hb^2;Sol^2/Sol^2$	BALB/cJ
$Hb^2/Hb^2;Sol^4/Sol^4$	C3H.B/St

[a]Sources of mice: Cum – Cumberland View Farms, Clinton, Tennessee; Ls – E. Les, J – Jackson, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine; Rl – W. Russell, Up – A. Upton, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.; and St – L. Strong, Roswell Park, Springville, New York.

Discussion. – Inbred strains (e.g., C57BL, C57BL/6, and C57L) that have a common ancestry usually have similar hemotypes (Table 22). However, strains A, BALB/c, C3H.B, and DBA/2 were derived from a common noninbred stock of mice; three *Sol* genotypes were found among these strains (Table 22), suggesting heterogeneity for *Sol* before the inbred lines were developed. These results provide a basis for selecting different mouse hemoglobins for future studies on allelomorphism and amino acid sequence. The inheritance of hemoglobins in mouse and man appear to be similar. However, the opportunity to program the mating of mice makes them useful experimental animals for investigating some hypotheses concerning the inheritance of hemoglobin.

ATTEMPTS TO PROPAGATE MOUSE MYELOID LEUKEMIA CELLS

Ernestine Teeter R. L. Tyndall
V. K. Jenkins

Investigation of the x-ray-induced myeloid leukemia of the RF mouse has been hampered by the lack of a consistently potent source of the causative agent, presumably a virus. Consequently, studies have been undertaken to attempt the *in vitro* propagation of the myeloid leukemic spleen cells and hence of the virus.

Results and Discussion. – Normal and leukemic spleen cells were seeded in Eagle's basal medium with a fourfold concentration of amino acids and vitamins. The normal spleen cultures exhibited a sharp drop in cell number during the initial 24 hr in culture. In contrast, myeloid spleen cultures showed an immediate increase in cell numbers, apparently due to the proliferation of erythrocytic precursors. After 3–10 days in culture, depending upon the size of the original inoculum, the cell number declined, owing to the eventual maturation and death of erythrocytes. A preliminary test of the leukemogenic potential of a 3-day culture of myeloid spleen cells resulted in the appearance of myeloid leukemia in 7 out of 10 mice injected with approximately 4×10^6 cells. Inoculation of a comparable number of uncultured spleen cells resulted in approximately 50% incidence of myeloid leukemia. The results to date suggest that the virus can survive and possibly multiply under the culture conditions so far tested and that the leukemic cultures proliferate differently from the controls. Further experiments are under way to promote the continued proliferation of leukemic cells and virus.

THE EFFECT OF ACTINOMYCIN D ON COXSACKIE B3 VIRUS REPLICATION

R. L. Tyndall Ernestine Teeter

Introduction. – Reich *et al.*[20] have postulated that Mengo virus and polio virus replication are independent of the function of host cell DNA. This hypothesis was based on the inability of actinomycin D to inhibit the production of these RNA viruses while suppressing 99% of the normal cell DNA-dependent RNA synthesis. Previous nutritional studies[21–23] have indicated differences, however, between the replication of polio virus and other enteroviruses. This study was undertaken to investigate the effects of actinomycin D upon the replication of an enterovirus other than polio virus, namely Coxsackie B3.

Methods and Results. – Three groups of HeLa cell cultures were tested for their ability to support the replication of Coxsackie B3 virus. The first group was overlaid with Medium 199 for 18 hr prior to virus infection. The second group of cultures was overlaid with Medium 199 for 18 hr prior to concomitant inoculation of actinomycin D (1 μg/ml) and Coxsackie B3 virus. The third group was overlaid with Medium 199 containing 1 μg/ml actinomycin D 18 hr prior to virus infection. All three groups were harvested 24 hr after infection and titrated for virus. The untreated cultures and those treated with actinomycin D at the time of virus inoculation supported the subsequent replication of Coxsackie B3 virus. However, cell cultures pretreated with actinomycin D failed to support virus production.

Discussion. – The inhibition of Coxsackie B3 virus production by actinomycin D resembles the effect of actinomycin upon NDV replication as recently reported by Kingsbury,[24] but differs markedly from the reports of Reich *et al.*[20] These

[20] E. Reich *et al.*, *Proc. Natl. Acad. Sci. U.S.* **48**, 1238 (1962).

[21] S. Chang, *J. Exptl. Med.* **109**, 229 (1959).

[22] R. L. Tyndall and E. H. Ludwig, *J. Bacteriol.* **80**, 90 (1960).

[23] D. Crowther and J. L. Melnick, *Virology* **15**, 65 (1961).

[24] D. W. Kingsbury, *Biochem. Biophys. Res. Commun.* **9**, 156 (1962).

differences may indicate two different basic mechanisms involved in the synthesis of viral RNA, one dependent on and one independent of host cell DNA.

UNMASKING VIRAL AND/OR VIRUS-INDUCED RNA

R. L. Tyndall K. B. Jacobson[25]
Ernestine Teeter

As Reich *et al.*[20] have shown that actinomycin D inhibits the synthesis of HeLa cell RNA, we have, likewise, noted that 5 hr after the addition of P^{32} and actinomycin to HeLa cell cultures, the RNA-P^{32} shows less than 10% of the radioactivity of comparable cultures not treated with actinomycin. In addition, we observed that concomitant inoculation of Coxsackie B3 virus and actinomycin did not greatly suppress subsequent virus replication. In this study, the RNA of actinomycin-treated virus-infected and uninfected HeLa cells has been analyzed.

Results. – One group of 48-hr HeLa cell cultures was overlaid with Medium 199 and a second group with Medium 199 containing 1 μg of actinomycin D per ml of medium. A third group was overlaid with Medium 199 containing 1 μg per ml of actinomycin D and Coxsackie B3 virus at a multiplicity >3.0. All three groups were then inoculated with approximately 40 μc of P^{32} per ml of medium. After 5 hr incubation, the RNA-P^{32} was extracted with 1 *M* NaCl at 100°C. The relative total RNA-P^{32} activities of the normal control culture, the actinomycin-treated virus-infected culture, and the actinomycin-treated uninfected culture were approximately 12, 3, and 1, respectively. Nearly equal amounts of P^{32} were distributed among the four RNA nucleotides of both actinomycin-treated cultures, whereas untreated cultures revealed a wider range of P^{32} distributions among the bases. Most significantly, however, the RNA fingerprints from both actinomycin-treated cultures exhibited a different pattern from those of the untreated control culture.

Discussion. – From these preliminary experiments, it appears that the virus-induced RNA synthesized in the presence of actinomycin resembles an RNA fraction in actinomycin-treated uninfected cells but differs from the RNA of control cells, which had not been treated with either virus or actinomycin. The significance of this difference in relation to viral replication is under study.

[25]Enzymology Group.

Cell Physiology

N. G. Anderson

Subcellular Physiology		Cell Biophysics	
N. G. Anderson	R. E. Canning	Peter Mazur	E. Jane Holloway
W. D. Fisher	R. Elizabeth Lybarger	J. G. Green[d]	
G. M. Padilla	C. T. Rankin, Jr.		
C. L. Burger[a]	Mary L. Anderson[b]		
B. T. Cole[b]	Regina Schuel[b]		
E. C. Horn[b]			
T. W. James[c]			
C. A. Price[b]			
Herbert Schuel[c]			

[a]Research participant.
[b]Consultant.
[c]USPHS Fellow.
[d]Research associate.

SURVIVAL OF YEAST CELLS SUBJECTED TO ULTRARAPID CENTRIFUGAL FREEZING

J. G. Green Peter Mazur N. G. Anderson

Introduction. – The apparatus and technique of ultrarapid centrifugal freezing have been described previously.[1] In the early experimentation a rather crude method for the transfer and thawing of frozen cells was employed. In this report an improved cell-transfer system which has yielded much higher survival values is described.

Experimental. – A transfer cap machined from methacrylate plastic has been fitted to the plastic rotor insert used in early experimentation, and the rotor has been modified to accept the cap. In addition, an angle head from an International PR-2 refrigerated centrifuge has been hollowed to accept a liter of thawing medium.

Cell suspensions in droplet form are frozen in the manner described in the previous report. The precooled transfer cap is fitted to the plastic insert using a silicone-based lubricant to seal mating surfaces. Part of the liquid nitrogen is allowed to evaporate, the insert and attached transfer cap are inverted, and the frozen droplets of cell suspension are expelled from the rotor insert into the thawing medium.

Best survival was obtained when the frozen droplets were expelled into 1 liter of 0.06 *M* potassium phosphate plating diluent spinning in the hollowed angle head. In preliminary experiments, survival values have averaged 7.6%, and individual trials have yielded survival values as high as 9.0%.

[1]N. G. Anderson and J. G. Green, *Biol. Div. Semi-ann. Progr. Rept. Aug. 15, 1962,* ORNL-3352, pp 115–16.

Discussion. – The preliminary results obtained from the use of the improved cell-transfer system confirm the practicability of ultrarapid, centrifugal freezing techniques in cell research and preservation. Further refinements in technique and apparatus are being formulated, and additional experimentation in survival is being performed.

INFLUENCE OF THE SUSPENDING MEDIUM ON THE ABILITY OF YEAST TO SURVIVE SUBZERO TEMPERATURES

Peter Mazur E. Jane Holloway

Introduction. – In previous work with *Saccharomyces cerevisiae,* the cells have been almost always suspended in distilled water.[2–4]

All evidence from this work points to a strong correlation between intracellular freezing and cell death. Conversely, factors such as slow cooling, which decrease the likelihood of internal ice formation, increase the percentage surviving. The present study is concerned with the survival of yeast in suspending media other than pure water (i.e., aqueous solutions). It is concerned with which media protect and what the relation is, if any, between protection and intracellular ice formation.

The solutes used were chosen because (1) they are relatively nontoxic to yeast at room temperature; (2) most of them are known to protect various cells; (3) information was available, or could be obtained, on their ability to permeate the cells; and (4) they are not rapidly metabolized by yeast.

Methods. – Liquid growth cultures (24 hr) of yeast were washed free of the growth medium (once in distilled water, twice in 1 *M* concentrations of the desired suspending medium) and were resuspended in the medium to yield a cell concentration of 2×10^8 per milliliter. One hour after suspending the cells in the solution, 0.1-ml portions in tubes were cooled in two stages: the tubes were held ~90 sec in a −30°C ethanol bath and then transferred to a −78°C dry ice–ethanol bath for 15 min. Subsequent warming was either slow (1°C/min) or rapid (~1000°C/min). Survival was determined from colony counts in a standard dilution–agar plate assay, and percentage survival was calculated as number of viable cells in the experimental/number of viable cells in the control. Controls were suspensions of cells in the medium held at room temperature during the experiment.

Results. – The percentage survivals are depicted by bar graphs in Fig. 25A (rapid warming) and Fig. 25B (slow warming). The tenfold difference in the scales of the ordinate should be noted. The following points are of interest.

1. Survivals were almost invariably higher with rapid warming. This is consistent with previous findings.[2]
2. Most of the solutions (although not all) yielded higher survivals than did pure water.
3. The relative ranking of the solutes with respect to protection was about the same with both rapid and slow warming, with two marked exceptions: galactose and dimethyl sulfoxide (DMSO).
4. Taken as a class, the sugars were the most protective, followed by the low-molecular-weight glycols.
5. Solutes did not have to penetrate the cell to be protective. In fact, rhamnose and lactose, which do not penetrate yeast, are among the most protective; formamide and propionamide, which penetrate most readily, are among the least protective.
6. The higher-molecular-weight nonpenetrating polyethylene glycols (PEG) and polyvinylpyrrolidone (PVP) did not protect, even though their weight concentration was comparable to the other solutes. Their molar concentration was considerable less than 1 *M*.

The approximate permeability of the yeast to the solutes is summarized in Table 23.

Discussion. – Lovelock[5] postulated that death at low temperature is due to the concentration of electrolytes occurring during freezing. From this he concluded that, to protect cells from injury, an added compound would have to be able to penetrate the cell; moreover, any solute that could penetrate, was nontoxic, and of low molecular weight should protect. Our results are in-

[2]Peter Mazur, *Biophys. J.* **1**, 247 (1961).

[3]Peter Mazur, *J. Bacteriol.* **82**, 662 (1961).

[4]Peter Mazur, to be published in the *Biophysics Journal,* 1963.

[5]J. E. Lovelock, *Biochim. Biophys. Acta* **11**, 28 (1953).

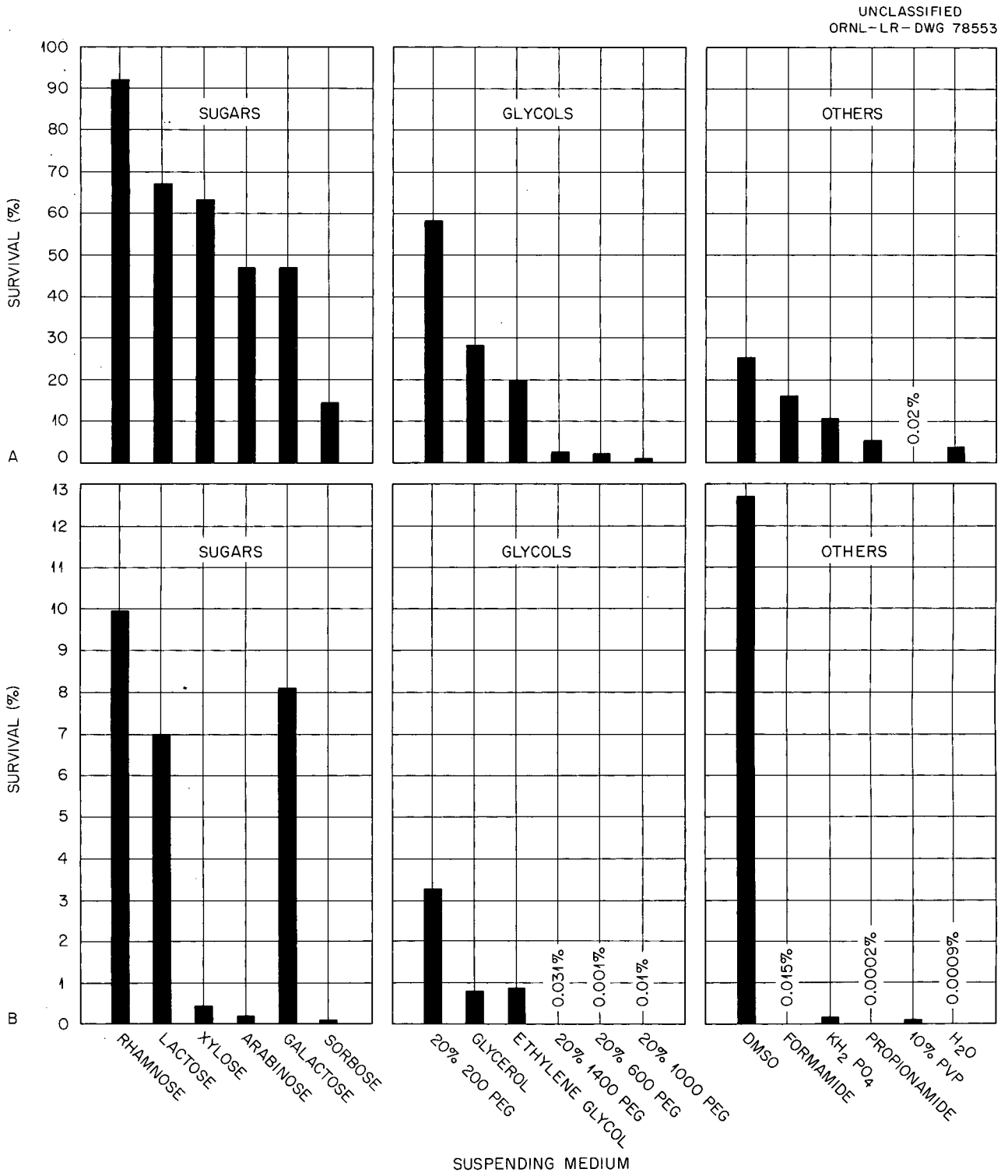

Fig. 25. Percentage of Yeast Surviving Two-Step Rapid Cooling in Various Suspending Media to –78°C and Subsequent Rapid Warming (A) or Slow Warming (B). PEG, DMSO, and PVP refer to polyethylene glycol, dimethyl sulfoxide, and polyvinylpyrrolidone respectively.

Table 23. Permeability of Yeast[a]

Solute	Literature	Hematocrit
Rhamnose	N.P.	N.P.
Lactose	N.P.	N.P.
Xylose	P.	P.
Arabinose	Conflicting	S.P.
Galactose	Conflicting	S.P.
Sorbose	P.	P.
PEG (200)[b]		S.P.
Glycerol		S.P.
Ethylene glycol		P.
PEG (600–1400)[b]		N.P.
DMSO		P.
Formamide	P.	P.
KH_2PO_4	N.P.	N.P.
Propionamide	P.	P.
PVP		N.P.

[a]Solutes producing and maintaining cell shrinkage for more than 2 hr were considered nonpenetrating (N.P.); those in 1 *M* producing no shrinkage after 5 min were considered penetrating (P.); those producing initial shrinkage followed by a restoration of the original cell volume over 15 min to 2 hr were called slowly penetrating (S.P.).

[b]Average molecular weight.

compatible with this theory. Lactose and rhamnose did not penetrate, but they protected; sorbose, ethylene glycol, formamide, and propionamide penetrated readily but produced little or no protection.

The cells in these experiments were cooled rapidly, and when suspended in pure water are believed, from previous work, to have undergone intracellular freezing. The question remains whether protection by the added solutes was brought about by a reduction in internal ice formation and, if so, what was the mechanism by which it was brought about.

KINETICS OF WATER LOSS FROM CELLS AT SUBZERO TEMPERATURES AND ITS RELATION TO INTRACELLULAR FREEZING

Peter Mazur

Introduction. – In the preceding semiannual report,[6] a differential equation was derived relating the volume of supercooled intracellular water (V) remaining in a cell to temperature (T) and to a number of parameters: the cooling rate (B), the known permeability of the cell (K_g) at a given temperature (T_g), the temperature coefficient of the permeability constant (b), the area of the cell (A), and the number of moles of solute in the cell (n_2). This equation is

$$Te^{2.303b(T_g-T)}\frac{d^2V}{dT^2} - \left[(2.303bT+1)e^{2.303b(T_g-T)} - \frac{ARK_g n_2}{B(V+n_2v_1^0)}\frac{T^2}{V}\right]\frac{dV}{dT} = \frac{L_f AK_g}{Bv_1^0}, \quad (1)$$

where R, L_f, and v_1^0 are the gas constant, latent heat of fusion, and molar volume of water respectively.

Results and Discussion. – This equation is not solvable analytically, but was solved numerically. Solutions were obtained by M. T. Harkrider of the Mathematics Division, and I am most grateful to him for his essential role in the study.

Table 24 lists the units and some values for the various parameters typical of living cells. Figure 26 shows the solutions obtained for an "average" spherical cell of 6 μ diameter cooled at five rates ranging from 1 to 10,000°C/min. The unbracketed cooling rates assume a permeability to water (K_g) of 0.3, which is typical of many cells;[7] those in parentheses assume a tenfold higher constant of 3.0, which is approximately that of mammalian erythrocytes. The relation plotted is the percentage of the original intracellular water remaining in the cells at temperatures from −1 to −28°C. The equilibrium curve shows the percentage of water remaining in cells cooled infinitesimally slowly; that is, it represents the water content of cells that have lost sufficient water to bring the vapor pressure of the internal water into vapor-pressure equilibrium with external ice, or, what is equivalent, to its freezing point.

[6]Peter Mazur, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 125–28.

[7]D. A. T. Dick, *Intern. Rev. Cytol.* **8**, 433 (1959).

Table 24. Variables, Parameters, and Constants in Eq. (1)

Symbol	Meaning	Units	Typical Values
V	Volume of water in cell	μ^3	Dependent variable
T	Temperature	°K	272 to 242
T_f	Freezing point of cytoplasm	°K	272
V_i	Volume of internal water at $T \geqq T_f$	μ^3	88
b	Temperature coefficient of permeability constant	degrees^{-1}	0.0141
B	Rate of temperature change	°K/min	−1, −10, −100, −1000, and −10,000
A	Area of cell	μ^2	107
R	Gas constant	μ^3 atm mole^{-1} deg^{-1}	82.057×10^{12}
K_g	Permeability constant at temperature T_g	$\mu^3\ \mu^{-2}$ min^{-1} atm^{-1}	0.3, 3
T_g		°K	293
m	Molality of protoplasm	moles/1000 g of H_2O	0.5
n_2	Osmoles of solute in cell	moles	4.4×10^{-14}
v_1^0	Molar volume of pure water	μ^3/mole	18×10^{12}
L_f	Latent heat fusion of ice	μ^3 atm^{-1} mole^{-1}	5.95×10^{16}

At a cooling rate of 1°C/min, the calculated water content remains very close to the equilibrium curve and joins it at −3°C. Since small quantities of water almost never freeze unless supercooled several degrees below the freezing point, the intracellular water would almost certainly not freeze at this cooling rate. However, with increasing cooling velocity, the curves depart more and more from equilibrium. The intracellular water is not able to leave the cell rapidly enough to maintain vapor-pressure equilibrium with the surrounding ice, and it becomes supercooled (the degree of supercooling is the horizontal distance between a point on a given curve and the equilibrium curve). Nucleation theory predicts that the likelihood of freezing increases exponentially with supercooling; therefore, intracellular freezing becomes increasingly probable at the higher cooling velocities.

Since a higher permeability constant means that water can leave a cell more rapidly under a given vapor-pressure differential, the intracellular water content ought to be lower at any given temperature with a given cooling rate. Figure 26 supports this idea. A tenfold increase in permeability to water shifts each cooling velocity curve to the left.

Experimentally, the survival of most cells to low temperatures is found to be higher with slow cooling than with rapid, and the range of cooling velocities which most affects survival coincides with the cooling velocities that most affect cell water content according to Eq. (1). Furthermore, the response of a few cells to low temperatures has been observed microscopically. Slow cooling has been found to produce cell dehydration and to prevent intracellular freezing. With more rapid cooling, dehydration does not occur but intracellular freezing does. Asahina,[8] for example, found that sea urchin eggs undergo dehydration when cooling is at 1°C/min but freeze internally when cooling is at 10°C/min. The solutions to Eq. (1), when appropriate values are used for

[8] E. Asahina, *Nature* **191**, 1263 (1961).

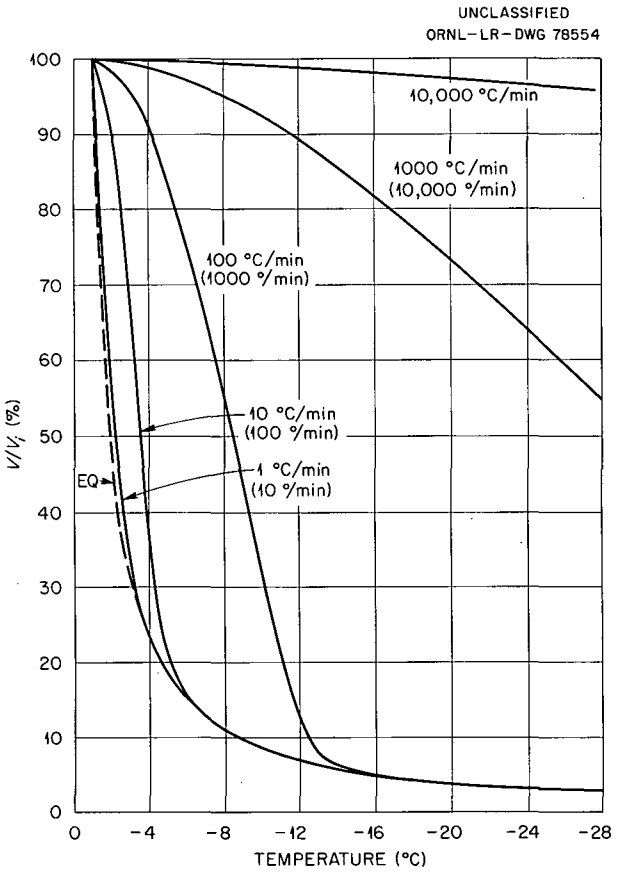

Fig. 26. Calculated Percentages of Supercooled Intracellular Water Remaining in 6-μ-Diam Spherical Cells Cooled at Several Rates. The unbracketed cooling velocities are for $K_g = 0.3$; the values in parentheses are for $K_g = 3$; the values for the other parameters are those given in Table 24.

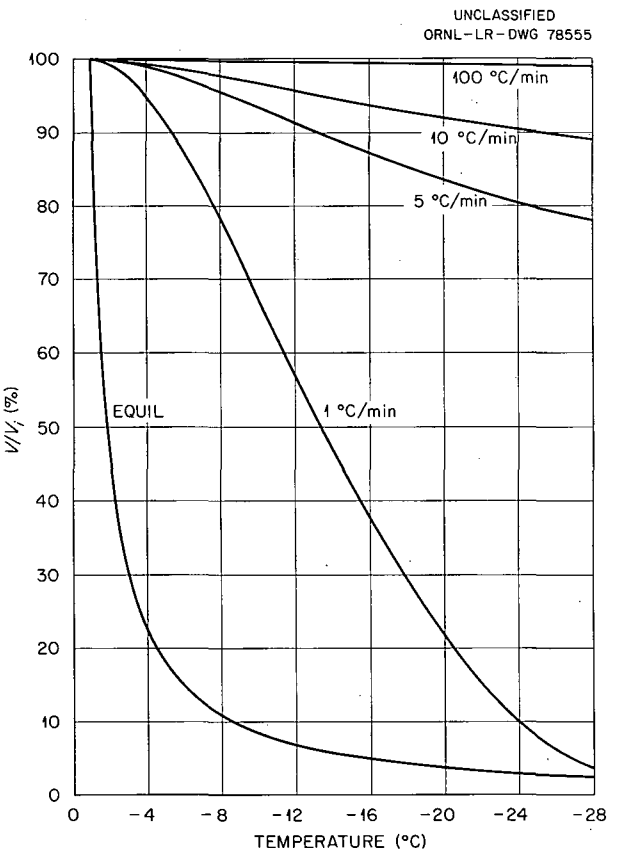

Fig. 27. Calculated Percentages of Intracellular Water Remaining at Indicated Temperatures in Unfertilized *Arbacia* Eggs Cooled at Indicated Rates. The values for those parameters applicable to *Arbacia* were $V_i = 2.08 \times 10^5$, $A = 1.86 \times 10^4$, $b = 0.0449$, $K_g = 0.11$, and $n_2 = 1.04 \times 10^{-10}$. The values of the other parameters are given in Table 24.

sea urchin eggs, are consistent with these observations (Fig. 27). Equation (1), therefore, appears to provide quantitative physicochemical support for the view that cooling velocity affects survival by affecting the likelihood of intracellular freezing.

STUDIES ON SYNCHRONIZED CELLS

T. W. James

Introduction. – Studies on cell division in the colorless euglenoid flagellate, *Astasia longa,* over the past 6 months have included the development of an experimental division synchronizer, radioautographic investigation of the cell membrane during the fission process, and microscopic examination of the mitochondria during the mitotic period. A variety of exploratory investigations have also been performed to determine the qualitative nature of the free nucleotides and the feasibility of particulate isolations from cells over the synchronized cell cycle by employing the zonal ultracentrifuge.

Development of Experimental Cell Synchronizer. – An experimental cell-division synchronizer has been developed and is in daily operation. It has a 5-liter capacity and is capable of a yield of 2.5 g of synchronized cells per day. A similar unit will be placed in operation before March 1.

The unit that is now in operation contains several devices that may prove to be of considerable importance to the general area of cell growth as well as to synchronization studies. The first of these is a cell-number monitor, which is designed to plot automatically the true cell number, irrespective of cell size, of a logarithmically growing or synchronized culture of cells. It consists of an electronic unit that senses the cells passing through a small orifice or window and presents these as electrical pulses, which are then counted. This unit is similar to the Coulter cell counter except that it can be operated continuously (several days) without the orifice becoming clogged with the usual debris that will accompany even the most meticulously prepared culture medium. The unit constantly withdraws, dilutes, and counts the cells from the culture and displays the count rate on a strip-chart recorder. The count rate is displayed against time as either a linear or a logarithmic function, and the accuracy and precision of such a display can be readily evaluated by the usual counting statistics employed for radioisotope work. We are presently using this device with the cell synchronizer to determine the effects of the rate of temperature change on the duration of the synchronous burst of cell division in the synchronizer. It permits us to see the exact shape of the burst as a population event in the culture.

A second device used in the experimental cell synchronizer is an oxygen electrode that monitors its oxygen tension over the entire synchronous cycle. Since from previous batch measurements on synchronized cells we have established that oxygen consumption shows a marked increase (2X) at the time of the cell-division burst, we came to believe that the oxygen tension may fall to a level in the culture that is deleterious to the completion of the division burst. Recent recordings of oxygen tension, temperature, and cell number on the same synchronous culture bear out this suspicion and indicate that, despite rapid stirring, diffusion of oxygen is not rapid enough to prevent anaerobic conditions from arising in the culture. Since aeration tends to wash out metabolic carbon dioxide and injure the cells, it will be necessary to monitor carbon dioxide with a CO_2 electrode along with oxygen tension and to make adjustments to increase the yield of synchronized cells. A high yield is necessary if extensive biochemical analyses are to be undertaken.

Studies on Cell Fission. – Several investigations more directly related to the division of these cells have been carried out. One of these has been concerned with the fission process and its relation to the synthesis of the cell pellicle, or membrane, and the other is related to the correlation between the increase in oxygen consumption of the cells and the morphological changes in the mitochondria at the time of mitosis.

The supposition that the synthesis of the cell pellicle occurs at the time of cell fission is deduced from the fact that the cell divides longitudinally, and the cleavage appears to follow along two of the strial grooves on the cell surface. Since the number of striae seems to be constant (36) during interphase, and since cell fission must reduce this by one-half (18), new cell pellicle must be laid down close to the time of fission. Radioautography at the time of cell fission has been used to test this hypothesis, but to date all the isolated membranes appear to show a uniform label. Several modifications of the labeling technique are now being tested to determine if this finding is true under other conditions of labeling.

The change in the mitochondrial morphology at the time of mitosis is also under investigation. Previously obtained electron micrographs reveal that at the time of cell division the mitochondria occur in the form of long chains and differ in size and shape from the interphase mitochondria. The fact that this change is also accompanied by an increase in respiratory activity leads us to postulate that this period in the cell cycle may yield some major clues on the origin and development of these particles. Recent results using light microscopy provide additional support to these findings.

LOW-SPEED ZONAL ROTOR A-V

N. G. Anderson R. E. Canning
C. T. Rankin, Jr.

Introduction. – Rotor A-V was designed to examine the possibility of separations at relatively low speeds (500–1000 rpm). The feasibility of loading and unloading type B (zonal) rotors at

very much higher speeds had already been demonstrated.[9,10] At very low speeds, however, the stabilizing effects of centrifugal force are much less, and the possibility of mixing during introduction of the sample layer correspondingly increased.

At low speeds, where the pressures and stresses are relatively small, large-diameter rotors of transparent materials may be considered.

Results. – The rotor is made of methacrylate resin, and its interior is divided into eight sector-shaped compartments, each 2.72 cm deep, with maximum and minimum radii of 13.60 and 3.49 cm. The upper and lower end plates are of 3.26-cm-thick clear plastic. The internal volume is 1300 ml.

Since all parts of a zone of one density in a zonal centrifuge constitute a paraboloid of revolution about the axis of rotation, a single conical-section core which allows the sample layer to slope 45° or more as it approaches the rotor center has been used. The rotating seal was constructed of filled Teflon; the static fluid-line seal was constructed of stainless steel and was flexibly mounted to allow alignment with the rotating seal during operation. Chilled water was circulated through the upper seal to remove frictional heat.

By adding water to the rotor in 100-ml increments and determining the position of the meniscus carefully at 1000 rpm, a chart of volume as a function of radius was constructed. The position of any portion of the gradient recovered during unloading could then be related to its original position in the rotor. All work was performed in an International Equipment Co. model PR-2 refrigerated centrifuge with a transparent plastic lid.

To determine whether density gradients could be introduced into the rotor and recovered without extensive mixing, a density gradient was analyzed refractometrically both before introduction into the rotor and after spinning in the rotor for 15 min at 1000 rpm. Negligible differences between the two gradients were seen.

A sample zone introduced into the rotor is widened by laminar flow through the tubing and rotor core, by any turbulence or convection in the rotor, and by diffusion. To determine how much widening will occur in practice, a sample zone containing 4% bovine serum albumin (BSA) having a total volume of 20 ml was introduced over a sucrose gradient. When moved to a position just external to the core, the sample zone should have a calculated width of 0.1 cm. The rotor was spun 20 min at 1000 rpm at 5°C (152 × g at R_{max}, 39 × g at R_{min}). When the gradient was recovered, the absorbency recording of the sample zone showed a peak whose width at half-height was equivalent to a zone width of 0.3 cm in the centrifuge. This amount of broadening is not considered excessive.

Experimental. – Three types of experiments were performed to determine the resolution obtainable with the rotor. In the first, ragweed pollen grains were used to form a sharp band at their isopycnic point; in the second, the sedimentation of rat RBC was followed. In the third series of experiments,[11] a method for isolating calf thymus nuclei, which retained their ability to incorporate amino acids, was developed.

Preliminary experiments with ragweed pollen suggested that more uniform behavior is obtained if the grains are first fixed in alcohol. Two hundred milligrams of pollen was therefore suspended in 10 ml of 95% ethanol, centrifuged briefly, and resuspended in 10 ml of 8.5% sucrose. This volume of material was used as the sample layer and was introduced centripetally to a 1200-ml gradient extending from 17% to 55% (w/w) sucrose. Fifty-five percent sucrose was also used as the "cushion" outboard of the gradient. After 10 min at 1000 rpm, the gradient was displaced with 55% sucrose through the core and the monitoring system and into 40-ml collecting tubes. Photographs of the rotor during the course of the run are shown in Fig. 28. The fractions were examined by dark-field phase-contrast microscopy. The peak at the sample zone level contained no particles and was produced by the leaking of soluble ultraviolet-absorbing materials from the pollen. All pollen particles were observed at the same level; none were found "scattered" throughout the gradient. It is quite evident that particle zones separated in the centrifuge can be recovered with very little loss in resolution.

[9] N. G. Anderson and C. L. Burger, *Science* **136**, 646 (1962).

[10] N. G. Anderson, *J. Phys. Chem.* **66**, 1984 (1962).

[11] W. D. Fisher and G. B. Cline, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 117.

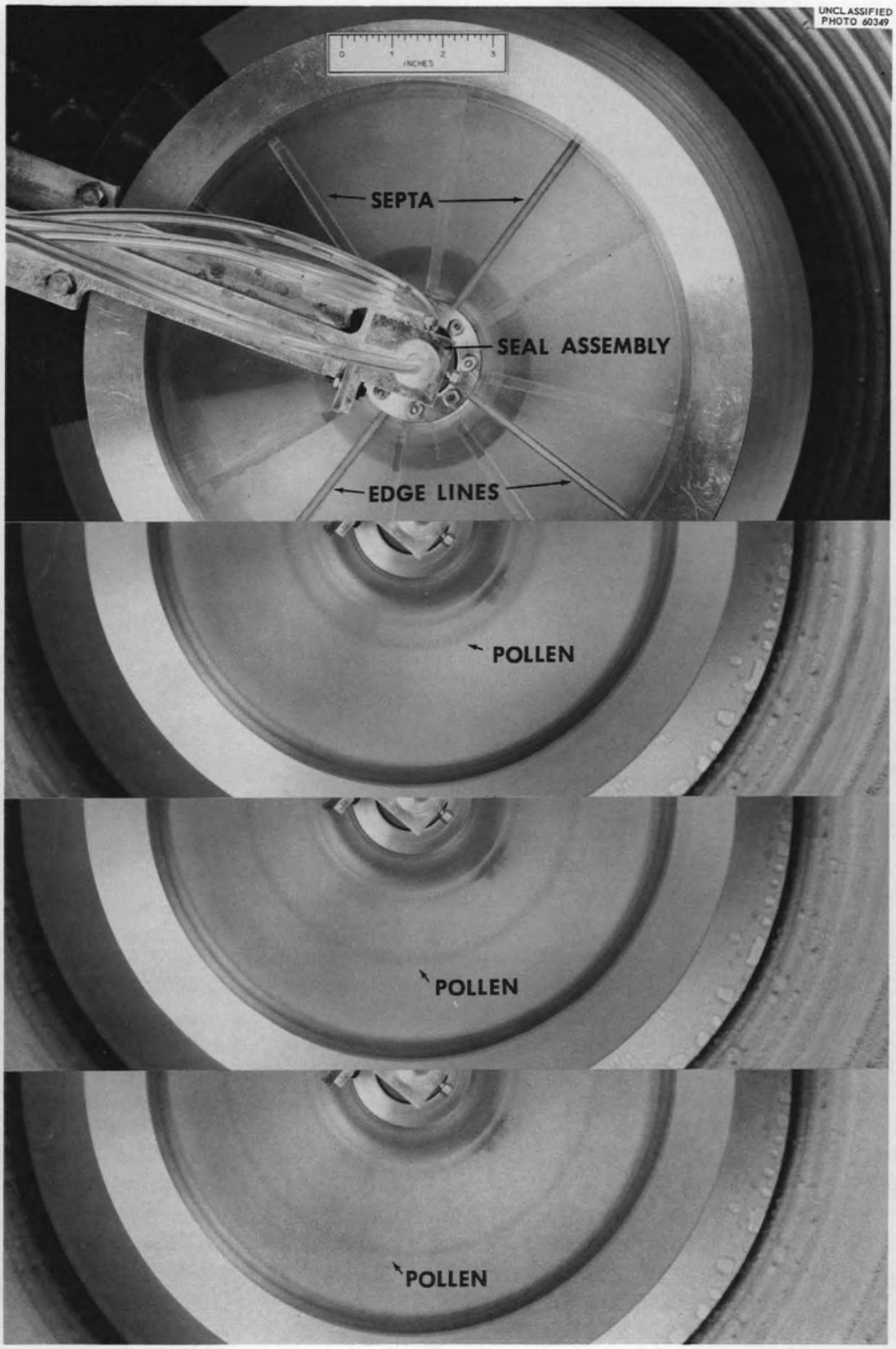

Fig. 28. Sedimentation of Ragweed Pollen in Rotor A-V.

ROTOR A-VI

N. G. Anderson H. P. Barringer*
C. E. Nunley*

Introduction. – Rotor A-VI is the largest zonal centrifuge rotor presently contemplated. It is designed to explore large-scale nuclear isolation and the separation of different cell types in a mixture as occurs, for example, in bone-marrow cell suspensions.

Discussion. – Rotor A-VI has a 3-liter capacity and is designed for operation at 6000 rpm and a maximal centrifugal force of 7100 × *g*. The design specifications were fixed by the speed, permissible diameter, and thrust-bearing capacity of the International model PR-2 which was used to drive the rotor. The design is not an optimum one from the point of view of the strength of the rotor material (7075-T6 aluminum). The rotor and seal system assembled for use is shown in Fig. 29.

The initial intent was to provide a fairly uniform centrifugal field by using a large-diameter central core to keep the entire gradient close to the rotor edge. The length was held to a minimum to give a ratio of $I_{spin}/I_{transverse} > 1$ for the moments of inertia, thus providing a configuration which spins about its most stable axis. The central core diameter is 9 in., and the inside diameter is 14 in., giving a gradient chamber length of $2\frac{1}{2}$ in. At the design speed of 6000 rpm, with a sucrose density gradient in position, a rather large radial growth (approximately $\frac{1}{64}$ in.) occurs. Since the end caps increase in diameter by only

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Fig. 29. Rotor A-VI and Seal Assembly Mounted in PR-2 Centrifuge. The rotor is visible during operation through the plastic top of the centrifuge.

a few thousandths of an inch, a mechanism was needed to center the rotor chamber wall with the end caps and maintain concentricity to the drive spindle. Plated brass plates, one in each end cap, were employed. The end caps expanded during centrifugation at a rate intermediate between that of the rotor wall and the end plates. Without the brass plates the runout (departure from circularity) increased from the normal 0.004 sec in total indicator reading (TIR) to 0.010 sec in TIR, demonstrating the effectiveness of the brass plates.

The necessity of balancing the rotor has been completely avoided by very accurate machining. The only metals in contact with fluid are anodized aluminum, nickel, Monel, or stainless steel. O rings are used to seal the rotor chamber and all components in the liquid lines.

Three methods have been investigated for obtaining flow into and out of the centrifuge at operating speed. In the first method skimmers were used. These did not prove successful at the relatively low peripheral speed at the edge of the skimmer disks. In the second approach, a self-aligning ball-and-socket rotating seal was tested. This seal was ineffective owing to distortion of the ball during attachment to the drive and to cold-flowing of the reinforced Teflon of the static seal when under pressure. In the third method a flat, modified face seal proved effective.

One top end cap for rotor A-VI has been fabricated from Lucite to permit viewing of the rotor contents completely across the rotor diameter. At the center of the rotor a large nut limits deflection of the cap and reduces the stress level. The rotor contents can be observed in detail with a stroboscopic light.

Power limitations of the PR-2 drive motor have restricted the top test speed in practice to 5160 rpm. Critical speeds of the spindle system, which occur at 700 to 900 rpm, have been negotiated with no difficulty.

At the end of this report period, studies using biological materials had just begun. It is contemplated that rotor A-VI will be extremely useful for the isolation of nuclei, amphibian egg yolk platelets, and paramylum granules and for the separation of cells that differ in size or density. The possibility of separating sperm cells on the basis of their containing X and Y chromosomes is also being considered.

REORIENTING-GRADIENT (REOGRAD) ZONAL CENTRIFUGES

N. G. Anderson C. A. Price
W. D. Fisher C. L. Burger

Introduction. – Theoretical considerations suggest that zonal centrifuges might be built in which the gradient and sample zones are introduced and recovered at rest and in which swinging buckets are not employed. The advantages of such systems are extreme simplicity of operation and the possibility of operation at very high speeds where the rotating fluid-line seals employed in the A-V, A-VI, and B-II rotors might not be usable because of frictional heating.

Theoretical Considerations. – The shearing forces occurring in a liquid confined in a closed cylinder during transition from rest to a stable orientation in a high-centrifugal-force field may be considered in a qualitative manner by the examination of Fig. 30. The horizontal lines indicate levels or surfaces of equal density in a continuous density gradient.

During acceleration, each isodense surface becomes part of a paraboloid of revolution. The equation for the intersection of an axial plane with the paraboloid of revolution is given by

$$L = \frac{\mu^2 w^2}{2 \times 980},$$

where L is the height from apex to paraboloid of a given point on the osodensity surface, and μ is the radius at that point. At a given rotational speed all isodensity curves are identical, being merely transposed vertically. Acceleration results in a series of configurations shown diagrammatically in Fig. 30 (b, c). At high speed, where the ratio between the centrifugal force and the acceleration due to gravity is very high, the isodensity surfaces will approach verticality, as is shown in Fig. 30 (d).

Deformations occurring at the various levels may be best understood by describing the changes occurring in layers originally at the top, middle, and bottom of the rotor. The fluid originally against the upper rotor cap becomes squeezed into a small paraboloid of revolution during acceleration [Fig. 30 (b, T)] and then occupies the center of the rotor at high speed [Fig. 30 (d, T)].

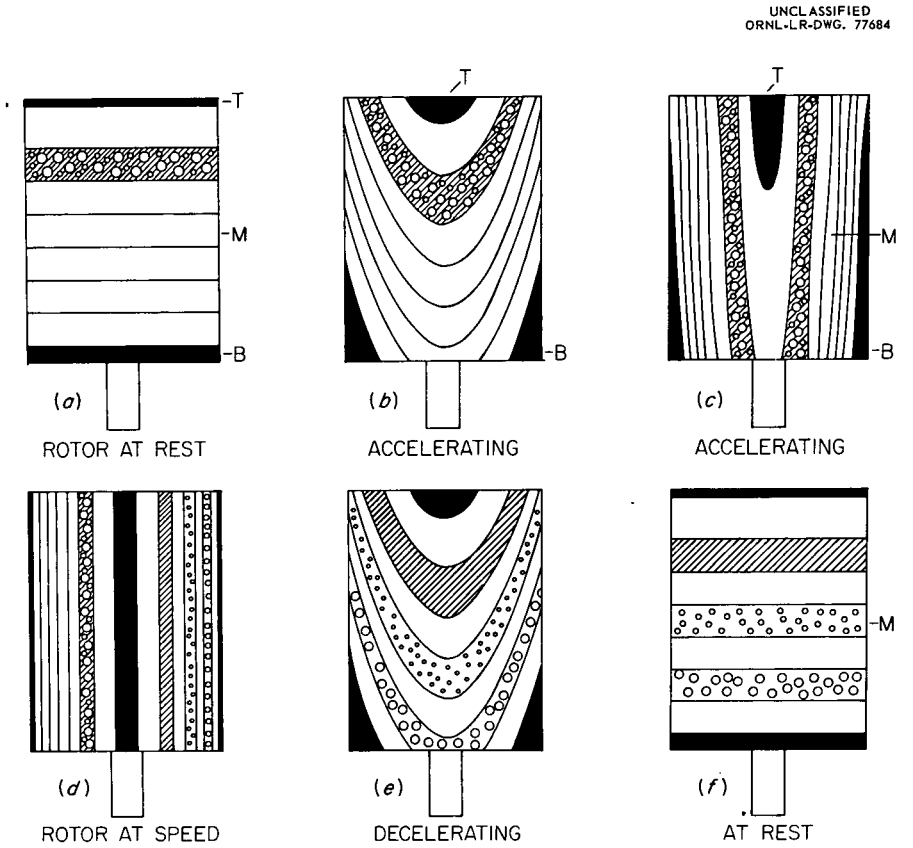

Fig. 30. Schematic Diagram of Reograd Rotor System. (*a*) Rotor is filled at rest with density gradient and sample layer. To indicate extremes of zone deformation, a thin upper layer *T* and bottom layer *B* are also indicated. (*b*) During acceleration each zone forms a paraboloid of revolution about the axis. Note *T* and *B*. (*c*) Near operating speed the zones approach a vertical orientation. (*d*) At a sufficiently high speed the zones become nearly vertical. (*e*) During deceleration, zones again form paraboloids of revolution. (*f*) At rest, various zones may be recovered by draining rotor contents out the bottom or displacing the gradient out through the top.

A zone in the middle of the rotor [Fig. 30 (a, M)] at rest increases in area during acceleration and then decreases in area slightly as an approximately vertical position is approached. The zone at the very bottom of the rotor at rest [Fig. 30 (a, B)] decreases markedly in surface area during acceleration [Fig. 30 (b, B)] but covers the entire surface of the rotor wall at high speed [Fig. 30 (d, B)]. The greatest area changes, therefore, occur in those zones near the top and bottom when at rest but near the center and the edge at high speed.

The reoriented gradient before the particles have sedimented appreciably is shown on the left side of Fig. 30 (d); the gradient after sedimentation is shown on the right side. The distribution during deceleration is shown in Fig. 30 (e); the distribution at rest is shown in Fig. 30 (f). The separated zones are recovered by draining the gradient out of the bottom of the rotor or displacing it out the top.

Discussion. – A mathematical analysis of the areas of isodensity surfaces (Fisher, Price, and Anderson, in preparation) shows that very little shearing occurs in the center of the gradient in a sector-compartmented rotor. Although increases and decreases in area occur, the difference in rate of increase or decrease in the areas of adjacent zones is rather small. By placing a dense "cushion" in the bottom and an overlay of light fluid above the sample layer at the top, the sample layer and density gradient may be

restricted to that part of the rotor where least shearing occurs. As the fluid layers change position during acceleration and deceleration, their tangential velocity will change, since the velocity at any point in the rotor is a function of both the rotational speed and the radius of the point. Fluid in the upper layer, originally near the edge of the rotor, decreases in tangential velocity relative to previously underlying fluid during acceleration, for example. Vertical septa, therefore, are considered necessary to prevent swirling during reorientation of the gradient.

ZONAL ROTOR A-VII

N. G. Anderson W. D. Fisher
R. E. Canning C. A. Price
C. T. Rankin, Jr.

Introduction. – To test the reorienting gradient concept,[12] a small methacrylate rotor was constructed and mounted in a brass holder attached to a heavy flywheel rotor. The large mass allowed smooth, controlled acceleration and deceleration in the International PR-2 centrifuge. This rotor, designated rotor A-VII, has a capacity of 127 ml. The gradient, when reoriented vertically during rotation, has a thickness of 1.27 cm.

Experimental. – To test the rotor, 27 ml of a pH 7.5 sodium phosphate–NaCl buffer, $\mu = 0.1$ (ref 13), was pipetted to the bottom of the rotor at rest. An 80-ml linear density gradient was formed using a two-cylinder gradient apparatus and was pumped to the bottom of the rotor using a Technicon proportioning pump with a flow rate of 1.89 ml/min. The gradient extended from 17% to 60% (w/w) sucrose. A 20-ml underlay of 60% sucrose was pumped in after the gradient. After an interval of 15 min, the gradient was pumped out of the rotor from the bottom using the same pump and flow rate and was collected in 5-ml volumes. The same experiment was performed with the rotor slowly accelerated to 3000 rpm and decelerated to rest over a 15-min interval. The gradients were analyzed for sucrose content refractometrically. It is evident that very little disturbance in the gradient occurred during the transition from rest to 3000 rpm and back to rest.

To determine whether a sample zone could be maintained in position during the rest-to-rotation transition, 1 ml of an 8% solution of bovine serum albumin was introduced to the bottom of the rotor between the overlay and the sucrose gradient. After acceleration to 3000 rpm and deceleration to rest over a 15-min period, the rotor contents were pumped out through an ultraviolet absorption analyzer, which was set at 280 mμ with a 0.2-cm light-path flow cell.[14] The results, replotted in terms of rotor radius with the rotor volume also included, are shown in Fig. 31. The sample, as introduced, had a calculated width of approximately 0.1 mm. After acceleration to 3000 rpm and deceleration to rest, the width of the sample peak at half-height, as observed by the monitoring system, was 1.0 mm. This amount of broadening, which is probably due to both diffusion and

[12] N. G. Anderson *et al.*, this report.

[13] G. L. Miller and R. H. Golden, *Arch. Biochem.* **29**, 421 (1950).

[14] N. G. Anderson, *Anal. Chem.* **33**, 970 (1961).

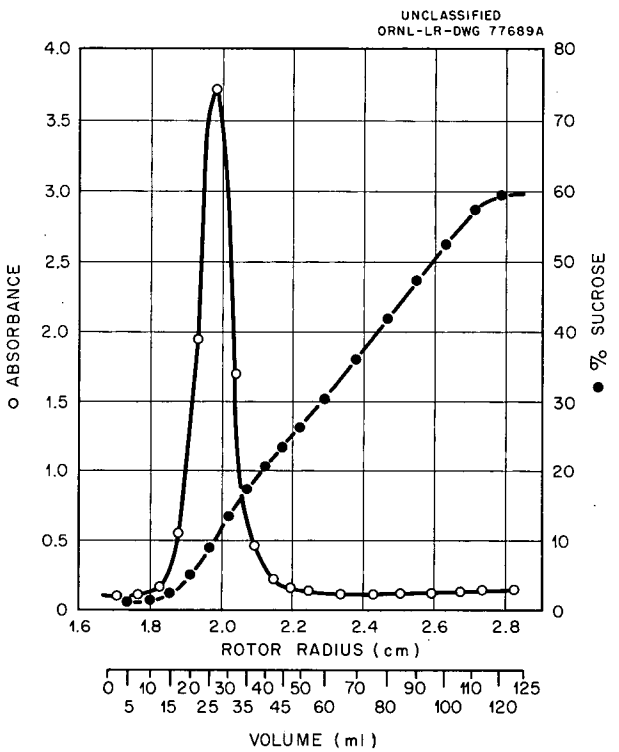

Fig. 31. Stability of Sample Layer During Acceleration to 3000 rpm and Deceleration to Rest. The sample layer was 1.0 ml of 8% BSA layered over a sucrose gradient.

laminar mixing in the fluid lines, is not prohibitively large for the purpose described in the discussion.

To see whether a zone of material banded sharply further down the gradient could be recovered with good resolution, 40 mg of ragweed pollen in 2 ml of 8% sucrose was used as the sample layer. The experimental conditions were identical with those described for the bovine serum albumin experiment. After the deceleration from 3000 rpm to rest, the absorbance at 280 mμ and the sucrose concentration were determined as shown in Fig. 32. A clear, yellow pigment released from the pollen grains accounted for nearly all the absorbance in the region of the starting zone. Extraction of the pigment from the pollen grains as they sedimented through the gradient probably accounts for the skewed tail observed in the starting zone curve. A small number of pollen grains were found through the starting zone. These may have been cells that had begun to swell in the sucrose solution. The vast majority of the cells banded in a narrow zone at the 45% (w/w) sucrose level. The width of the peak at half-height corresponds to a zone thickness of 0.87 mm in the rotor during rotation. In these experiments the pollen was not treated with alcohol, as is generally done when the grains are used as standards for particle counters.

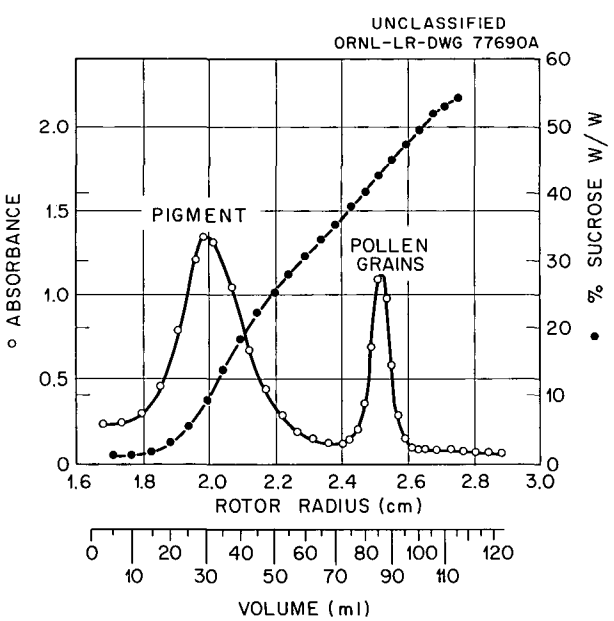

Fig. 32. Banding of Ragweed Pollen Grains in a Sucrose Gradient.

Discussion. – Since the diameter of a high-speed rotor is determined and limited by the strength of available materials, large rotor capacity can only be obtained by using elongated cylindrical configurations. A zone occupying a narrow band in the density gradient during rotation will, when the rotor is returned to rest, be physically thicker as its cross sectional area is decreased. This is true even though the zone occupies the same volume of fluid during rotation and at rest. Band widening by diffusion and by laminar mixing along the wall during unloading is therefore minimized.

In these experiments the density gradients have been preformed. It is evident that reograd rotors may be used with preformed gradients for separations based either on sedimentation rate or on density alone by isopycnic banding and that the gradients for the latter type of separation may be formed in the rotor by the centrifugal field.

HIGH-SPEED ZONAL ROTOR C-II

H. P. Barringer N. G. Anderson

Introduction. – Speeds in excess of 60,000 rpm and force fields of more than 250,000 × *g* have not been generally available for biological studies. The purpose of the C-II rotor system has been to provide an extremely simple centrifuge system, requiring no critical adjustments, which may be routinely operated at extremely high speeds by untrained personnel. Since the rotor is intended primarily for work with cesium chloride solutions, a fluid density of 1.7 has been used in the design calculations. When solutions of this density are used, the SW 39 rotor is limited to a total capacity of approximately 12 ml at 33,000 rpm, while the Spinco analytical AN rotor is operated at 44,770 rpm with approximately 0.7 ml of solution. It did not appear feasible to explore much higher speeds if the rotor volume would be cut down at the rate suggested by the capacities of currently available equipment. Fortunately, the reograd concept[12] makes the use of tubular sector-compartmented rotors feasible. Since it was considered important for future planning to know as soon as possible whether or not this concept,

configuration, and drive system would be suitable for biological studies, the C-II rotor was designed, constructed, and tested to 90,000 rpm in somewhat less than 3 weeks.

Results and Discussion. – Rotor C-II has a design speed of 141,000 rpm (310,000 × g at R_{max}) when loaded with 100 ml of fluid having a density of 1.7. With lighter fluids, proportionately higher speeds may be obtained.

The rotor is a hollow, reorienting gradient type of zonal centrifuge which is loaded and unloaded at rest. The density gradient may be formed with a suitable gradient engine and introduced together with sample zone at rest for rate-zonal molecular sedimentation studies, or the gradient may be formed by the centrifugal field, as is often done in isopycnic zonal centrifugation.

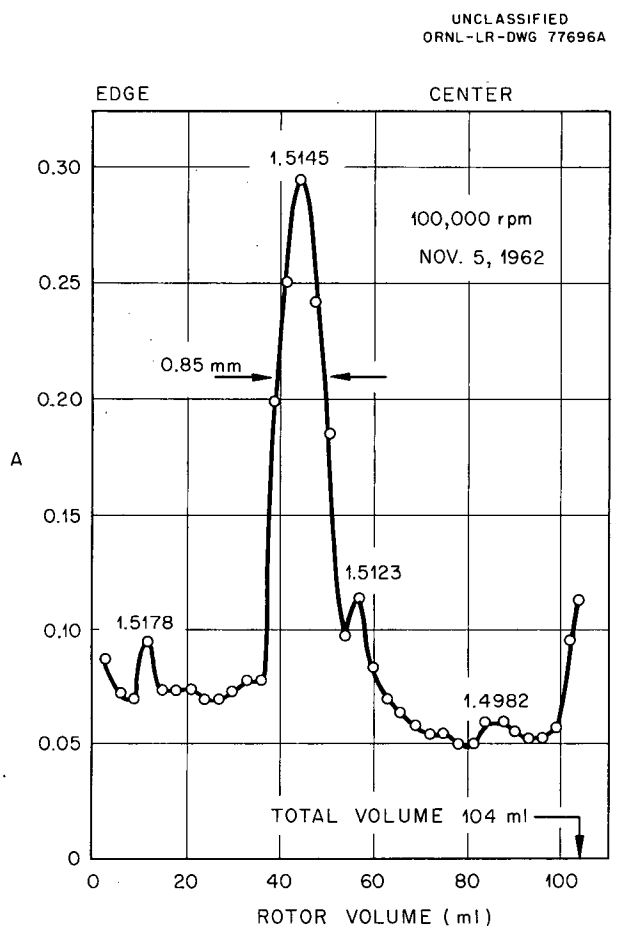

Fig. 33. Banding of T3 Phage in Cesium Chloride in Zonal Rotor C-II.

The rotor chamber is divided into four compartments by a single-piece core so that angular momentum can be imparted to the fluid mass during acceleration and deceleration without stirring or mixing.

The rotor is operated *in vacuo* with suitable cooling coils to remove any heat generated at high peripheral speeds. Both the cooling coils and the rotor surface have high emissivity values to provide excellent radiant heat transfer.

Over 300 hr of run time at various speeds between 98,400 and 141,000 rpm have been accumulated. The rotor is estimated to have a life in excess of 8000 hr at 141,000 rpm.

The C-II rotor has been tested using cesium chloride gradients formed by the centrifugal field to band DNA and virus particles at speeds up to 141,000 rpm. Since the large rotor volume (100 ml) allows milliliter or larger fractions to be recovered, sufficient material is available for accurate refractometric determination of cesium chloride concentration. Banding of T3 phage particles in 9 hr at 100,000 rpm is illustrated in Fig. 33. The band, as recovered, was 0.85 mm thick at half-height, calculated back to the band position in the rotor.

In similar experiments at speeds up to 141,000 rpm, two bands have been observed with milligram quantities of calf and rat thymus DNA. These experiments will be reported in more detail in the next report.

This work makes it possible to determine easily whether or not a labeled drug or other substance becomes attached to DNA in the cell.

ULTRAHIGH-SPEED ROTOR SYSTEMS FOR EQUILIBRIUM CENTRIFUGATION

N. G. Anderson H. P. Barringer

Introduction. – Molecular equilibrium centrifugation or "banding" in cesium chloride or other salt solutions is successful with presently available centrifugal forces because of the very low diffusivity of DNA and other large macromolecules. With lower-molecular-weight substances the bandwidth with a homogeneous substance may become excessively large, making it difficult to resolve two components differing slightly in density. With a given system (such as DNA, cesium chloride, and water), resolution is not increased with

increasing centrifugal force – as the steepness of the gradient is increased, bands of DNA differing in density move closer together and also narrow. To increase resolution markedly, the centrifugal field must be greatly increased without a proportional increase in gradient steepness. By using deuterium oxide and mixtures of salts or organic substances of lower molecular weight than cesium chloride, the steepness of the gradient in a given centrifugal field can be decreased and still include regions having the desired density. Since the centrifugal field may then become the limiting factor in achieving high resolution in a reasonable time, it is of interest to explore the limits set by existing materials and technologies on the construction of ultrahigh-speed rotors.

Discussion. – The first rotor of this series, D-I, will have a design speed of 400,000 rpm and produce 1.2 million × *g* for extended periods of time. At these speeds the volume of fluid must be restricted to 10 ml. For the initial tests the D-I rotor will be operated like the C-II rotor, with the fluid being sealed in the rotor chamber during operation.

The D-II rotor will utilize several components of the D-I rotor, but rather than steel end caps, the end enclosures will be transparent windows permitting observations and optical measurements.

A third rotor design, D-III, with fairly large-diameter quartz, ultrahigh-strength glass, or sapphire windows is being considered. Rotor D-III will be useful for basic studies on the formation and capacity of density gradients.

For maximal centrifugal field studies, rotor D-IV is being considered. It has transparent end windows, a 0.75-ml capacity, maximal centrifugal force of 2.5 million × *g* at 840,000 rpm (275 m/sec inner peripheral velocity), and an inside diameter of 1 mm. This rotor would be useful for studies on the effects of high hydrostatic pressure on the density of macromolecules.

THEORY OF ZONE CAPACITY

N. G. Anderson

Introduction. – A density gradient stabilizes a particle zone by preventing convective disturbances. As was first explicitly shown by Svensson *et al.*,[15] a stable sample zone can exist only as long as all portions of the gradient remain positive (i.e., increase in density in a centrifugal direction). At a level in the gradient where the particles have the same density as the solvent (the isopycnic level), the gradient capacity is equal to the volume, that is, a sheet of packed particles may be supported. At other levels the capacity is a function of the steepness of the gradient, and the difference between the density of the particles and solvent. Assuming that the particles are impermeable and nonosmotic, the theoretical capacity of a gradient may be approximated. Such an approximation is shown graphically in Fig. 34. If the rotor radius is plotted along the ordinate and the density is plotted along the abscissa, a linear gradient may be indicated, as shown. A sample zone may be represented by a triangle whose horizontal length *L* equals the maximum volume fraction of particles times *C*, which is the distance along the density abscissa from zero to the isopycnic level. The density (from zero to the value at the isopycnic level) is plotted along line *L*, and line *L* is shifted horizontally until the intersection of line *L* and the gradient curve is at a point on the scale of line *L* equal to the density at the point of intersection. Since no zone with a density exceeding that of any point on the gradient underneath it is allowed, it is evident that a thin, dense particle zone must be supported by a decreasing, or negative, gradient of particles underneath it. As shown in Fig. 34, the maximum capacity indicated in the upper zone occurs when the density does not change through a zone. By dropping a perpendicular from *L*, as shown, a triangle is formed which indicates the distribution of particles as a function of rotor radius. The area to the right of the density curve is proportional to the excess density, while the dark area to the left is proportional to the buoyancy. As the zone sediments, the intersection of *L* and the gradient density curve shifts until, at the isopycnic point, no excess density exists. When a zone is placed below the isopycnic level a reverse situation, with an excess buoyancy, occurs.

The following factors favor the use of convex gradients:

[15] H. Svensson, L. Hagdahl, and K-D. Lernes, *Sci. Tools* 4, 1 (1957).

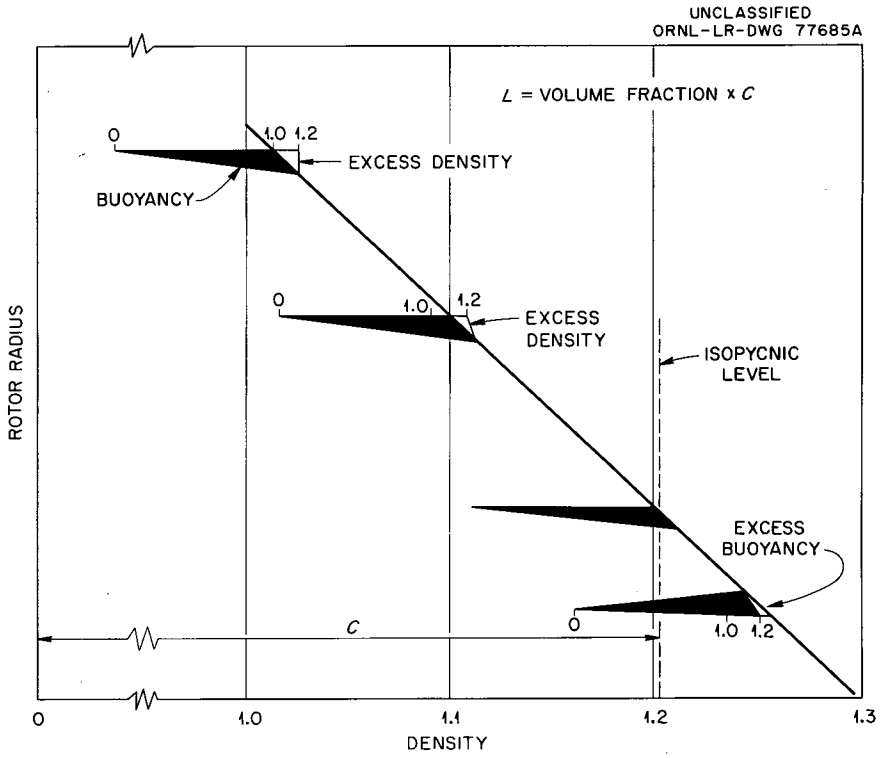

Fig. 34. Graphic Representation of Gradient Capacity. Particle zones are represented as triangles whose length L is equal to the particle volume fraction multiplied by C, the distance along the abscissa from zero density to the particle isopycnic point. Along L is plotted a scale from zero to the isopycnic density number. L is shifted horizontally until the intersection between L and the gradient curve is at the gradient density (as read on scale L) existing at the level of intersection. Displacement buoyancy is indicated in black, excess density or buoyancy in white.

1. The capacity of a gradient increases as the difference between the particle and solvent density increases. If the capacity of a gradient is to be the same along its length, as would be desirable when a single virus species is being purified, then dc/dx should decrease as $\rho_p - \rho_s$ decreases.

2. In a multicomponent system a series of zones will leave the starting zone with little separation between them, but these will become widely distributed throughout the gradient during centrifugation. It is evident that the greatest gradient capacity is required through and just below the sample zone, that is, the gradient should be convex.

Concave gradients may be employed when it is desirable to band a rapidly sedimenting or dense particle at its isopycnic point while allowing a slowly sedimenting component to sediment through a shallow gradient. Complex gradients have been employed for cell-component isolation in the present work when a linear gradient ends in a second small, steep gradient, as in many tissue separations.

The capacity of a zone in terms of the amount of particulate material supported in a thin, stable zone in a zonal centrifuge may be calculated approximately using Eq. (1) (derived from an equation of Svensson *et al.*[15] and Brakke[16] for gradients in tubes of circular cross section). It is based on the assumption that the negative concentration gradient of a particle zone may increase until the solvent plus particle gradient approaches zero.

$$A_i' = \frac{\pi L h^2 (x_0 + 0.29h - cn)\,[(d\rho_1'/dx) - (d\rho/dx)_e]}{[1 - (\rho_{1x}'/\phi_1')]} . \quad (1)$$

[16] M. K. Brakke, *Advan. Virus Res.* **7**, 193 (1960).

The symbols with prime marks indicate values in sucrose solutions, and symbols without prime marks refer to values under standard conditions (i.e., water at 20°), ρ'_{1x} is the density of the sucrose solution, ρ'_x is the density of the bottom (outer edge) of a zone, ϕ_i is the effective density for sedimentation in solution of component i, h is the thickness of the zone, c is the thickness of one septum, n is the number of septa, L is the height of the internal rotor chamber, and x_0 is the distance from the axis of rotation to the top (inner edge) of the particle zone. The term $(d\rho/dx)_e$ is a correction factor for the minimum overall density gradient necessary for gravitational stability. Theoretically, it should approach zero for centrifugation but should have a small positive value for electrophoresis. One-half of the particles in a wedge distribution of particles lie above a point $0.29h$ distance down the zone. The area of the zone perpendicular to the direction of sedimentation is taken to be $2\pi L(x_0 + 0.29h - cn)$. The 2 in the denominator of the Svensson equation thus cancels out when applied to zonal rotors.

Discussion. – In practice, the amount of material which may be supported in a zone of given thickness is much smaller than predicted by Eq. (1). The reasons for this are not understood, and series E rotor systems are proposed specifically for studying this and several other problems in connection with gradient theory.

RIBOSOME SEPARATIONS IN THE ZONAL ULTRACENTRIFUGE

N. G. Anderson R. E. Canning
C. T. Rankin, Jr.

Introduction. – The operation of the B-II rotor in the zonal ultracentrifuge has been previously described. This rotor has been used to separate ribosomes from *Escherichia coli*, and the results are reported here.

Experimental. – Rapidly growing *E. coli* cells were chilled, concentrated by centrifugation, and broken in a constant-pressure French press. The ribosomes were concentrated by centrifugation

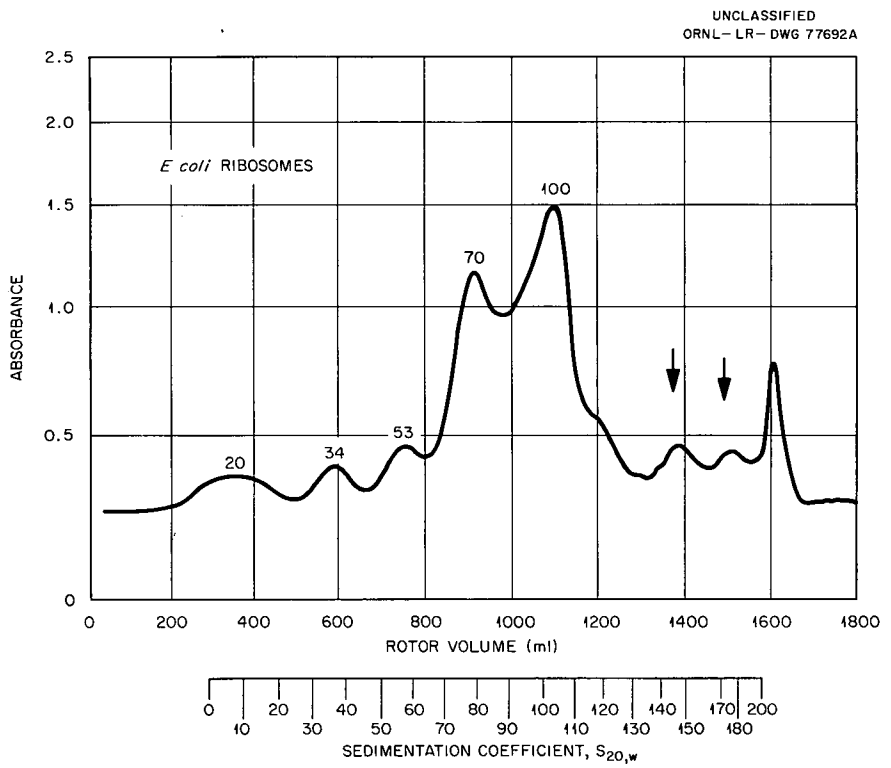

Fig. 35. Separation of *E. coli* Ribosomes in B-II Zonal Rotor.

and resuspended in 0.01 *M* $MgCl_2$–Tris Cl (pH 7.5) buffer, and layered over a 10 to 30% sucrose gradient in rotor B-II. The rotor was run at 24,600 rpm for 6 hr and then unloaded. The results are shown in Fig. 35.

Discussion. – The results agree extremely well with the pattern obtained in the analytical ultracentrifuge. Note peaks to the far right. These may very well indicate polyribosomes. This work opens up many new possibilities in the biophysical study of ribosomes.

More extensive experiments on ribosome separations are in progress.

HIGH-PRESSURE ION EXCHANGE CHROMATOGRAPHY OF NUCLEOTIDES AND RELATED COMPOUNDS

C. L. Burger

Introduction. – The chief experimental difficulty in chromatographing acid-soluble nucleotides and related compounds has been the nonreproducibility of commercially available resins. After testing all batches of Dowex 1-X8 produced during the past 6 months, this problem appears to have been finally solved, and a large batch of satisfactory 400-mesh resin was prepared. We now have to increase the efficiency of the system by cutting down on the time required for analysis.

Experimental and Discussion. – The time required for chromatographing a tissue extract for acid-soluble materials is approximately 30 hr, using glass columns 150 cm in length with internal diameters of 0.9 cm and employing pressures of less than 70 psi. The eluting system is a sodium acetate gradient, linear from 0.14 *M* to 3.0 *M*. The same run has been performed with acceptable resolution and reproducibility in 10 hr using pressures of 150 to 200 psi and glass columns 135 cm in length, inside diameter of 0.5 cm. The latter is the Technicon system.

To test the concept of using narrower columns and extremely high pressures, a length of 0.2-cm (ID) stainless steel tubing was fitted to the base of a French pressure cell, packed with resin, and loaded with a standard sample. Buffer was pumped through the column from the French cell, and the column effluent was analyzed at 260 and 280 mμ using a DB spectrophotometer. This run was carried out at 2800 psi and at room temperature; normally such runs are carried out at 40°C. Though longer lengths of tubing were used initially, a 24-in. segment proved most efficient. The resin used in all cases was through 400 mesh BioRad 1 × 8.

The volume of buffer required to elute the sample of standards was 11 ml; the time required was 30 min. This compares to 131 ml and 180 min in the first system developed[17] and to 12.5 ml and 25 min in the Technicon system, at the pumping rates employed.

Though resolution in this very high-pressure system needs to be improved, the experiments nevertheless demonstrated that such separations could be made in extremely short times using small sample volumes (0.2 ml). Development of a suitable high-pressure system (up to 10,000 psi) for use with the newly obtained resin is now being explored.

AGGREGATION REACTIONS IN RAT LIVER HOMOGENATES

Herbert Schuel Regina Schuel

When the surface of a cell is ruptured, the emerging cytoplasm usually is not dispersed into the medium, since a new membrane is quickly formed and the flow is thus halted. This process is known as the "surface precipitation" reaction. The addition of calcium ions to initially decalcified sea urchin egg and rat liver breis produces a series of reactions similar to those known to take place in cells undergoing "surface precipitation" and other "sol-gel" reactions; that is, activation of proteolytic enzyme(s), production of acid, liberation of TCA-soluble nitrogen, increase in viscosity, and aggregation of ribonucleoprotein particles.[18–20] Heilbrunn[21] suggested that the molecular mechanisms responsible for these phenomena in cells might be similar to those involved in blood coagulation. As the first part of a long-range program, a comparison

[17] N. G. Anderson *et al.*, to be published in *Analytical Biochemistry*, 1963.

[18] T. Hultin, *Exptl. Cell Res.* **1**, 272 (1950).

[19] P. R. Gross, *Trans. N.Y. Acad. Sci.* **20**, 154 (1957).

[20] N. G. Anderson, T. Makinodan, and C. B. Norris, *Exptl. Cell Res.* **22**, 526 (1961).

[21] L. V. Heilbrunn, *Dynamics of Living Protoplasm*, Academic Press, New York, 1956.

was made of the effects of calcium and magnesium on acid production and aggregation in rat liver breis.

Experimental. – The supernatant (15,000 × *g* for 30 min) was obtained from 25% homogenate (prepared in 0.150 *M* KCl) of rat liver that had been perfused with an ice-cold 1:1 mixture of calcium-free Locke's solution and 0.109 *M* sodium citrate. Acid production and aggregation were measured following the addition of $CaCl_2$ or $MgCl_2$ (final concentration, 0.025 *M*) to the supernatant. As a control, KCl (final concentration, 0.075 *M*) was added to produce the same ionic strength. The preparations were strongly self-buffering at pH 6.9 to 7.0. The addition of calcium resulted in a rapid drop of pH (0.3 to 0.5 unit). Most of the protons appeared within the first minute, and subsequently the pH continued to fall very slowly. The amount of the pH drop increased as the temperature was raised from 26°C to 37°C. The velocity of the initial drop was not decreased by lowering the temperature to 4°C. Magnesium produced an equally rapid, but smaller initial, pH drop, while subsequently the pH rose very slowly (0.05 to 0.1 unit within 20 min). The pH changes induced by magnesium did not appear to be affected by temperature. Titration of the supernatant with 0.01 *N* HCl indicated a great excess of calcium and magnesium ions added over protons released. Aggregation was monitored by following the absorbance of the brei supernatant at 650 mμ. Within the temperature range of 30 to 38°C, the rate of the aggregation reaction induced by calcium was much faster than that induced by magnesium (Fig. 36). The curve for KCl represents the effect of dilution on the brei supernatant. Storage of the brei supernatant in a deepfreeze did not affect the cation-induced pH changes, while this treatment resulted in an acceleration of the aggregation reaction. This suggests that some inhibitor of the aggregation step fails to survive freezing. Neither acid production nor aggregation reaction could be detected in control samples exposed to KCl under these conditions.

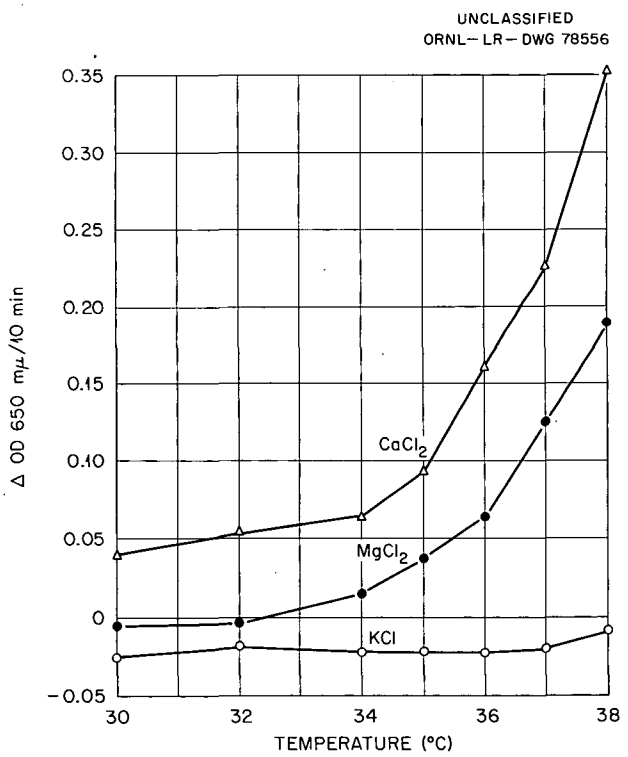

Fig. 36. The Effect of $CaCl_2$, $MgCl_2$, and KCl on Turbidity of Rat Liver Brei Supernatant (15,000 × *g*/30 min). Data expressed as Δ optical density at 650 mμ for the first 10 min of incubation.

Discussion. – These observations provide additional evidence in support of the validity of this model system, since in intact cells magnesium is far less potent than calcium in triggering the "surface precipitation" reaction.[21]

FURTHER DEVELOPMENT OF AN AUTOMATED CARBOHYDRATE ANALYZER

J. G. Green N. G. Anderson

Introduction. – The principles and basic mechanical components of an automated carbohydrate analyzer under development have been reported previously by the authors.[22] Several mechanical improvements, which have been incorporated into the analyzer, and the identity of the eluted peaks of a standard mixture of sugars are reported here.

Experimental. – Efforts to improve the resolving capability of the Dowex 1-X8 column and borate elution system have been expended in the areas of elution temperature, gradient concentration, and resin particle size effects. Increasing the

[22] J. G. Green and N. G. Anderson, *Biol. Div. Semi-ann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 128–30.

column temperature from 55°C to 72°C in a series of experiments resulted in little improvement in column resolution and adversely affected resin durability. In addition, at temperatures over 65°C, some sugars (notably fructose) were partially degraded during passage through the column. The chosen operating temperature of 55°C appears to afford optimal temperature-dependent resolution with minimal sample alteration and acceptable resin durability.

It was reported previously that gradients involving a tenfold to sixfold concentration increase of the borate elution system varied the position of the eluted peaks and yielded acceptable resolution. An optimal gradient involving a concentration increase of 3.4 times to a final molarity of 0.32 *M* borate at a total volume of 1 liter has proved to yield the best resolution in the present system.

The most promising increase in resolving power of the column resulted from the use of a 14- to 48-μ resin rather than the 48- to 72-μ resin employed in preliminary developmental studies. However, top-of-column pressures of 70 to 80 psi were encountered and proved to be the maximal tolerable pressure range for the standard columns and fittings employed. Subsequently, a column has been designed and fabricated to support a 0.45-cm-diam, 92-cm-long resin column. Although this column has not been proof-tested, it is expected to yield very high resolution at a top-of-column pressure not exceeding 200 psi with a through 400-mesh-grade resin. The improved resolution should permit the analysis of complex mixtures of sugars in a minimum time.

Several refinements have been incorporated into the colorimetric system described in the earlier report. Concentrated sulfuric acid is being dispensed by a Beckman model 746 solution-metering pump rather than the larger and heavier Lapp Pulsafeeder pump. The phenol and sulfuric acid mixing system is being redesigned. Air, the colorimetric reagent, and the column effluent are combined into a single stream in a glass manifold that permits air-purging of the entire system by means of suitable valving. The colorimetric reaction goes to completion in an air-moderated heat exchanger, which has replaced the insulated-coil system previously employed. The spectrophotometric monitoring system remains unchanged.

The elution sequence and volumes of the various sugars of the standard test mixture have been determined. For convenience, the first three letters of the name of each sugar are used to refer to the compound. Elution patterns of the

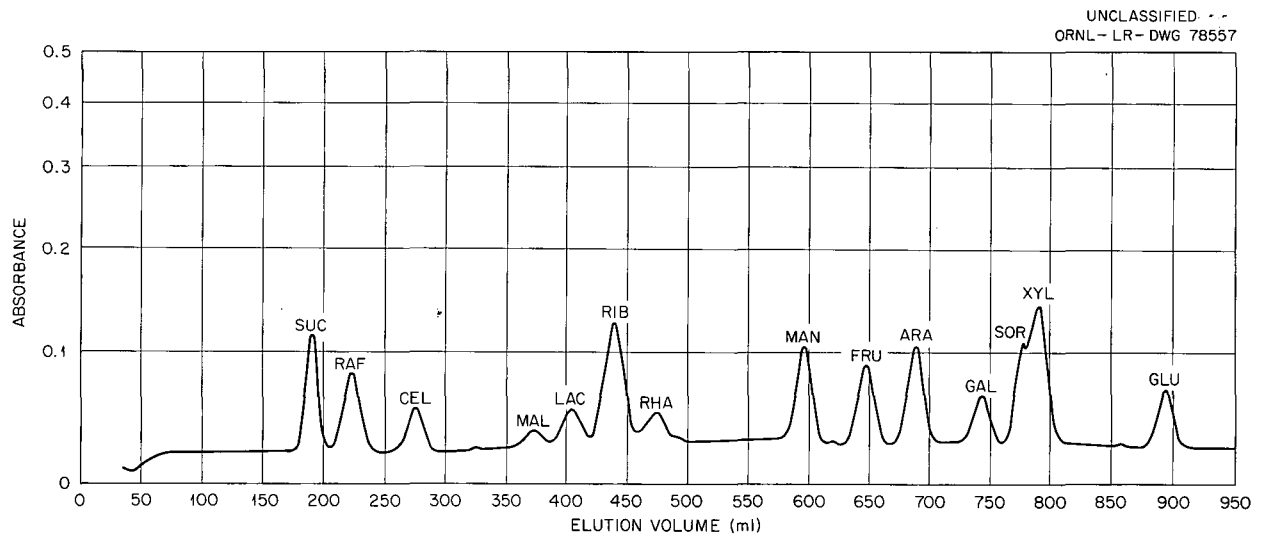

Fig. 37. Separation of a Standard Mixture on a Long Column. Mixture contains 150 μg of each of 14 sugars on Dowex 1-X8 in a column 0.9 × 150 cm.

standard mixture are depicted in Fig. 37 for a long-column analysis and in Fig. 38 for a medium-length-column analysis. Although resolution is inferior in the latter case, analytical time is reduced.

Discussion. – In its present form the analyzer is a serviceable instrument. Some design improvements, especially in the monitoring system, remain to be accomplished, and sample preparation techniques must be evaluated.

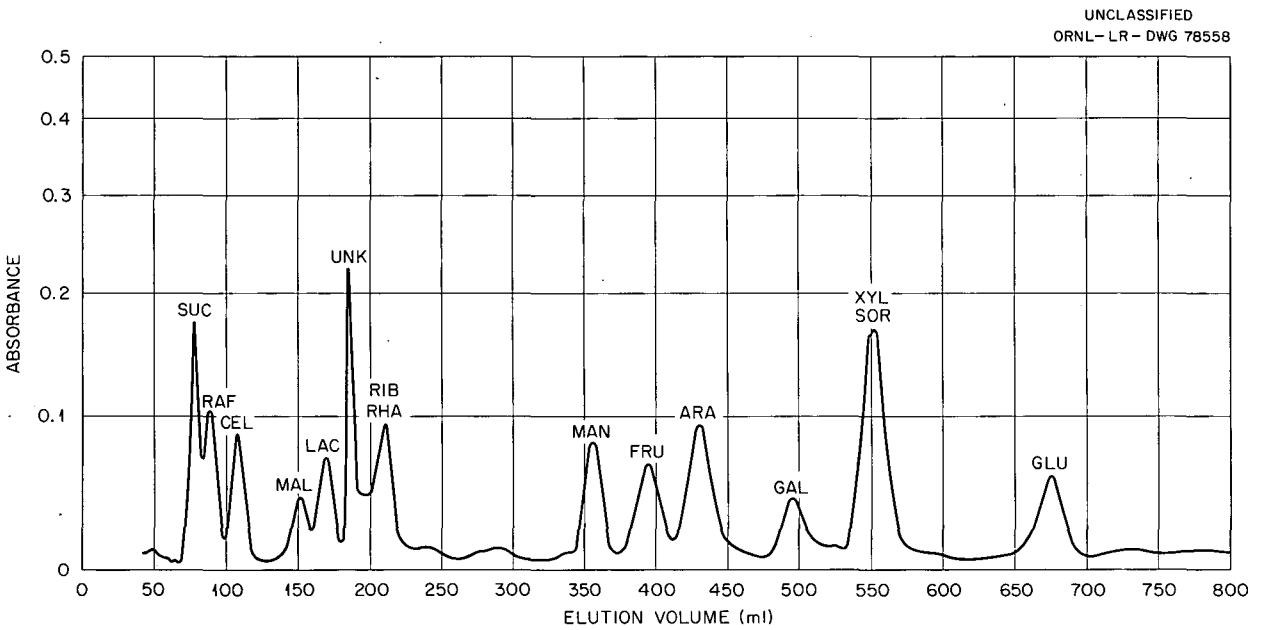

Fig. 38. Separation of a Standard Mixture on a Medium-Length Column. Mixture contains 150 μg of each of 14 sugars on Dowex 1-X8 in a column 0.9 × 60 cm.

Nucleic Acid Chemistry

Nucleotide Chemistry	Molecular Biology
W. E Cohn[a] A. Jeannine Bandy	F. J. Bollum G. E. Houts Amleto Castellani[b] Patricia A. Williams Masahiko Yoneda[b]
[a]Leave of absence (October 1962—September 1963). [b]Visiting investigator from abroad.	

PURIFICATION OF DNA POLYMERASE FROM CALF THYMUS GLAND

Masahiko Yoneda F. J. Bollum

A number of anion- and cation-exchange adsorbents were examined for their usefulness in the DNA-polymerase preparation, and only two were of exceptional interest, phosphocellulose (P-70) and diethylaminoethylcellulose (DEAE). P-70 has a high capacity for specific adsorption of DNA polymerase from the crude thymus extract. Thus 150 liters of crude extract from 30 kg of fresh thymus gland, clarified at pH 6.2 and readjusted to pH 6.7, may be pumped through a 10-liter P-70 column, and essentially quantitative adsorption of enzyme activity results. All the extract turbidity and 90% of the total protein pass through the column. The DNA polymerase is subsequently eluted in good yield at pH 7.0, passed through a 3-liter DEAE column for RNA removal, and then concentrated by $(NH_4)_2SO_4$ fractionation. The specific activity of the concentrated P-70 eluate is comparable to fractions obtained by a more tedious method,[1] and the DNase and diesterase contamination (appreciable in the P-70 fraction) may be reduced by chromatography on hydroxylapatite. A large-scale column step has, therefore, considerably simplified the early stages of enzyme purification, but the product obtained is not as free of DNase and diesterase as fraction E obtained by the older, more tedious route.

Chromatography of partially purified enzyme on DEAE columns has been carefully reexamined to see if a practical separation of end-addition activity and replication activity might be devised. While earlier results[2] on this separation are readily reproduced, activity recoveries are low, apparently as a result of aggregation and enzyme inactivation on the column. Thus a practical separation on this exchanger must await some insight into the problem of protein aggregation.

NATURE OF DNA POLYMERASE PRODUCTS

F. J. Bollum G. E. Houts
A. Jeannine Bandy

Calf thymus DNA polymerase has an absolute requirement for denatured DNA primer[3] and produces only a doubling of primer DNA. Removal of residual primer material is a major problem in examining the nature of the DNA-polymerase products. Earlier attempts to effect the primer-product separation on methylated albumin columns were not satisfactory

[1]F. J. Bollum, G. E. Houts, and Patricia Williams, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 134.

[2]E. Groeniger and F. J. Bollum, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 139.

[3]F. J. Bollum, *J. Biol. Chem.* **234**, 2733 (1959).

because the separation was not complete. The problem has now been solved through use of the aqueous polymer phase systems described by Albertsson.[4] A simple ten-stage countercurrent distribution experiment, which uses 7% Dextran : 4.4% polyethylene glycol : 0.01 *M* sodium phosphate, pH 6.8 demonstrates (Fig. 39) the quantitative conversion of denatured H^3-*Escherichia coli* (15-T⁻) primer DNA to a hybrid material (P^{32} coming from $dXTP^{32}$ substrate), which distributes like native DNA. The DNA-polymerase product may be recovered from the neutral polymer solutions by adsorption and elution from methylated albumin columns. The eluate is then centrifuged in cesium chloride density gradients to determine the physical nature and distribution of isotope in the hybrid product.

Examination of a product prepared from H^3-DNA primer and $dATP^{32}$, $dGTP^{32}$, $dTTP^{32}$, and dBrCTP substrates is presented in Fig. 40. As expected, the product is hybrid, containing P^{32}, H^3, and bands at ρ^{25} = 1.77 g/cc as expected for DNA which contains Br·C in one strand (normal *E. coli*

[4] P. Å. Albertsson, *Arch. Biochem. Biophys.* (suppl 1), 264 (1962).

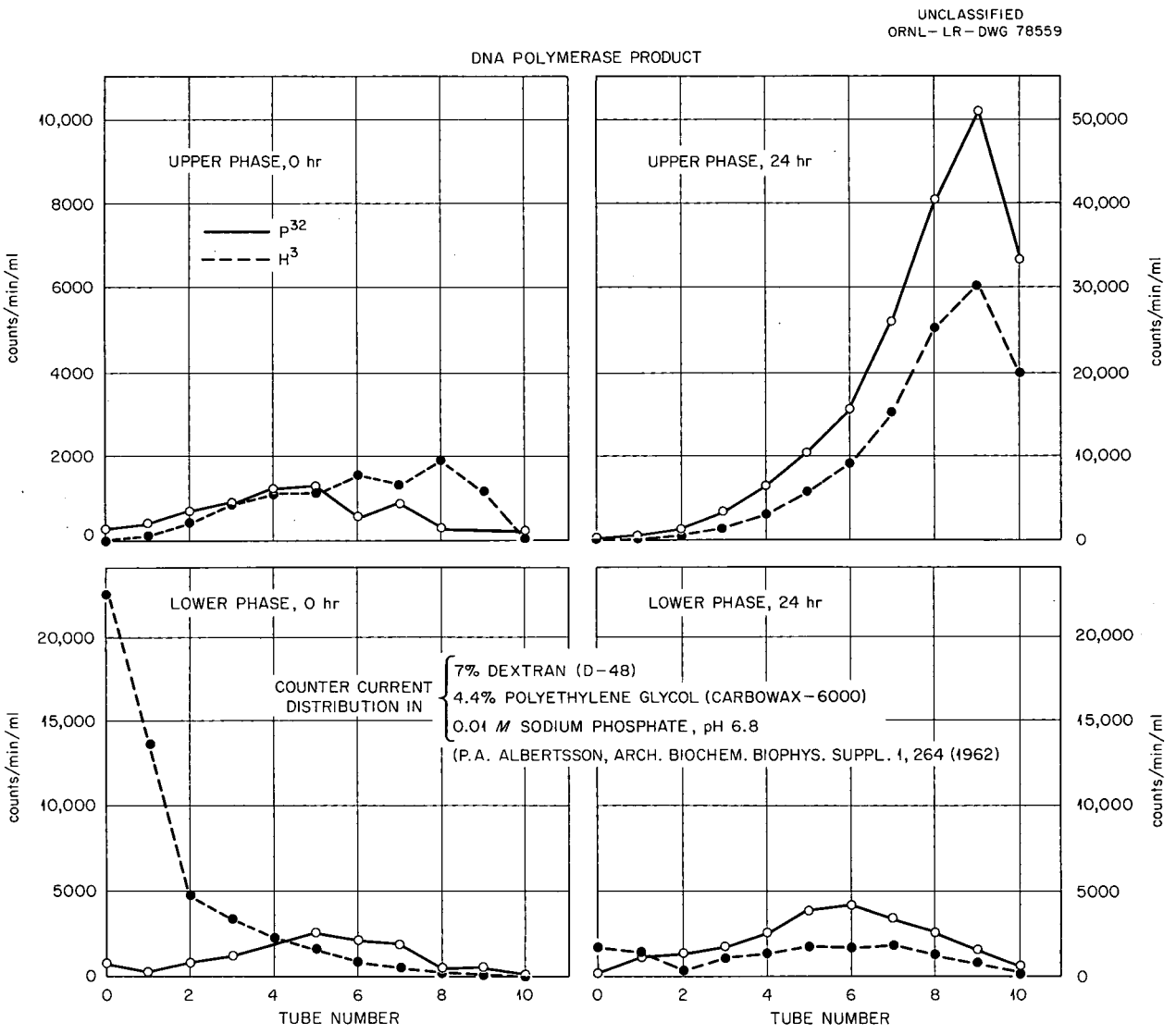

Fig. 39. Countercurrent Distribution of H^3-DNA Primer.

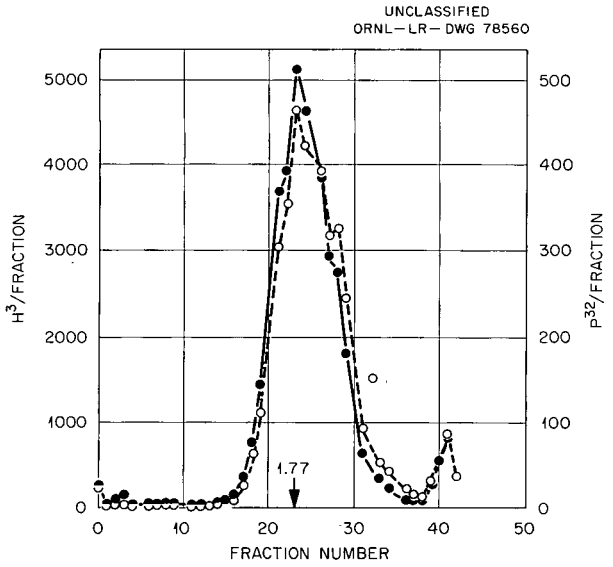

Fig. 40. Cesium Chloride Density Gradient of BrC-P^{32}-H^3 *E. coli* **DNA, Heat-Denatured Product.** Centrifuged 72 hr at 33,000 rpm. Bottom of centrifuge tube is fraction 1.

DNA bands at ρ^{25} = 1.71 g/cc). From the bandwidth it may be deduced that the hybrid product has not suffered molecular weight degradation. Heat denaturation of this DNA-polymerase product in salt solution or in the presence of formaldehyde does not, however, result in strand separation (that is, P^{32}-Br·C strand from H^3-primer strand) in the manner observed for "natural" DNA. In addition, the heat-denatured polymerase product does not distribute like denatured "natural" DNA in the aqueous polymer phase system; neither does it distribute like unheated "polymerase" DNA.

We are thus in a position to state that DNA replication in a purified enzyme system produces a product remarkably similar to native "natural" DNA – but not identical. This product undergoes a denaturation reaction similar to "natural" DNA, but strand separation does not occur. Other experiments[5] have demonstrated some biological activity in DNA-polymerase products, but this activity was not as high as anticipated from the total synthesis occurring.

Chemical, physical, and biological studies on the products of the enzymatic replication process catalyzed by calf thymus DNA polymerase have uncovered, therefore, two interesting aspects at the macromolecular level:

1. The necessity for a modification of native DNA before it can act as a primer for replication.[6]
2. A necessity for some modification of the polymerase product before it becomes physically identical to natural DNA and perhaps before it may act in information transfer.

Schematically,

$$\text{Native "natural" DNA} \longrightarrow \text{primer DNA}, \quad (1)$$

$$\text{Primer DNA} \xrightarrow[\text{dXTP, } Mg^{2+}]{\text{DNA polymerase}} \text{DNA-polymerase product}, \quad (2)$$

$$\text{DNA-polymerase product} \longrightarrow \text{native "natural" DNA}. \quad (3)$$

It is hoped that future experiments will clarify steps 1 and 3 in the complete replication scheme.

[5]F. J. Bollum and Jane K. Setlow, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 135.

PROTEIN DISTRIBUTION IN AQUEOUS POLYMER SYSTEMS

F. J. Bollum Patricia A. Williams

The aqueous polymer systems described by Åbertsson[6] provide a mild, general method of protein fractionation which can be translated easily

[6]P. Å. Albertsson, *Partition of Cell Particles and Macromolecules*, Wiley, New York, 1960.

Table 25. Distribution Constants in 7% Dextran : 4.4% PEG : 0.01 *M* Sodium Phosphate, pH 6.8

Protein	Molarity of Added NaCl			
	0	0.1	1.0	2.5
Human serum albumin	0.57	0.13	0.18	0.41
Human serum azo albumin	0.80	0.19	0.32	0.84
Bovine serum globulin	0.80	0.45	0.96	5.90
Liver catalase	0.45	0.12	0.74	14.10
Spleen ferritin	1.64	0.09	0.34	51.7
Human hemoglobin	0.21	0.24	0.69	2.38
Egg white lysozyme	0.59	1.56	3.09	10.22
Calf thymus histone	0.11	3.30	6.85	27.90

into countercurrent distribution equipment. Prior to a study of the distribution of calf thymus DNA polymerase in the system 7% Dextran : 4.4% polyethylene glycol (PEG), we extended the studies of Albertsson to a variety of commercially available pure proteins to see if any general trends might be recognized. The results are presented in Table 25. These data will be extended as more types of proteins are tested, but it is clear that basic proteins or complex proteins with a basic *apo* protein do not have a minimum in K_d at 0.1 *M* NaCl. This observation may be useful in fractionating basic proteins (e.g., DNase and RNase) from cell extracts.

Our first attempts to distribute DNA polymerase in this system resulted in precipitation of the DNA polymerase in the presence of the PEG component of the phase system. Thus the ready aggregation of the active protein again becomes a major problem. Other phase systems and conditions (e.g., high salt concentrations) will be tested in the hope that the aggregation reaction might be avoided.

Nucleic Acid Enzymology

Elliot Volkin	
Microbial Systems	**Developmental Biochemistry**
Elliot Volkin J. X. Khym J. T. Fairley[a] M. Helen Jones	F. J. Finamore C. G. Mead N. G. Sansing[b] Rose Feldman
[a]USPHS Fellow. [b]Research associate.	

BACTERIOPHAGE T2 DNA AND PROTEIN SYNTHESIS

Elliot Volkin J. L. Fairley
M. Helen Jones

Previous work[1] with the analogue, 5-fluorodeoxyuridine (FUdR), demonstrated that the processes of T2-specific RNA, protein, and DNA synthesis can be dissociated, and, when carried out in this order, viable phage produced. This system allows us to gain further insight into two major problems concerned with viral infection: the fate of parental phage DNA after infection, and the nature of the terminal processes in the assembly of viral protein and DNA into an infectious viral particle. Our investigations in these areas are still in the preliminary stages.

The results on the fate of parental DNA seem to substantiate Stent's[2] conclusions that a nonrandom fragmentation and distribution of the T2 DNA occurs upon infection. Studies are under way which are designed to define the biochemical nature of the DNA fragments.

The assembly of T2 protein probably involves the utilization of relatively small protein subunits. These can "mature" to some extent in the absence of T2 DNA synthesis, but having matured, are no longer available for the incorporation into viable particles. It is possible to accumulate such protein under conditions of FUdR inhibition, then remove the inhibitor and demonstrate the utilization of the synthesized protein. In this manner, the kinetics of formation of the protein and its rate of utilization can be estimated. Our results indicate that 15 to 20 min are required for a maximal production of usable T2 protein. The rate of utilization of such protein seems to be between 5 and 10 min.

[1]Elliot Volkin and A. Ruffilli, *Proc. Natl. Acad. Sci. U.S.* **48**, 2193 (1962).

[2]G. S. Stent, G. H. Sato, and N. K. Jerne, *J. Mol. Biol.* 1, 134 (1959).

CHEMICAL SYNTHESIS OF P^1 P^4 DIGUANOSINE 5′-TETRAPHOSPHATE LABELED WITH P^{32}

F. J. Finamore Rose Feldman

Introduction. – To study the fate of the symmetrical pyrophosphate ester, P^1 P^4 diguanosine 5′-tetraphosphate (Di-GDP), during the development

of the brine shrimp, *Artemia salina*, we attempted to incorporate P^{32} into the compound in the egg. *In vivo* experiments with P^{32} orthophosphate or P^{32} pyrophosphate as substrates failed to label the compound; furthermore *in vitro* experiments using egg extracts also failed to yield labeled Di-GDP.

We therefore tried to synthesize chemically Di-GDP^{32} by the use of P^{32} orthophosphate, GMP, and the dicyclohexylcarbodiimide reagent first described by Khorana and Todd.[3]

Results and Discussion. – Initial experiments revealed that Di-GDP^{32} could be prepared in low yields starting with GMP, H_3PO_4, P^{32} orthophosphate, and dicyclohexylcarbodiimide in a nonaqueous solvent system. In addition to Di-GDP^{32}, larger quantities of GDP^{32} and GTP^{32} were formed as well as labeled inorganic pyrophosphates of various chain lengths. We observed that over 90% of the labeled inorganic pyrophosphates could be converted to inorganic phosphate by treatment of the Di-GDP preparation with bacterial alkaline phosphatase (Worthington) for 3 hr. To date, Di-GDP^{32} has been obtained in yields of 5 to 8% starting with GMP.

To prove that Di-GDP^{32} was synthesized by the dicyclohexylcarbodiimide procedure, the labeled preparation, after treatment with alkaline phosphatase, was mixed with unlabeled Di-GDP prepared from the eggs, and the mixture was separated by column chromatography. It was found that Di-GDP was indeed labeled and that the radioactivity followed the ultraviolet absorbancy through the peak. To substantiate further the synthesis of Di-GDP^{32}, the compound was subjected to the action of snake venom phosphodiesterase for 5 min, and the resulting equimolar quantities of GMP and GTP were separated by column chromatography. As expected, GMP was unlabeled, whereas GTP contained all the radioactivity.

These results indicate that one of two mechanisms is operating in the chemical synthesis of Di-GDP starting with GMP:

$$\begin{aligned} &GMP + P^{32} \rightarrow GRPP^{32}\ (GDP^{32}), \\ &GRPP^{32} + GRPP^{32} \rightarrow \\ &\qquad GRPP^{32}P^{32}PRG\ (\text{Di-}GDP^{32}), \end{aligned} \tag{1}$$

$$\begin{aligned} &GMP + P^{32} \rightarrow GRPP^{32}\ (GDP^{32}), \\ &GRPP^{32} + P^{32} \rightarrow GRPP^{32}P^{32}\ (GTP^{32}), \\ &GRPP^{32}P^{32} + GMP \rightarrow \\ &\qquad GRPP^{32}P^{32}PRG\ (\text{Di-}GDP^{32}). \end{aligned} \tag{2}$$

Both mechanisms yield a product labeled in exactly the same positions, and on hydrolysis with snake venom phosphodiesterase, the Di-GDP^{32} formed would yield GTP^{32} but unlabeled GMP.

Currently we are attempting to improve the yield of Di-GDP^{32} by systematically varying the concentration of reagents and the reaction times.

[3]H. G. Khorana and A. R. Todd, *J. Am. Chem. Soc.* **75**, 2257 (1953).

USE OF LIQUID ANION EXCHANGE FOR THE EXTRACTION OF NUCLEIC ACID DERIVATIVES FROM AQUEOUS SOLUTIONS

J. X. Khym

The technique of liquid anion exchange[4] is being further developed as a means for the investigations of nucleic acids and associated lower-molecular-weight components. The mechanism of extraction with this technique is not completely understood. However, the analogy with conventional ion exchange involving sorptions by alkylammonium groups in solid anion exchangers is very striking. For instance, different salt forms of an amine show particular affinities in their extractability for a given compound and, as the charge in a particular class of compounds is increased, correspondingly higher extraction coefficients are obtained.[5] In an extraction sequence, a series of decreasing extraction coefficients can be obtained when the aqueous salt concentration is increased.[5] Also, conversion of an amine salt to free amine immediately releases extracted compounds and prevents the amine from extracting any additional material.[5]

All the foregoing observations fit qualitatively an equation which can be derived for the liquid ion exchange reaction:

[4]J. X. Khym, Katherine H. Stephenson, and Elliot Volkin, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1962*, ORNL-3267, p 120; *Aug. 15, 1962*, ORNL-3352, p 145.

[5]J. X. Khym, *Biochemistry*, in press.

$$a\text{R–NH}_3F(org) + X^{-a}(aq) \rightleftharpoons (\text{R–NH}_3)_a\, X(org) + aF^-(aq)\ ,$$

$$K = \frac{[(\text{R–NH}_3)_a X]\,[F^-]^a}{[X^{-a}]\,[\text{RNH}_3F]^a},$$

$$E_A^O = \frac{C_x \text{ in org}}{C_x \text{ in aq}} = \frac{[(\text{R–NH}_3)_a X](org)}{[X^{-a}](aq)}\ ,$$

$$E_A^O = \frac{K(\text{R–NH}_3F)^a}{(F^-)^a}\ .$$

Thus, if F is equal to formate and X is equal to a substance such as UTP (where C_x = concentration of UTP in a given phase), then at a given formate concentration, the E_A^O for UTP would be expected to be proportional to some power of the concentration of free amine salt used as the extractant. At a given amine concentration the E_A^O should vary inversely to the formate concentration. At pH 3.5, UTP would be expected to have a negative charge of at least 3, and, therefore, the extraction coefficient should vary as the cube of the free amine salt concentration. However, the extraction coefficient of UTP and ATP varies directly to the first power of the amine salt concentration.[5] Also, as shown in Fig. 41, a limiting association number of about 7 moles of amine to 1 mole of triphosphate was determined from extraction isotherms. Thus, a simplified interpretation of liquid ion exchange does not adequately explain the data, but the reactions considered as liquid ion exchange have been useful in designing and correlating the various extraction experiments.

With a given amine-solvent system, inherent differences in extraction coefficients are readily obtained on the lower-molecular-weight phosphate compounds, so that a given pair of compounds, for example, ADP-ATP or $(pA)_2$-$(pA)_5$, can be separated easily with a few batch extractions.[5] For other pairs or groups of compounds with separation factors of much lower magnitude (<50), countercurrent distribution would be necessary to effect practical separations.

Another possibility of extending the usefulness of this technique would be to fix the amine salt–solvent system in an inert carrier in accordance with the principles of partition chromatography. Experiments are in progress to test the feasibility of this approach.

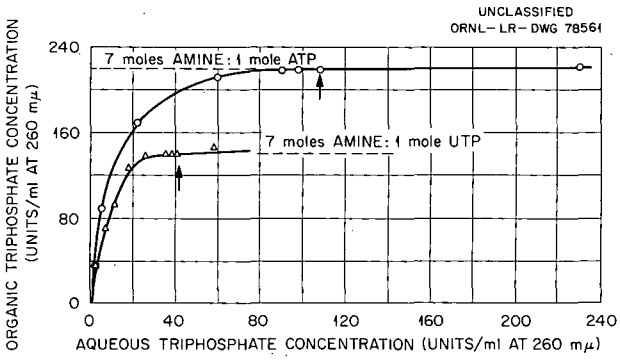

Fig. 41. Nucleoside Triphosphate Isotherms. Cascade extractions (organic phase contacting successive volumes of aqueous phase) at phase ratio 1:1 and contact time of 10 min/stage by wrist-action shaker. O, ATP in 0.5 *M* formate buffer pH 3.5 vs 0.1 *M* 1-(3-ethylpentyl)-4-ethyloctyl amine formate in butyl ether; △, UTP in 0.25 *M* formate buffer pH 3.5 vs 0.1 *M* 3-ethyl-1-isobutyloctyl amine formate in butyl ether. Arrows indicate stage at which aqueous phase concentration of nucleoside triphosphates equals initial concentration. Additional points after arrows are the concentrations of a different solution of triphosphates in which the aqueous phase far exceeded initial stock concentrations.

HETEROCHROMATIC DNA

C. G. Mead

Heterochromatic chromosomes, by definition, have cytological and genetic properties distinct from euchromatic chromosomes. The DNA of the heterochromatic Y chromosome of *Drosophila melanogaster* has been examined indirectly for gross differences in nucleotide composition, molecular weight, and secondary structure as compared with the DNA of the combined X chromosome and autosomes. The DNA was isolated from adults by the Aerosol procedure from (*a*) a mixture of males (XY) uniformly labeled with P^{32} and a fivefold excess of unlabeled females (XX) and (*b*) a mixture of females (XX) uniformly labeled with P^{32} and a fivefold excess of unlabeled males (XY). The DNA was first examined by chromatography on methylated albumin columns, which separate DNA molecules according to nucleotide composition and size. The elution patterns observed are illustrated in Fig. 42. Any fraction with a relatively high specific activity in (*a*) or low specific activity in (*b*) could be

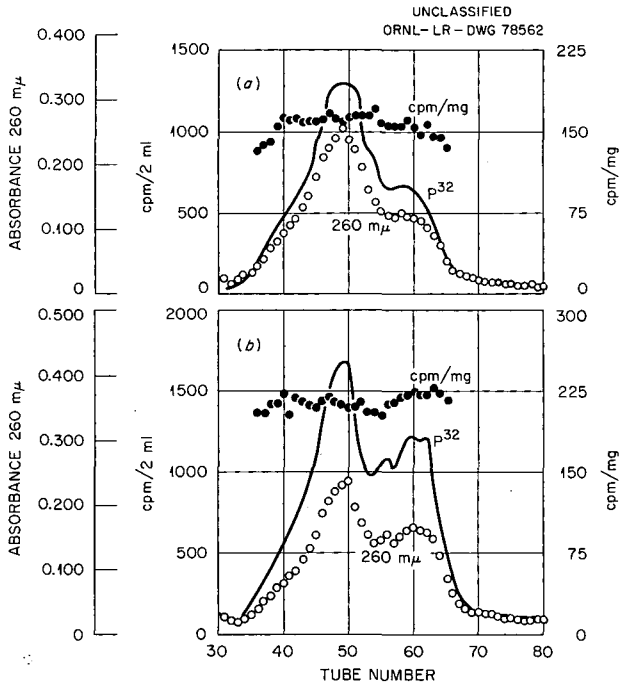

Fig. 42. Chromatography of Drosophila DNA on Methylated Albumin Columns. *a*, XY-P^{32} + XX-P^{31}; *b*, XX-P^{32} + XY-P^{31}.

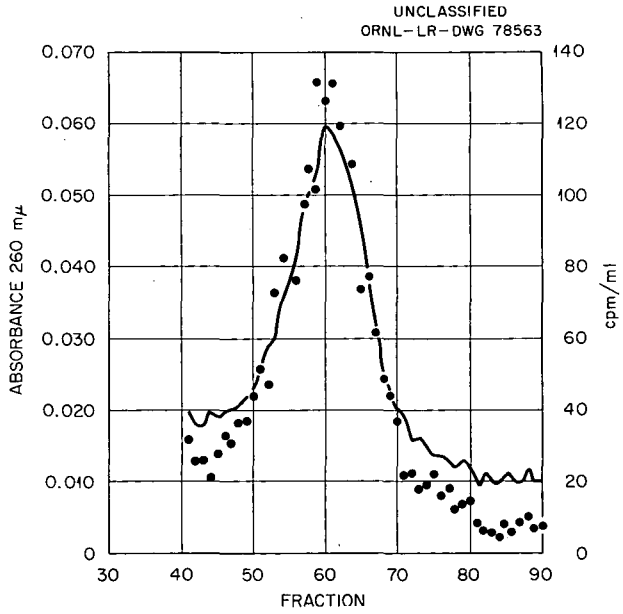

Fig. 43. Cesium Chloride Density Gradient Centrifugation of *Drosophila* **DNA (XY-P^{32} + XX-P^{31}).**

interpreted as a fraction enriched in Y-chromosomal DNA. The observed specific activities through the eluted peaks in both experiments do not differ significantly.

Preparation (*a*) was examined by cesium chloride density gradient centrifugation (Fig. 43). Separation of DNA molecules by this method is dependent on differences in nucleotide composition and secondary structure. The observed specific activities through the DNA peak were found to be constant.

It is concluded that the DNA of the heterochromatic Y chromosome does not contain any particular species of molecules that are markedly different from the DNA of the combined X chromosome and autosomes in nucleotide composition, molecular weight, or secondary structure.

Chemical Protection and Enzyme Catalysis

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CHEMICAL PROTECTION

D. G. Doherty J. D. Bales, Jr.
Martha A. Turner

Introduction. – Investigation in the chemical protection field has been divided between a search for more useful, active, and less-toxic protective compounds and a further examination of the effects of treatment prior to radiation with mixtures of glutathione and other protective agents.[1]

Results and Discussion. – Forty representatives of various classes of compounds have been tested intraperitoneally for protective activity in $BC3F_1$ mice (LD_{50} within 30 days, 720 r) by the sequential procedure with a lethal (900 r) radiation dose. Various gallic acid derivatives were completely ineffective, as were the sulfones, sulfoxides, and sulfides related to mercaptoethylamine and mercaptoethylguanidine. The seleno compounds[2] corresponding to cysteamine and aminoethylisothiourea (AET), although very toxic, were moderately protective at their maximum tolerated dose (6 and 16 mg/kg respectively). In the heterocyclic series, 2,4-diaminothiazole (600 mg/kg) gave complete 30-day survival, while the D-, L-, and DL-5-ethyl-2-aminothiazolines provided 70 to 90% survival at 30 mg/kg. Less than 50% survival was obtained with *s*-triazole and 3-mercapto-*s*-triazole, while the thiadiazoles[3] were completely ineffective. Several isothioureas related to AET that yielded complete 30-day survival at 900 r were examined for maximum protective activity. The values for LD_{50} within 30 days and the relative dose reduction factors (RDF) were as follows: AET, 360 mg/kg, 1500 r, RDF, 2.1; DL-2-aminopropylisothiourea, 190 mg/kg, 1275 r, RDF, 1.77; D-2-aminobutylisothiourea, 200 mg/kg, 1400 r, RDF, 1.95, 120 mg/kg, 1250 r, RDF, 1.74; DL-3-aminobutylisothiourea, 160 mg/kg, 1150 r, RDF, 1.6; and 1-(3,4,6,-tri-*O*-acetyl)2-deoxy-β-D-glucopyranosylisothiourea,[4] 480 mg/kg, 1200 r, RDF, 1.67. For purposes of comparison with regard to structure, the corresponding values were obtained for three compounds reported elsewhere to be protective: 2-aminoethylthiosulfuric acid, 320 mg/kg, 975 r, RDF, 1.35; 3-aminopropylthiosulfuric acid, 320 mg/kg, 825 r, RDF, 1.14; and dimethylsulfoxide, 4.6 g/kg, 925 r, DRF, 1.28. A new derivative of mercaptoethylamine-sodium, *S*-β-*N*-methylaminoethyl hydrogen phosphorothiolate·$4H_2O$ (ref 5), shows some promise of being a useful protective agent. The maximum tolerated dose to mice is 1 g/kg; at 300 mg/kg, the LD_{50} within 30 days is 1050 r, RDF, 1.46, while at 600 mg/kg the values are 1350 r, RDF, 1.88.

[1]D. G. Doherty, J. D. Bales, Jr., and C. M. Park, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 148.

[2]Supplied by H. Mautner, Yale University.

[3]Supplied by K. Potts, University of Louisville, Ky.

[4]Supplied by M. Wolfrom, Ohio State University.

[5]Supplied by Schwarz BioResearch, Orangeburg, N.Y.

Mixtures of mercaptoethylamine (MEA) and glutathione (GSH) were examined for protective activity like AET and glutathione,[1] and the following results obtained: 120 mg/kg MEA–1.6 g/kg GSH, LD_{50} within 30 days, 1550 r, RDF, 2.15; 160 mg/kg MEA–1.6 g/kg GSH, 1650 r, RDF, 2.15; and 200 mg/kg MEA–1.6 g/kg GSH, 1700 r, RDF, 2.25. Mixtures with oxidized glutathione were less effective than MEA alone although the toxicity of MEA was reduced, as is the case with AET.

ENZYME CATALYSIS

D. G. Doherty Martha A. Turner

The investigation into the specificity requirements of pepsin[1] has been extended to additional substrates. The kinetic constants for two model substrates with aromatic residues on both sides of the sensitive peptide bond were determined and found to be: acetyl-L-phenylalanyl-L-phenylalanine, $K_m = 1.05 \times 10^{-3}$, $k_3 = 1.88 \times 10^{-3}$; acetyl-L-phenylalanyl-L-tyrosine, $K_m = 6.0 \times 10^{-3}$, $k_3 = 20.0 \times 10^{-3}$. Although the K_m of the latter substrate is in reasonable agreement with that obtained by Baker,[6] the k_3 value is tenfold greater. Acetyl-L-phenylalanylphenylethylamide and carbobenzoxy-L-glutamylphenylethylamide were not split by pepsin, indicating the need for at least a carbonyl group on the distal side of the bond to be hydrolyzed.

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[6] L. E. Baker, *J. Biol. Chem.* **211**, 701 (1954).

Enzymology

G. D. Novelli

Protein Synthesis

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SOLUBLE AMINO ACID INCORPORATING SYSTEM ISOLATED FROM *ESCHERICHIA COLI*

Akira Kaji Hideko Kaji
G. D. Novelli

Introduction. – A partially purified *Escherichia coli* RNA-polymerase fraction isolated from a French pressure cell extract of *E. coli* essentially according to the methods of Chamberlin and Berg prior to the column step[1] was found to incorporate C^{14}-leucine *by itself* into a hot trichloroacetic acid (TCA) insoluble fraction. Since this fraction was isolated from the 105,000 *g* supernatant fluid, the characteristics of this system were studied.

Results and Discussion. – The following observations indicate that this system is different from the ordinary particle-supernatant system for amino acid incorporation.

1. The majority of the leucine-incorporating activity was found in the supernatant fluid after centrifugation at 105,000 *g* for 2 hr. Combination of the pellet and supernatant did not cause stimulation.

2. The only ribosome-like material found in this fraction was a trace of 30 to 35 S component.

[1]M. Chamberlin and P. Berg, *Proc. Natl. Acad. Sci. U.S.* **48**, 81 (1962).

3. Sucrose density gradient centrifugation showed that the incorporating activity was associated with the supernatant only, indicating that the trace of 30 to 35 S particle plays no role in the incorporating activity. A small amount of pellet obtained after the centrifugation did not stimulate the incorporation by the supernatant.

4. All the radioactive, hot TCA-insoluble fraction synthesized by this system was found in the supernatant.

The biochemical characteristics of this system are: (1) the incorporation is dependent on S-RNA and the ATP-generating system; (2) pancreatic RNase and puromycin inhibit the incorporation; and (3) the incorporated C^{14} is made acid-soluble by trypsin, chymotrypsin, or papain, but carboxypeptidase does not release more than 20% of the incorporated radioactivity. These observations suggest that the ribosome-free fraction of *E. coli* extract can incorporate amino acid into the hot TCA-insoluble fraction, and that this system could be used as a model for studying the mechanism of protein synthesis.

CELL-FREE BIOSYNTHESIS OF THIOGALACTOSIDE TRANSACETYLASE

Hideko Kaji Akira Kaji
G. D. Novelli

Introduction. – Zabin *et al.*[2] reported that thiogalactoside transacetylase may be related to the permease. Despite some suggested evidence for this hypothesis, there are several difficulties involved in assuming this enzyme to be permease. Nevertheless, the finding of this cell-free synthesizing system will be interesting since genetic information for the formation of this enzyme is carried in the "lactose" region of the bacterial chromosome which controls β-galactosidase formation.

Results and Discussion. – Fractions isolated by ammonium sulfate from *Escherichia coli* extract according to Zabin *et al.*[3] showed thiogalactoside transacetylase activity. This partially purified fraction was incubated with C^{14}-acetyl CoA and was chromatographed on DEAE paper. One-third of the radioactivity remained at the origin, indicating that C^{14}-acetyl thiogalactoside was formed in the presence of isopropyl β-D-thiogalactoside (IPTG). When methyl β-D-thiogalactoside (TMG) or IPTG-induced particle and supernatant fluid were incubated in the presence of IPTG, a 10 to 30% increase in thiogalactoside transacetylase activity was noted. Increasing the concentration of particle resulted in a decrease of enzyme synthesis when TMG-induced particle and supernatant fluid were used. When TMG was used instead of IPTG as a reaction component for the particle-supernatant incubation mixture, no appreciable amount of the enzyme was formed. Further studies on biochemical characteristics of this system are in progress.

[2] I. Zabin, A. Kepes, and J. Monod, *Biochem. Biophys. Res. Commun.* **1**, 289 (1959).

[3] I. Zabin, A. Kepes, and J. Monod, *J. Biol. Chem.* **237**, 253 (1962).

RESISTANCE OF *ESCHERICHIA COLI* S-RNA TO RIBONUCLEASES IN THE PRESENCE OF MAGNESIUM ION

Susumu Nishimura G. D. Novelli

Introduction. – It is well known that bovine pancreatic RNase and RNase from *Bacillus subtilis* are not markedly inhibited by divalent cations such as magnesium ion when enzymatic activity is determined by measuring an increase in acid-soluble material by the use of high-molecular-weight RNA as a substrate.[4,5] The present report deals with a marked resistance of *Escherichia coli* S-RNA to hydrolysis by RNase, coupled with a striking shift of the melting curve to higher temperatures when S-RNA is mixed with magnesium ion.

Results and Discussion. – S-RNA was prepared from *E. coli* B stationary cells by the procedure of Berg and Ofengand[6] except that a DEAE-cellulose column was used instead of an ECTEOLA-cellulose column. Yeast RNA (insoluble in 1 *M* NaCl) was prepared as described by Crestfield *et al.*[7] Enzymatic digestion of S-RNA by both RNases was markedly inhibited by 0.01 *M* Mg^{2+},

[4] S. R. Dickmann, J. P. Aroskar, and R. B. Kropf, *Biochim. Biophys. Acta* **21**, 539 (1956).

[5] Susumu Nishimura, *Biochim. Biophys. Acta* **45**, 15 (1960).

[6] E. J. Ofengand, M. Dieckmann, and P. Berg, *J. Biol. Chem.* **236**, 1741 (1961).

[7] A. M. Crestfield, K. C. Smith, and F. W. Allen, *J. Biol. Chem.* **216**, 185 (1955).

whereas only 50% inhibition was observed with high-molecular-weight yeast RNA as substrate. Other divalent cations, such as manganese and calcium, gave similar results. When loss of amino acid acceptor function of S-RNA is taken as a measure of hydrolysis, the inhibition by Mg^{2+} is more striking, as previously described.[8] About 100 times as much *B. subtilis* RNase was required to obtain the same loss of leucine acceptor activity when Mg^{2+} was present. The same results were obtained with RNase T1 from Takadiastase (Samkyo Co., Ltd.) which produces only 3′-guanylic end products. However, with bovine pancreatic RNase, magnesium failed to protect the leucine acceptor activity. It seems likely that the terminal nucleotide chain, the sequence

adenylyl 5′ ⟶ 3′ cytidylyl 5′ ⟶ 3′ cytidylyl 5′,

at the amino acid acceptor end is not hydrogen bonded and easily hydrolyzed by pancreatic RNase, which produces 3′ pyrimidine nucleotide end products. The *B. subtilis* RNase, on the other hand, produces mainly 3′ purine nucleotide end products and thus cannot attach the terminal nucleotide sequence.

Since the inhibition of hydrolysis by the two RNases was most pronounced with S-RNA as substrate in the presence of Mg^{2+}, a possible explanation could be that Mg^{2+} causes a change in the physical configuration of S-RNA. This concept led to the finding of a striking shift of the thermal denaturation curve of S-RNA to higher temperatures when heated in the presence of 0.01 *M* magnesium. ΔTm was about 17°C. It is of interest to note that the melting curve obtained in the presence of Mg^{2+} is not parallel to the original curve like that obtained by increasing salt concentration.[9] A shift in the melting curve by Mg^{2+} was also observed in the case of high-molecular-weight RNA, but in this use the new curve was parallel to the original curve. It is possible that Mg^{2+} may affect the physical state of S-RNA, causing it to assume a more rigid secondary structure and thereby rendering it more resistant to attack by RNases.

[8]Susumu Nishimura and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 153.

[9]A. Tissières, *J. Mol. Biol.* 1, 365 (1959).

We have also studied the loss of the amino acid acceptor function of S-RNA with other amino acids such as arginine, aspartic acid, glutamic acid, serine, threonine, and tryptophan with *B. subtilis* RNase. Even in the presence of Mg^{2+}, S-RNA loses its acceptor function for these amino acids more readily than for leucine, which shows that resistance in the presence of Mg^{2+} varies with different amino acids. Resistant leucine S-RNA molecules behave as if they have a decreased content of guanine on gel-filtration and chromatography on methylated albumin. This suggests that a portion of the nucleotide chain in the leucine S-RNA molecule that is essential for its amino acid acceptor function is more resistant to *B. subtilis* RNase than some nonessential part of the chain.

CELL-FREE AMINO ACID INCORPORATION BY EXTRACTS OF *SACCHAROMYCES FRAGILIS*

A. K. Williams G. D. Novelli

Introduction. – A cell-free system consisting of particles sedimenting at 105,000 *g* and a supernatant fraction obtained from *Saccharomyces fragilis* was found to be capable of incorporating radioactive leucine into acid-insoluble protein. In an effort to increase the incorporating activity of this system, various methods of preparing the microsomal and supernatant components were investigated. Since, in several amino acid incorporating systems, the addition of S-RNA results in a marked increase in incorporation, the effect of this material was also tested.

Results and Discussion. – When *S. fragilis* was grown in a medium consisting of inorganic salts, yeast extract, and glucose, the cells were found to be extremely resistant to rupture in the French pressure cell. For this reason, a procedure which consisted of stirring the cells with glass beads in a Waring blender was employed. It was found that this treatment resulted in preparations with rather low activity. However, when the cells were grown in a medium which contained peptone, treatment in the French cell at 10,000 psi pressure resulted in a high degree of cell rupture. Microsomal and supernatant fractions isolated from these cells had about three times the incorporating activity of the glass-bead-treated cell preparations.

The incorporating system from yeast was found to be stimulated by the addition of S-RNA to the

reaction. S-RNA at a concentration of 1.0 mg/ml resulted in twice as much isotope incorporation as reactions containing no added S-RNA. At concentrations above 1.0 mg/ml, S-RNA strongly inhibited the incorporation reaction. By utilizing these more active preparations, we are now attempting to characterize more definitively the amino acid incorporating system from *S. fragilis*.

PREPARATION OF A DNA-DEPENDENT RNA POLYMERASE FROM *ESCHERICHIA COLI*

L. Rosen A. Marie Manning
G. D. Novelli

Introduction. – As mentioned in a previous report,[10] the limiting factor in many of our cell-free protein synthesizing systems is the RNA polymerase that specifically copies genetic information from DNA that can be used to synthesize specific proteins. Thus, the present investigation is concerned with working out a good procedure for the isolation and purification of this enzyme that can be applied on a large scale in our new biochemical processing area.

Results and Discussion. – The assay for RNA polymerase is based on the DNA-dependent synthesis of RNA from the ribonucleoside triphosphates and is most conveniently followed by determining the conversion of one of the ribonucleoside triphosphates to acid-insoluble material. This determination is most readily made if the triphosphate is labeled with a radioactive isotope which will permit the counting of millimicromoles of acid-precipitable material. For this purpose, it is desirable to have available a triphosphate precursor with relatively high specific radioactivity. Although $8'$-C^{14}-ATP is commercially available, it is often desirable to have a nucleoside triphosphate labeled in the $5'$ ester phosphate with P^{32}. We have thus adopted the synthesis of AMP^{32} from P_i^{32} and the isopropylidine derivative of adenosine according to Weiss.[11] The phosphorylated derivative is separated from orthophosphate by adsorption and elution from charcoal. The AMP^{32} is converted to ATP with a kinase system from rat liver, and the ATP is purified by column chromatography. This procedure gives us about a 40% yield of P^{32}-ATP.

We have investigated a large number of procedures to attempt to remove ribosomes without the use of a Spinco centrifugation. These procedures have been uniformly unsuccessful. Several improvements in the procedure have been made since the last report.[10] We have obtained a new supply of protamine sulfate that seems to work much better than previous samples, and the protamine step is now a good one to use in the purification. We have also found that extracts prepared with a Biosonic 20 kc oscillator will fractionate the same as French cell extracts with about the same yield of enzyme. This procedure gives us a large time-saving step, since we can now prepare 2 liters of extract in 1 hr. By use of the French cell, this would have taken 4 to 6 hr. By the use of a combination of the procedures described by Hurwitz[12] and Berg[13] and their collaborators together with some of our own modifications, we now have a method that will give us about a fortyfold purification with a 50% yield. We are currently stockpiling enzyme at this point before going on to work out other steps.

PURIFICATION AND CHARACTERIZATION OF THE pH 7.5 ESTERASE FROM MAIZE

Kathrine H. McGrath G. D. Novelli

Introduction. – In previous studies, Schwartz and McGrath[14] have demonstrated that the *E* gene in maize controls the synthesis of the pH 7.5 esterase. Three alleles of this gene, E^F, E^N, and E^S have been described[15] that specify isoenzymes with different electrophoretic migration rates. In addition, mutant forms of the E^F and E^S alleles, designated $E^{F'}$ and $E^{S'}$, have been recognized.[16]

[10] E. Glassman *et al.*, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 157.

[11] S. Weiss, *Proc. Natl. Acad. Sci. U.S.* **46**, 1022 (1960).

[12] J. J. Furth, J. Hurwitz, and M. Anders, *J. Biol. Chem.* **237**, 2611 (1962).

[13] M. Chamberlin and P. Berg, *Proc. Natl. Acad. Sci. U.S.* **48**, 81 (1962).

[14] D. Schwartz and Kathrine H. McGrath, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 43.

[15] D. Schwartz, *Proc. Natl. Acad. Sci. U.S.* **46**, 1210 (1960).

[16] D. Schwartz and Kathrine H. McGrath, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 38.

These mutant genes appear to be turned off early in the development of the endosperm.

The purpose of the present investigation is to work out a procedure for the isolation and purification of the esterase product of these alleles. When purified enzyme is available, chemical structural studies will be undertaken to determine which factor(s) may be determining the differences in electrophoretic mobility of the different esterases. We also intend to prepare specific antisera to the various isozymes to obtain a reagent to be used for measurement of the cell-free synthesis of the enzyme in the maize incorporating system we have developed.[17] The ultimate aim of this investigation is to study the biosynthesis of the esterase isozymes in a cell-free system to obtain information concerning at which point the genetic control mechanisms involved in the regulation of their synthesis and activity affect the system. The main prerequisite to embarking on such a program is to be able to isolate and purify these enzymes from various types of maize tissue. Thus the initial stages of this investigation are mainly concerned with purification studies.

Results and Discussion. – One of the immediate problems concerns the fact that maize tissues contain a number of proteins that exhibit esterase activity. The pH 7.5 esterase can only be distinguished from the others by electrophoresis in starch gels, but this is not a quantitative assay. Accordingly, a considerable effort has been expended in working out the variables in the quantitative assay for esterase. A preliminary fractionation procedure has been developed. This consists of an acid precipitation followed by an ammonium sulfate fractionation. After dialysis, the material is applied to a cellulose acetate column that is first washed with a dilute buffer, then with water. The esterase is recovered by a gradient elution with phosphate buffer at pH 8.0. This procedure, applied to germinating seedlings from 50 lb of maize kernels, gives a thirty- to fiftyfold purification. What is more important, however, is that the procedure gets rid of all the other esterases, thus allowing us to use the quantitative assay. We now intend to stockpile the esterase prior to working out other purification procedures.

[17] R. J. Mans and G. D. Novelli, *Biochim. Biophys. Acta* **50**, 287 (1961).

STUDIES ON THE MOUSE LIVER AMINO ACID INCORPORATING SYSTEM

Piero Cammarano G. D. Novelli

Introduction. – The mouse liver amino acid incorporating system previously described by Lea and Novelli[18] has been reinvestigated as a preliminary step to the study of the same system in mice with liver hepatomas. The object of the study is to determine whether any profound changes occur in any of the components of the liver incorporating system as the mouse develops hepatoma. Several changes in the procedure for the preparation of the cell-free system have been introduced that have resulted in a marked increase in incorporating activity.

Results and Discussion. – When ribosomes are prepared directly from a 25,000 *g* supernatant of mouse liver homogenate by adding deoxycholate (DOC) at this point rather than through the conventional procedure of prior isolation of microsomes and subsequent treatment with DOC, they are 4 to 10 times more active in incorporating C^{14}-leucine into protein. A comparative study of the two types of ribosomes has been made. Ribosomes prepared by the conventional procedure were inert to the addition of S-RNA from *Escherichia coli* or from rat liver. Likewise, they failed to respond to RNA isolated from mouse liver ribosomes. On the other hand, ribosomes prepared directly from the 25,000 *g* supernatant were inhibited by *E. coli* S-RNA and stimulated by rat liver S-RNA and RNA isolated from mouse liver ribosomes. A reason for these differences is not yet apparent, but preliminary examination of the ribosomes in the analytical ultracentrifuge revealed that conventional ribosomes gave two peaks with different sedimentation constants, whereas those prepared directly from the 25,000 *g* supernatant gave three such peaks. Attempts are being made to determine whether the additional peak is responsible for the increased activity.

During this investigation, we had occasion to determine the ability of the preparations to activate amino acids through the formation of amino acyl-S-RNA. We made the surprising finding that mouse liver supernatant has a very low content of S-RNA, and the bulk of the S-RNA is associated

[18] C. L. Robinson and G. D. Novelli, *Arch. Biochem. Biophys.* **96**, 459 (1962).

with the ribosomes. This represents independent confirmation of the similar observation made by Smith[19] and published while this investigation was in progress.

STUDIES ON THE CONTROL OF THE SYNTHESIS OF DNA POLYMERASE IN CELL-FREE PREPARATIONS FROM RAT LIVER

Giovanni Giudice G. D. Novelli

Introduction. – Normal rat liver has a relatively low content of the enzyme DNA polymerase. After hepatectomy, DNA polymerase is synthesized at an increasingly rapid rate reaching 10 times greater a level than normal about 40 hr after partial hepatectomy.[20] This and other observations suggest that in the normal, fully developed liver the synthesis of DNA polymerase may be under some kind of repression control. After partial hepatectomy, when the liver must grow rapidly to repair the surgical damage, the repression of synthesis of DNA polymerase is apparently released, thus allowing a more rapid synthesis of this enzyme.

The present investigation was undertaken to learn which factor(s) are involved in this regulatory mechanism. We used a cell-free amino acid incorporating system from normal rat liver, where presumably the synthesis of DNA polymerase occurs at a slow rate, and we added various components from regenerating rat liver to see if the synthesis of this enzyme could be increased.

Results and Discussion. – It was necessary first to learn how to assay for the activity of DNA polymerase and then to develop an active amino acid incorporating system from normal rat liver. F. J. Bollum was most helpful with the assay for DNA polymerase in regenerating rat liver. During development of this assay, two significant observations were made. To observe the high levels of DNA polymerase in regenerating rat liver previously observed by Bollum,[21] it is necessary to use rats that are between 1 and 2 months of age. Older rats failed to exhibit the rise in DNA polymerase after hepatectomy and indeed behaved like normal animals. In addition, even with younger animals, there is considerable variability in the response of DNA polymerase to hepatectomy.

Although a rat liver amino acid incorporating system had been described by Zamecnik,[22] we desired to improve the system. By taking advantage of previous observations in our laboratory[23,24] that ribosomes prepared directly from the 25,000 *g* supernatant are more active than those prepared by prior isolation of microsomes, we have been able to develop a rat liver incorporating system that is 7 to 14 times more active than the ribosomes prepared by conventional procedures.

A number of attempts have been made to further increase the incorporating ability of the system through various additions. Occasionally, the addition of nuclear RNA, prepared by the method of Hiatt,[25] after 30 min gave a slight but variable stimulation. However, with a system presumably saturated with supernatant fluid, the further addition of this fluid after 30-min incubation consistently leads to a stimulation of incorporation. Generally the incorporation process has leveled off after 30 min, and our previous experience has shown that only further addition of ribosomes could alter the plateau. This is the first time that a supernatant fraction has exhibited this effect. The factor responsible for this effect is under investigation.

FURTHER STUDIES ON THE AMINO ACID INCORPORATING SYSTEM ISOLATED FROM MAIZE SEEDLINGS

R. J. Mans

Introduction. – Studies are continuing with the maize incorporating system directed toward the biosynthesis of a specific protein by this cell-free system. Being aware of the demonstrated requirements for the β-galactosidase synthesizing system from *Escherichia coli*,[26] there are three areas

[19] K. Smith, *Biochemistry* **5**, 842 (1962).

[20] F. J. Bollum *et al.*, *Cancer Res.* **20**, 138 (1960).

[21] F. J. Bollum and V. R. Potter, *J. Biol. Chem.* **223**, 478 (1958).

[22] P. C. Zamecnik and E. B. Keller, *J. Biol. Chem.* **209**, 337 (1954).

[23] C. J. Wust and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 159.

[24] G. Giudice and G. D. Novelli, this report.

[25] H. Hiatt, *J. Mol. Biol.* **5**, 217 (1962).

[26] J. M. Eisenstadt, T. Kameyama, and G. D. Novelli, *Proc. Natl. Acad. Sci. U.S.* **48**, 652 (1962).

of refinement and supplementation of the maize system currently being pursued:

1. fractionation of the soluble component; the role of S-RNA in the incorporating system;
2. isolation and characterization of the most active fraction of the ribosomal component;
3. isolation and purification of the DNA-dependent RNA polymerase from maize.

Results and Discussion. – (1) Previously we described the inhibition of the maize amino acid incorporating system by the addition of S-RNA isolated from maize.[27] We have since determined that the inhibition is due to component(s) that are removed by passage of the nucleic acid fraction over DEAE-cellulose followed by overnight dialysis against 10^{-3} *M* Versene. A twofold stimulation of leucine incorporation by the addition of purified S-RNA is routinely obtained. Approximately 10% of the purified material is charged with leucine (on a molar basis) and it is also charged with all ten of the amino acids tested. Furthermore, 30% of the leucine of isolated leucyl-S-RNA is transferred to acid-precipitated protein in the presence of added ribosomes, high-speed supernatant fraction, GTP, Mg^{2+}, and β-mercaptoethanol.[28]

(2) The availability of large amounts of particle protein from pilot plant materials[29] makes possible the isolation of 250- to 500-mg quantities of deoxycholate-treated (DOC-treated) ribosomes. Such preparations have been characterized by activity measurements coupled with ultracentrifuge and electron microscope studies. The DOC-treated preparation is heterogeneous with respect to the latter criteria. Consequently, efforts to fractionate the ribosomal population by sucrose gradient centrifugation for the most active component are now being undertaken. Direct isolation and further characterization of this "most active" particle component will then be attempted.

(3) An RNA-polymerase-type activity has been demonstrated in the chromatin fraction isolated from maize seedlings. In preliminary experiments, the activity measured (65 to 100 micromicromoles of labeled ribonucleotide per mg of protein per 10 min) is comparable with that reported by Huang and Bonner[30] in similar preparations from peas. The reaction is measured as the parallel incorporation of UTP and ATP into acid-precipitated material dependent upon the presence of all four ribonucleotide triphosphates in the reaction mixture. Dependence on added DNA cannot be demonstrated with this crude preparation, which contains 90% of the DNA of the cell. Efforts to enhance the magnitude of the reaction and to demonstrate a DNA requirement by fractionation of the crude chromatin for RNA polymerase are now in progress. It is hoped that this operation will yield a preparation active enough to permit the isolation of a specific RNA product for addition to the maize incorporating system.

CELL-FREE AMINO ACID INCORPORATING SYSTEM FROM MAIZE TISSUE CULTURE

J. E. Graebe G. D. Novelli

Introduction. – An amino acid incorporating cell-free system has been prepared from the microsomal fraction of large-scale tissue culture of maize endosperm. The tissue was grown submerged with aeration on a completely synthetic medium as described earlier.[31] The microsomal fractions used in the experiments were obtained from 500-g batches of tissue after approximately 2 months of growth from small inocula.

Results and Discussion. – The amino acid incorporation shows the typical characteristics of classical protein biosynthesizing systems. Thus it requires, of cell components, a fraction sedimenting at 105,000 *g* as well as a small amount of supernatant fraction from the same-speed centrifugation. As the supernatant fraction obtained from the tissue culture cells is inactive, this fraction has been prepared from maize seedlings. The system has an absolute requirement for an energy source and for Mg^{2+} or Mn^{2+} ions. The optimum concentration of ATP is 1 to 3 micromoles/ml,

[27] C. M. Purcell and R. J. Mans, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962,* ORNL-3352, p 160.

[28] R. J. Mans and G. D. Novelli, presented at the Southeastern Regional Meeting of American Chemical Society, Gatlinburg, Tenn., Nov. 1–3, 1962.

[29] R. J. Mans, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962,* ORNL-3352, p 161.

[30] R. Huang and J. Bonner, *Proc. Natl. Acad. Sci. U.S.* **48,** 1216 (1962).

[31] J. E. Graebe and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962,* ORNL-3352, p 162.

above which ATP becomes inhibitory. Phosphoenolpyruvate, used with its kinase as an ATP generator, is very strongly inhibitory in concentrations above the optimum 1 micromole/ml. The Mn^{2+} can be substituted for Mg^{2+} but with less efficiency. β-Mercaptoethanol (10^{-2} *M*) in the incubation mixture increases the activity twofold, and its inclusion in the homogenization mixture increases the activity threefold. As might be expected in a crude system, the incorporation is not stimulated by the addition of GTP or K^+ ion.

The system is 95% inhibited by 2 μg of RNase per ml and 82% inhibited by 10^{-3} *M* puromycin. No effect has been obtained with DNase (20 μg/ml), o-fluorophenylalanine (2×10^{-2} *M*), or chloramphenicol (400 μg/ml). The plant tissue is easily disrupted, and usually the homogenates have been prepared with a glass homogenizer and loose-fitting pestle. Results have also been obtained with a French pressure cell, in which the tissue can be broken at a very low pressure.

Work is in progress to increase the activity of the system, to find the reason why the supernatant fraction is inactive, and to separate the different phases of protein biosynthesis. The highest activity obtained is 3000 counts/min or 100 micromicromoles of C^{14}-leucine incorporated per mg of particle protein during 30 min. Since the tissue culture under the present conditions is slow-growing, it is likely to accumulate dead or metabolically inactive cells. Efforts directed toward an increased growth rate are, therefore, expected to result in more active cell-free preparations as well. Earlier reported studies on a fraction sedimenting at 10,000 *g* have been discontinued.[31]

STUDIES ON ANTIBODY SYNTHESIS

C. J. Wust

Introduction. – The amino acid incorporating system previously reported[32] has been characterized further, and attempts to use the system in antibody synthesis have been initiated.

Results and Discussion. – Rat spleen ribosomes obtained after treatment of the 32,000 *g* supernatant fluid with 0.65% deoxycholate (DOC) and centrifugation at 105,000 *g* for 90 min can be stored at −192°C (liquid nitrogen) for 5 days. After this time, there is a decay of their ability to incorporate C^{14}-leucine that is labeled into TCA-precipitable material. This decay had a half-life of 3.6 days. Assay of RNase activity during the period of storage indicated that latent enzyme was activated at a rate of 0.045 μg per mg of ribosomal protein per day. After 5 days, the level of RNase activity was sufficient to inhibit the incorporating ability of the ribosomes. RNase (1.5 μg/ml) added to freshly prepared ribosomes completely inhibited incorporation.

An RNA fraction of rat liver 105,000 *g* supernatant fluids can be prepared by treatment of the fluid with 1.25% streptomycin sulfate. The RNA-to-RNA and protein ratio of this fraction has been found to vary from 0.09 to 0.21. When this fraction was added to 0.75 saturated $(NH_4)_2SO_4$ protein concentrate of supernatant fluids, there was an increase in the formation of amino acyl-RNA and of incorporation. The formation of amino acyl-RNA occurred at a rate tenfold greater than incorporation. Whereas addition of the RNA fraction enhanced the formation of amino acyl-RNA proportional to the amount of RNA added, there was an optimal concentration for maximal incorporation. Further addition over the optimum inhibited incorporation.

Two enzymes were purified and used as antigens in rats. β-Galactosidase was purified from *E. coli* strain ML308, constitutive for the enzyme, by the procedure of Kameyama and Novelli.[33] Alkaline phosphatase was prepared after the procedure of Garen and Levinthal[34] from *E. coli* strain K-12. These enzymes are antigenic in the rat, although dose levels for optimal response are not known and constitute the current area of study.

STUDIES ON THE INCORPORATION OF C^{14}-5-FLUOROURACIL

W. E. Barnett[35] C. J. Wust

Introduction. – The pyrimidine analogue 5-fluorouracil (5FU) is incorporated into RNA and has

[32] C. J. Wust and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962,* ORNL-3352, pp 159–60.

[33] T. Kameyama and G. D. Novelli, *Arch. Biochem. Biophys.* **97**, 529–37 (1962).

[34] A. Garen and C. Levinthal, *Biochim. Biophys. Acta* **38**, 470–83 (1960).

[35] Fungal Genetics Group.

been shown to affect protein synthesis specifically. Its effect has been attributed to incorporation into the "informational" or "messenger" RNA. The ability of 5FU and other purine and pyrimidine analogues to phenotypically revert mutants of *Neurospora* has been studied, and the evidence suggests that incorporation into at least two different RNA's may effect protein synthesis. It was considered important, therefore, to examine the various RNA fractions of *Neurospora* with respect to their ability to incorporate 5FU.

Results and Discussion. – Log-phase cultures of *Neurospora* were exposed to C^{14}-5FU for various periods of time, harvested, and the RNA's were separated by centrifugation into three crude fractions: (1) nuclear RNA (RNA isolated from a nuclear pellet), (2) ribosomal RNA, and (3) soluble RNA (RNA not sedimented by centrifugation at 105,000 *g* for 90 min). Since all three fractions are certainly contaminated with RNA from the others, we are examining each by sucrose gradient centrifugation to compare relative rates of incorporation into the various species of RNA as identified by sedimentation rates.

Preliminary results reveal that all these fractions are rapidly labeled with C^{14}-5FU. Isolation of transfer RNA of high specific activity has been achieved, and it is clear that 5FU incorporation is not restricted to a single class of RNA molecules. A complete analysis of the various fractions is in progress.

RIBOSOMAL-BOUND TRANSFER RNA FROM *NEUROSPORA*

W. E. Barnett[35] C. J. Wust

In vitro experiments designed to measure the enzymic production of C^{14}-amino acyl-transfer-RNA by extracts of *Neurospora crassa* led to the finding that most of the "soluble" or transfer RNA is bound to the ribosomes. In addition, we have found that, unlike most analogous systems, transfer RNA has no stimulatory effect upon *in vitro* amino acid incorporation. To demonstrate that typical transfer RNA is indeed associated with the ribosomes, RNA was prepared from washed ribosomes by the phenol procedure and fractionated by DEAE paper chromatography. An amount of transfer RNA was isolated which amounted to 10% of the total ribosomal RNA. Calculations based on an assumed molecular weight of 30,000 for transfer RNA indicated that 5% of the RNA molecules existed as leucyl-transfer-RNA.

We have examined the effect of centrifugation time upon the amount of ribosomal-bound transfer RNA, and we find that it is constant. Thus far, our observations are consistent with the hypothesis that the transfer RNA involved in amino acid incorporation in cell-free extracts of *Neurospora* is bound to the ribosomes.

TRANSFER RNA CHROMATOGRAPHY ON DEAE-CELLULOSE PAPER

K. B. Jacobson Susumu Nishimura

Introduction. – Results from chromatographing various types of RNA on paper sheets of DEAE-cellulose were reported previously.[36] Further studies on transfer RNA (or S-RNA) have shown that it will resolve into a variety of fractions which are characterized by different amino acid specificities.

Results and Discussion. – The chromatographic behavior of transfer RNA which had been charged with four different radioactive amino acids is shown in Fig. 44. The behavior of eight other amino acid-specific RNA's has also been determined. The RNA's are specific for valine, lysine, and alanine in the isoleucine-RNA region. In addition to glycine-RNA, RNA's that are specific for leucine, histidine, and arginine are found migrating more slowly. Ordinarily, glutamic acid–RNA and aspartic acid–RNA are somewhat slower than glycine-RNA, while phenylalanine-RNA and serine-RNA are slower yet. The conditions for chromatography, such as salt concentration and temperature, have pronounced effects on the rate of migration of RNA; the presence of a low concentration of magnesium ion increases the rate of RNA migration.

This technique may find application in isolating an amino acid-specific transfer-RNA molecule. Perhaps it will be more useful as a rapid means of (1) screening the transfer RNA on different species or (2) determining the specificity of charging enzymes from different species for an RNA from a single species.

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[36] K. B. Jacobson and A. L. Dopirak, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962,* ORNL-3352, p 165.

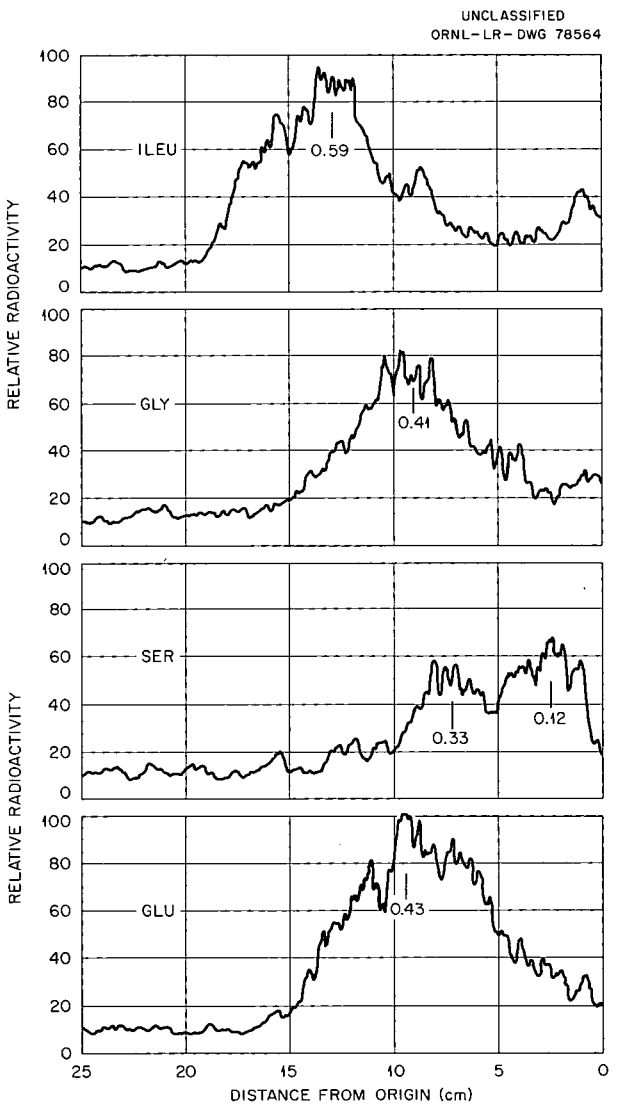


Fig. 44. Chromatography of Amino Acid Charged RNA on DEAE-Cellulose Paper. *E. coli* transfer RNA was charged with the radioactive amino acid indicated and chromatogrammed at 4° as previously described.[36] The RNA was moved in 0.60 *M* NaCl–0.02 *M* acetate buffer (pH 5.6).

INACTIVATION OF S-RNA BY UV RADIATION

P. A. Swenson

Introduction. – Ultraviolet (UV) radiations cause the formation of uracil dimers in polyuridylic acid; then dimers are broken by short-wavelength (2380 A) UV light.[37] Ultraviolet light is known to inhibit the charging of soluble RNA (S-RNA) by amino acids.[38] This reaction is one of the first of a series in the synthesis of protein. On the basis of the photochemistry of polyuridylic acid, it was postulated that the formation of uracil dimers in S-RNA is responsible for its inactivation by UV light.

Results and Discussion. – The charging by ten different C^{14}-labeled amino acids was tested with S-RNA irradiated at 2650 A by use of doses up to 2.0×10^5 ergs/mm^2. Inhibition of charging ranges from 25% for valine to 85% for phenylalanine and lysine. Intermediate in sensitivity are the charging processes for glutamic acid, glycine, aspartic acid, arginine, alanine, serine, and isoleucine. Reversal of the inhibition by 2380 A was attempted for the phenylalanine, lysine, and glutamic acid charging processes. No reversal was obtained using doses up to 2.5×10^4 ergs/mm^2. Optical studies on irradiated S-RNA gave similar results. Irradiation at 2650 A produced a 10% decrease in absorbance at 2600 A, and the absorption decrease was not reversed by 2380 A radiations. It is concluded that uracil dimer formation plays a minor role in the inactivation of S-RNA by UV light. The possibility of other photochemical changes to account for the inhibition is being investigated.

PHOTOCHEMICAL REACTIONS OF URACIL IN POLYURIDYLIC ACID

P. A. Swenson R. B. Setlow[39]

Introduction. – A large fraction of the biological inactivation of DNA by ultraviolet (UV) light has been shown to result from the formation of dimers between adjacent thymine residues in polynucleotide chains.[40] Since dimer formation between adjacent uracils might be expected to be the major cause of the UV inactivation of RNA, the photochemistry of uracil dimers and poly-U has been

[37] P. A. Swenson and R. B. Setlow, this report.

[38] J. F. Scott and A. R. Tutter, presented at the 6th Annual Meeting of the Biophysical Society, Washington, D.C., 1962.

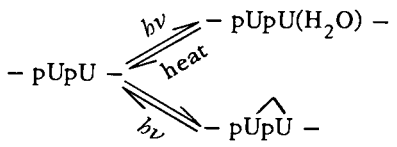
[39] Biophysics Group.

[40] R. B. Setlow and Jane K. Setlow, *Proc. Natl. Acad. Sci. U.S.* **48**, 1250 (1962).

studied. The numbers of dimers formed in poly-U by different wavelengths and doses have been determined.

Results and Discussion. – (*a*) *Uracil Dimers.* – Irradiation of a solution of dimers causes a rapid absorbance increase that arises from the production of uracil from the split dimers. The sensitivity for dimer splitting is high at 2300 A and decreases rapidly with increasing wavelength.

(*b*) *Poly-U.* – Ultraviolet light may form dimers ($\widehat{UU}$) between adjacent uracils, or it may cause hydration of individual ones. The formation of both types of photoproducts results in a loss in absorbance; but the effects may be separated since the dimers may be split by short-wavelength irradiation; and hydration is reversed by heating at low pH. The quantitative observations of absorbance after irradiation at a number of wavelengths and heat treatment have been interpreted quantitatively in terms of these reactions:



The number of dimers formed by UV irradiation reaches a maximum of 15% at 20×10^4 ergs/mm^2, 2650 A, and then decreases at higher doses because the small amount of dimer splitting by 2650 A is followed by hydration in a process that removes uracils from the dimerization pathway.

CELL-FREE AMINO ACID INCORPORATING SYSTEM FROM UTERINE TISSUE

D. L. Greenman F. T. Kenney

Introduction. – Functional sex reversal of genotypic males following the administration of estrogenic substances, as demonstrated by Yamamoto[41] and others, suggests that the action of the estrogens may be rather closely linked with the specific expression of the DNA code, since a complete reversal in the normally expected translation of the genetic material has been effected by the presence of the hormone. To examine the area of DNA expression with respect to the mechanism of action of estrogenic hormones, a cell-free amino acid incorporating system has been prepared from uterine tissue of the adult rat.

Results and Discussion. – Initial preparations of the cell-free uterine system, which were found to have little incorporating ability, did promote amino acid incorporation in crossover experiments with an active rat liver preparation. However, either uterine ribosomal or supernatant fraction inhibited incorporation when added to the complete liver system. High levels of RNase activity were found in both uterine fractions, and treatment of either fraction with bentonite removed much of this enzyme. Subsequent preparation of ribosomal and supernatant fractions in the following manner resulted in an active amino acid incorporating system. Frozen uteri were pulverized under liquid nitrogen by grinding with a mortar and pestle for 20 min. The powder was briefly homogenized in four volumes of Medium A (ref 42) containing mercaptoethanol (5×10^{-3} *M*) and 1 mg of bentonite per ml of medium. The soluble fraction was then obtained by centrifugation for 1 hr at 105,000 *g*. The pellet thus obtained was homogenized in seven volumes of Medium A (brought to 0.7 *M* with respect to sucrose), and deoxycholate was added to 0.5%. Following a 30-min centrifugation at 20,000 *g*, ribosomes were obtained by centrifuging the supernatant fraction 2.5 hr at 105,000 *g*. The ribosomal pellet was washed once and suspended in Medium A; and then both ribosomal and supernatant fractions were stored in liquid nitrogen.

The incorporating system thus prepared showed a requirement for Mg^{2+} (10 m*M*, optimum). The ratio of ribosomal protein to soluble protein for maximum incorporation of C^{14}-leucine was 1:2. Dialysis of the supernatant fraction for 6 hr against Medium A markedly increased the maximum incorporation but changed the optimum ratio of ribosomal to soluble protein to 1:4. After further characterization, this amino acid incorporating system will be used in studying the mechanism of action of the estrogenic hormones.

[41] T. Yamamoto, *Genetics* **44**, 739 (1959).

[42] C. L. Robinson and G. D. Novelli, *Arch. Biochem. Biophys.* **96**, 459 (1962).

HORMONAL CONTROL OF HEPATIC ENZYME SYNTHESIS

F. T. Kenney F. J. Kull

Introduction. – It is now well established that adrenocortical hormones effect large increases in the rate of synthesis of certain hepatic enzymes. Our analysis of the mechanism of this hormonal control has been directed toward the nucleic acid components thought to direct specificity in protein synthesis, as discussed in a previous report.[43]

Results and Discussion. – Changes in RNA metabolism that follow hydrocortisone administration *in vivo* have been studied with a view toward determining both the time course and the subcellular location of the presumed alterations. Adrenalectomized rats were given hydrocortisone and at intervals were given 1 mc of P^{32}. The animals were killed 20 min after isotope administration, and their livers were fractionated into nuclear and cytoplasmic components by centrifugation. Specific radioactivity determinations were made on RNA freed of P^{32}-containing contaminants by exhaustive washing with trichloroacetic acid and with lipid solvents. These standard techniques have yielded somewhat erratic results in these experiments, and we are investigating some chromatographic procedures designed to overcome this difficulty. If minor variations owing to these procedural difficulties are ignored, the following picture emerges: (1) the rate of synthesis of *cytoplasmic* RNA (measured as P^{32} incorporated in 20 min) is unchanged up to 4 hr after hormone administration; (2) the rate of synthesis of *nuclear* RNA is constant up to about $1\frac{1}{2}$ hr post-hydrocortisone and then increases sharply, doubling by 2 hr; and this increased rate is maintained without further change up to 4 hr; and (3) enzyme induction follows almost exactly the same kinetics as the altered synthesis of nuclear RNA, except that enzyme continues to increase after 2 hr.

The nature of the nuclear RNA synthesized in response to hormonal stimulation has been analyzed by centrifugation in a sucrose gradient. When nuclear RNA from untreated animals is prepared by phenol-detergent extraction[44] and analyzed, the bulk of the RNA fractionates into 34, 22, and 6 S components. Newly synthesized (P^{32}-labeled) RNA is diffused throughout almost the entire gradient and thus consists of components ranging from >40 S to 8 S. Preliminary experiments indicate that the hormonally stimulated RNA is similarly heterogeneous, but this point requires further experimentation.

These findings are consistent with the fact (but do not yet establish it) that enzyme induction is associated with an increased rate of synthesis of "messenger" RNA, as predicted by the gene activation theory of induction. Unequivocal proof of this assertion would require that "messenger" activity be demonstrated in the RNA synthesized due to hormonal stimulation. Toward this end we have prepared and characterized an active amino acid incorporation system from rat liver. This system, as prepared, is not responsive to "messenger" addition; and we are attempting various treatments to make it so. When this is accomplished, we shall use this system to assay "messenger" activity in RNA preparations synthesized under hormonal control *in vivo*.

The observation that the kinetics of enzyme induction are almost identical to those of altered nuclear RNA synthesis suggests that there is little or no lag between RNA production and its utilization in enzyme synthesis. That there is no change in the rate of labeling of cytoplasmic RNA is consistent with this suggestion. Thus there is probably no marked accumulation of "messenger" RNA *in vivo*, and it may be necessary to experimentally force its accumulation, if this is possible. We are investigating the possibility of using puromycin treatment in this fashion.

STUDIES ON LACTIC DEHYDROGENASE ISOZYMES

P. J. Fritz K. B. Jacobson

Introduction. – It has been generally accepted that lactic dehydrogenase (LDH) exists in five electrophoretically distinguishable forms in various animals. A theory to account for these five isozymes has been proposed by Markert[45] and Kaplan.[46] The theory is based upon the idea that

[43] F. T. Kenney and F. J. Kull, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 164–65.

[44] H. Hiatt, *J. Mol. Biol.* **5**, 217 (1962).

[45] E. Apella and C. L. Markert, *Biochem. Biophys. Res. Commun.* **6**, 171 (1961).

[46] R. D. Kaplan *et al.*, *Science* **136**, 962 (1962).

the LDH molecule is a tetramer involving five different combinations of two distinct monomers. Electrophoresis of mouse muscle homogenates in polyacrylamide gels in this laboratory has revealed the presence of at least 15 distinct bands with LDH activity. A theory to explain these bands within the framework of the Kaplan-Markert hypothesis was proposed.[47]

Results and Discussion. – When mouse muscle homogenates were subjected to electrophoresis at pH 8.5 in polyacrylamide gels at 86 v/cm in the presence of 0.005 *M* 2-mercaptoethanol or at 26 v/cm in the absence of this reagent, the five major LDH bands were observed to divide into minor bands in the following way: band 1, the electrophoretically slowest band, gave five minor bands; band 2, four minor bands; band 3, three minor bands; band 4, two minor bands; and band 5 did not divide. It was proposed that the minor bands were a manifestation of differential coenzyme binding in the two different monomers, and preliminary experiments with muscle homogenates seemed to support the hypothesis.

It was realized that more definitive results would be obtained with a pure enzyme; therefore, experiments were performed with crystalline rabbit muscle LDH. The crystalline enzyme also showed five major bands upon electrophoresis. At pH 8.5, band 1 was found at the origin, band 2 split into two minor bands, band 3 split into four minor bands, band 4 split into three minor bands, and band 5 split into two minor bands when the electrophoresis was carried out at 26 v/cm. Dialysis of the crystalline enzyme preparation against charcoal to remove bound nicotinamide adenine dinucleotide (NAD) and subsequent electrophoresis at high voltage revealed that the minor bands were still present. This result would seem to contradict the hypothesis that the multiple bands arise as a result of coenzyme binding.

If the multiple bands cannot be explained as before, an alternative idea is that each minor band represents a separate and discrete protein.

[47] P. J. Fritz and K. B. Jacobson, *Science*, in press.

MECHANISM OF ACTION OF ADENOSINE 5′-PHOSPHOSULFATE REDUCTASE FROM *DESULFOVIBRIO DESULFURICANS*

H. D. Peck, Jr. J. T. Davidson

Introduction. – Adenosine 5′-phosphosulfate (APS) reductase from *Desulfovibrio desulfuricans* catalyzes the nucleotide-dependent oxidation of SO_3^{2-} to APS as follows:

$$AMP + SO_3^{2-} \rightleftharpoons APS + 2e\ .$$

The purified enzyme, essentially homogeneous in the ultracentrifuge, contains flavin adenine dinucleotide (FAD) and iron; however, it has not been possible to demonstrate that these components are required for activity. Iron and FAD have been indirectly implicated in the activity of the enzyme by the observation that *p*-hydroxymercuribenzoic acid, which inhibits activity and fragments the enzyme, also releases these two components from the protein. In addition, sulfite reduces the enzyme-bound flavin. Other observations now suggest that the oxidation is a two-step reaction involving an "enzyme-sulfate" as an intermediate.

Results and Discussion. – Enzyme-bound flavin in purified preparations of APS-reductase, dialyzed against charcoal to remove nucleotides, is reduced by sulfite without the concomitant formation of APS. This observation suggested that an "enzyme-sulfate" intermediate was involved in the reaction and that it should be possible to demonstrate the formation of S^{35}-labeled enzyme after exposure of the reductase to $S^{35}O_3^{2-}$. When purified APS-reductase was reacted with $S^{35}O_2^{2-}$ and passed through Sephadex to separate free and protein-bound $S^{35}O_3^{2-}$, it was possible to demonstrate the formation of S^{35}-labeled protein. However, it could not be determined by this technique whether the protein was labeled by a chemical reaction between disulfide bonds and $S^{35}O_3^{2-}$ (ref 48).

A soluble, stable, labeled protein was prepared by reacting the reductase with sulfite and then inactivating with *p*-hydroxymercuribenzoic acid. After removing the free $S^{35}O_3^{2-}$, the stability of

[48] J. M. Swan, *Nature* **180**, 643–45 (1957).

the sulfur label in the enzyme protein was compared with that of *S*-sulforibonuclease which is labeled with SO_3^{2-} exclusively by reaction with disulfide bonds.[49] Stability studies with the sulfur label in these two proteins indicate that a major portion of the sulfite bound to the enzyme protein is not present as *S*-sulfonate and must be assumed to be bound to the protein of the enzyme by another type of linkage. As sulfite can be nonenzymatically incorporated into protein only by reaction with disulfide bonds to form an *S*-sulfonate, it seems that the S^{35} found in the reductase must result from the enzymatic activity of the enzyme. These results further substantiate the idea that an "enzyme-sulfate" is involved as an intermediate in the mechanism of action of APS-reductase, and the nature of the bond between the enzyme and sulfite is being investigated.

FORMIC DEHYDROGENASE ACTIVITIES OF *DESULFOVIBRIO DESULFURICANS*

Joy P. Williams H. D. Peck, Jr.

Introduction. – Cell-free extracts of *Desulfovibrio desulfuricans* catalyze the cytochrome C_3-dependent decomposition of formate to CO_2 and H_2 (formic hydrogenylase) and exhibit formic dehydrogenase activity with the artificial electron acceptors, methylene blue and benzyl viologen. These formic dehydrogenase activities are being investigated to ascertain their role in the formic hydrogenylase reaction of *D. desulfuricans.*

Results and Discussion. – The formic dehydrogenase with benzyl viologen has been partially resolved into two protein components, both of which are required for maximum activity. Crude extracts, prepared by the French pressure cell, were treated with streptomycin sulfate (5 mg/ml) and fractionated with $(NH_4)_2SO_4$. Fractions precipitating with low concentrations of $(NH_4)_2SO_4$ showed little activity, but a fraction obtained with a higher concentration of $(NH_4)_2SO_4$, exhibited formic dehydrogenase activity with methylene blue and low-activity benzyl viologen. Combination of fraction 6 with a broad fraction (fraction 1) precipitating with low concentrations of $(NH_4)_2SO_4$ restored benzyl viologen activity to that observed with crude extracts; however, there was no stimulation of formic dehydrogenase with methylene blue. Boiling of either fraction destroys the activity observed with benzyl viologen as well as that observed with methylene blue. Fraction 6 can be saturated by fraction 1 with respect to activity with benzyl viologen, and under these conditions, formic dehydrogenase activity with benzyl viologen is twice that shown by methylene blue. Since all active preparations of fraction 6 have shown formic dehydrogenase activity with methylene blue, it is postulated that this fraction contains the enzyme formic dehydrogenase and fraction 1 contains a protein required for transfer of electrons to benzyl viologen. However, it will be necessary to purify these two protein fractions further before the role of the two components in the formic hydrogenylase reaction and reduction of cytochrome C_3 can be determined.

[49] J. L. Bailey and R. D. Cole, *J. Biol. Chem.* **234**, 1733 (1959).

ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION IN THE "SULFATE-REDUCING BACTERIA"

Sister M. Regina Lanigan H. D. Peck, Jr.

Introduction. – Evidence derived from growth and inhibitor studies with *Desulfovibrio desulfuricans* indicates that this microorganism can produce a portion of the energy required for growth and the reduction of sulfate by means of an "anaerobic" oxidative phosphorylation. To study this system in detail, an attempt is being made to obtain the oxidative phosphorylation in cell-free preparations.

Results and Discussion. – Extracts of *D. desulfuricans* catalyze the cytochrome C_3-dependent reduction of sulfite and thiosulfate in the presence of molecular hydrogen. These reductions have been found to be particularly active in extracts prepared from cells grown in the presence of excess ferrous salts. As yet, it has not been possible to demonstrate a net esterification of orthophosphate during the reduction of thiosulfate or sulfite with hydrogen. However, a hydrogen-dependent exchange of P^{32}-labeled phosphate into ATP has been shown to occur in these preparations. As this exchange reaction may be due to the oxidative phosphorylation system, the nature of this reaction will be further investigated.

Microbiology

Enzyme and Tracer Studies on Bacterial Metabolism

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ALKALI MODIFICATION PRODUCTS OF NICOTINAMIDE ADENINE DINUCLEOTIDE

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As part of a program dealing with electron transport reactions, certain aspects of pyridine nucleotide chemistry are under investigation. Previous work from this laboratory[2] demonstrated that storage of commercial NAD solutions at −20°C and pH 11–11.5 caused the formation of a strongly fluorescent product not identical with that formed in 5 *N* alkali. Five modification products with closely related spectra have now been isolated from frozen, weakly alkaline solutions of NAD. One of the major modification products has been isolated in pure form (by chromatography on DEAE cellulose) and shown to be a dextrorotatory dinucleotide (molecular rotation, +27,500) in which normal AMP (i.e., 5′-AMP, in which the ribose has a β configuration) is linked in a pyrophosphate bridge to a modified form of NMN. The dinucleotide has a pK_a of 9.5, as demonstrated by spectrophotometric titration and by fluorescence-vs-pH curves. This pK_a is confirmed by paper electrophoresis.

A mononucleotide modification product, released on hydrolysis of the dinucleotide with venom diesterase, has also been prepared in pure form. Spectra in the near-ultraviolet and visible regions closely resemble those of the dinucleotide, except that the absorption peaks are shifted 6–7 mμ toward the ultraviolet. The mononucleotide has a pK_a at 9.7.

Examination of the pH-vs-fluorescence curves for the mono- and dinucleotide modification products points to the existence in the dinucleotide of an intramolecular complex between the adenine and pyridine rings. A similar situation has been described for the flavin coenzymes,[3] that is, complex formation between the adenine and isoalloxazine rings.

Tentative identification of the dinucleotide modification product has been made on the basis

[1]Enzymology Group.

[2]M. I. Dolin, *Biochim. Biophys. Acta* **63**, 219 (1962).

[3]G. Weber, *Biochem. J.* **47**, 114 (1950).

of (1) ultraviolet and infrared spectra, (2) elementary analysis of the mononucleotide product, and (3) mass spectrometry. Confirmation of structure must await NMR analysis and further study of several homologous model compounds that have recently been prepared.

EVIDENCE FOR THE PRESENCE OF A QUINONE IN *STREPTOCOCCUS FAECALIS*, 10Cl

R. H. Baum M. I. Dolin

Introduction. – Quinones have characteristically been found in organisms and tissues carrying out aerobic metabolism, and have been implicated in electron transport and oxidative phosphorylation. On the other hand, neither benzoquinones nor naphthoquinones have been detected by spectrophotometric examination of lipid extracts prepared from various anaerobic and lactic acid bacteria.[4-6] Since *Streptococcus faecalis*, 10Cl, when grown either aerobically or anaerobically, contains a functional flavoprotein oxidase system,[7] it was of interest to determine whether quinones could be detected in this organism and whether they play a role in its metabolism.

Experimental and Results. – Lipid extracts, prepared by the method of Brodie,[8] from anaerobically grown *S. faecalis*, 10Cl, were found to give positive Dam-Karrer and Schilling-Dam tests, suggesting the presence of a quinone. During chromatography on Decalso, the compound responsible for these colorimetric reactions accumulates mainly in the fraction eluted with 4% ether:96% pentane. This chromatographic fraction, when spotted on silicone-impregnated paper and reduced with borohydride, can reduce neotetrazolium to a formazan at *room temperature*. The latter result is thought to be characteristic of naphthoquinones.[9] Chromatography of the 4% ether:96% pentane fraction on silicone-impregnated paper revealed only one formazan-positive spot. The compound isolated at this R_f gives positive Dam-Karrer and Schilling-Dam tests and has an ultraviolet spectrum similar to that of naphthoquinones. The spectrum differs from that of the known naphthoquinones, however, in that there is a single peak at 265 mμ, instead of the expected peaks at 260 and 269 mμ. Reduction with borohydride causes bleaching in the region 225 to 280 (about 65% at 265 mμ), leaving an absorption peak at 246 mμ. Results of chromatography suggest that the compound is less polar than vitamin K_2 isolated from *Escherichia coli*.

The extent to which lipid extracts can be bleached with borohydride at 265 mμ (vitamin K_1 used as standard) indicates a quinone content of 5×10^{-5} to 10×10^{-5} μmole per mg dry weight of anaerobically grown cells.

Research currently under way is directed toward further purification and identification of the quinone found in *S. faecalis*, 10Cl, and subsequently toward the revelation of its function in the metabolism of the organism.

NEGATIVE ION MASS ANALYSIS OF DICARBOXYLIC ACIDS IN STUDIES ON THE MECHANISM OF THE METHYLMALONYL ISOMERASE REACTION

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

Introduction. – Marston[10] and Hegre[11] independently proposed that the cobamide coenzyme-dependent isomerization of methylmalonyl CoA to succinyl CoA occurs by an *inter*molecular reaction involving a cyclic dimer. We have previously reported an experiment designed to test the possibility of this mechanism.[12] We used positive ion mass analysis of the C^{13}-labeled succinic acid and found no evidence of an intermolecular

[4] R. L. Lester and F. L. Crane, *J. Biol. Chem.* **234**, 2169 (1959).

[5] B. K. Jacobsen and H. Dam, *Biochim. Biophys. Acta* **40**, 211 (1960).

[6] D. H. L. Bishop, K. P. Pandya, and H. K. King, *Biochem. J.* **83**, 606 (1962).

[7] M. I. Dolin, in *The Bacteria*, vol II, chap. 9, I. C. Gunsalus and R. Y. Stanier, eds., Academic Press, New York, 1961.

[8] A. F. Brodie, in *Methods in Enzymology*, vol 6, Academic Press, New York (in press).

[9] R. L. Lester and T. Ramarsarma, *J. Biol. Chem.* **234**, 672 (1959).

[10] H. R. Marston, J. A. Mills, and R. M. Smith, *Nature* **193**, 240 (1962).

[11] C. S. Hegre, S. J. Miller, and M. D. Lane, *Biochim. Biophys. Acta* **56**, 538 (1962).

[12] E. F. Phares, Mary V. Long, and S. F. Carson, *Biochem. Biophys. Res. Commun.* **8**, 142 (1962).

reaction. Kellermeyer and Wood[13] subsequently used positive ion analysis of C^{13}-labeled butadiene (derived from succinate) to study this reaction and obtained similar results. We have now carried out the complete analysis by the more conclusive negative ion method[14] using *intact* succinic acid.

Results and Discussion. – An equal mixture of C^{13}-carboxyl- and C^{13}-thioester-labeled methylmalonyl CoA was used as a substrate for the *Propionibacterium shermanii* isomerase. After hydrolysis of the CoA ester, the purified succinic acid was subjected both to positive and to negative ion mass analysis. Negative ion analyses of the *intact* molecules of succinic and of the initial and residual methylmalonic acids yielded acid anion spectra virtually identical with theoretical values for singly labeled acids. Mass spectrometer data of CO_2 derived from the complete combustion of the labeled acids are in agreement with the molecular spectra. An *inter*molecular transcarboxylation would label a predictable fraction of the product molecules with *two* C^{13} atoms. Thus the transcarboxylation occurs by *intra*molecular rather than *inter*molecular rearrangement, which rules out the cyclic dimer theory of Marston and of Hegre.

The simplicity of negative ion spectra compared to the positive ion spectra of the intact acid offers a tremendous advantage in ease of interpretation. The contrast of the negative ion method to the positive ion method involving the reduction of succinate to butadiene is even more pronounced. In cooperation with the Analytical Chemistry Division, we have examined the possibility of extending the intact acid analysis to other biologically important molecules. Of five dicarboxylic acids examined, methylmalonic, succinic, and malic acids had simple negative ion spectra only, while fumaric and oxalacetic acids had simple positive ion spectra only. Thus the method would appear to have fairly wide application.

[13]R. W. Kellermeyer and H. G. Wood, *Biochemistry* 1, 1124 (1962).

ELECTRON SPIN RESONANCE STUDIES ON COBALAMINE COENZYMES

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

Introduction. – Bernhauer[15] reported a magnetic moment for dimethylbenzimidazolylcobalamine coenzyme of 1.63 Bohr magnetons, which is close to the theoretical value of 1.73 μB for cobalt in the divalent state. These values were determined by magnetic susceptibility measurements. The vitamin or inactivated form of the coenzyme was not paramagnetic, however, and so biological activity appears to be associated with the complex which produces the magnetic moment. If the coenzyme undergoes oxidation or an appreciable shift of electron densities during the isomerization, then a change in the magnetic moment would be expected. If a sharp resonance band is contributing to the magnetic moment, a study of the effect of enzyme and substrate might be useful in understanding the role of the coenzymes in the methylmalonyl isomerase reaction and in other rearrangements.

Results and Discussion. – With the cooperation of the Biophysics Group of this Division and the Chemistry Division of ORNL, electron spin resonance spectra of concentrated solutions of the dimethylbenzimidazolyl- and adeninylcobalamine coenzymes were made. With the former compound, very broad and weak peaks were obtained in the $g = 2$ region at liquid-nitrogen temperatures. In the case of the latter, a very weak peak was obtained at room temperature. This resonance is in the region expected for divalent cobalt and supports the interpretation of Bernhauer's susceptibility measurements. However, the nature of the peaks makes their use in mechanism studies impractical with present equipment.

[14]G. A. Ropp and C. E. Melton, *J. Am. Chem. Soc.* **80**, 3509 (1958).

[15]Konrad Bernhauer *et al.*, *Biochem. Z.* **333**, 560 (1961).

Plant Physiology and Photosynthesis

Plant Physiology		Photosynthesis	
A. H. Haber	N. Ann Payne	W. A. Arnold	C. B. Kincaid
T. J. Long[a]	Helen J. Luippold[b]	W. F. Bertsch	

[a]USPHS Fellow.
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EVIDENCE FROM UNTREATED PLANTS FOR NONESSENTIALITY OF CELL DIVISIONS FOR DEGREE OF POLARIZATION OF GROWTH

A. H. Haber N. Ann Payne
Helen J. Luippold

Introduction. – With use of radiation-induced mitotic inhibition, it was shown previously that concurrent, oriented cell divisions play no role in determining the degree of polarization of growth of the first foliage leaf during development of wheat seedlings.[1] This study is concerned with the question of the general applicability of this conclusion. We consider morphogenesis in leaves and other determinate organs that have patterns of growth and meristematic activity different from those in the grass leaf and that have not received experimental treatments similar to irradiation.

Results. – Growth of tobacco leaf blades occurs from areas of approximately 0.25 to 100 cm^2 with a constant degree of polarization (i.e., ratio of the rate of increase in length per millimeter of length to the rate of increase in width per millimeter of width) equal to 0.8. During this growth there is steadily decreasing mitotic activity; in the final stages of development, cell number remains constant. When cell divisions do occur, they are not localized in any part or parts of the leaf. This pattern of mitotic activity thus contrasts with the intercalary meristem of the grass leaf. The divisions are usually oriented so that the new cell plates tend to be perpendicular to the axis of greater relative extension, as shown by the fact that cell shapes remained relatively constant while leaf shape was changing.

A literature survey furnished data providing the following specific examples of polarized growth with constant degree of polarization during which mitotic activity falls: leaves of *Aster multiflorus*[2] and *Callitriche intermedia*,[3,4] and fruits of *Curcurbita pepo*[5,6] and *Lagenaria vulgaris*.[5,6] The last two examples are of the same varieties of fruits for which Sinnott[7] demonstrated that the final orientations of mitotic spindles tended to be parallel to the axis of greater relative extension.

[1]A. H. Haber, *Am. J. Botany* **49**, 583 (1962).

[2]A. L. Delisle, *Am. J. Botany* **25**, 420 (1938).

[3]H. Jones, *Ann. Botany* **19**, 369 (1955).

[4]H. Jones, *The Growth of Leaves*, pp 93–106, Butterworths Scientific Publications, London, 1956.

[5]E. W. Sinnott, *Am. Naturalist* **70**, 245 (1936).

[6]E. W. Sinnott, *Am. J. Botany* **26**, 179 (1939).

[7]E. W. Sinnott, *Am. J. Botany* **31**, 388 (1944).

Discussion. – These findings illustrate several instances for which concurrent cell divisions are not essential for maintaining a degree of polarization. In contrast to our earlier study,[1] the organs studied have no intercalary meristem, and the plants were not irradiated. Thus, our earlier conclusion that cell divisions play no role in determining the degree of polarization[1] is confirmed using different plant material and different experimental methods. As in the earlier study, and as generally occurs during polarized growth, the majority of divisions tended to have an orientation such that the new cell plates are perpendicular to the axis of greater relative extension.[1,7] Thus, the degree of polarization is unaffected by the orientations, as well as by the extent, of cell divisions. Since oriented cell divisions do not contribute to the degree of polarization of growth, and since the degree of polarization is by definition a ratio of rates of extension per unit length along different axes, these findings indicate that concurrent cell divisions do not directly influence growth rate. Consequently, there is a fallacy in the concepts "growth by cell division" and "growth by cell enlargement" and in the usual and accepted manner of interpreting changes in organ size as being due to changes in cell size and number.

GROWTH OF EMBRYOS EXCISED FROM HEAVILY IRRADIATED AND UNIRRADIATED SEEDLINGS ON MIXTURES OF CARBOHYDRATES

T. J. Long

Introduction. – In a previous report,[8] the growth in darkness of seedlings excised from heavily irradiated (500 kr) grain (gamma-plantlets) and unirradiated grain was shown to be markedly dependent upon glucose concentrations from 0 to 15 g/liter when glucose was the sole carbon source. In the presence of 15 g/liter glucose, 5 g/liter galactose inhibited root and coleoptile growth irrespective of irradiation. In the presence of glucose, this concentration of galactose had no effect on growth of the gamma-plantlet leaves even though it severely inhibited growth of the unirradiated leaves.[8] This project has been extended to include further studies on the effects of galactose and several other sugars that are constituents of normally occurring polymers.

Results. – The findings, consistent with those previously reported for galactose,[8] were obtained from growth studies of two types: (1) study of the effects of various concentrations (0 to 5 g/liter) of galactose added to 15 g/liter glucose, and (2) a study of the time course of growth on 15 g/liter glucose in presence of either no galactose or 5 g/liter galactose. In all these experiments the addition of galactose to glucose inhibited growth of roots and coleoptiles irrespective of irradiation, but inhibited growth of leaves only in unirradiated plants; the length of the gamma-plantlet first leaf was unaffected by galactose.

Addition of xylose or ribose to glucose promoted growth of seedlings irrespective of irradiation. Addition of arabinose to glucose slightly inhibited growth irrespective of irradiation. When 5 g/liter mannose is added to 15 g/liter glucose, growth of the unirradiated coleoptile and first leaf is severely inhibited. On the other hand, the addition of mannose to glucose has no effect on the growth of the gamma-plantlet coleoptile or first leaf. Addition of mannose to glucose inhibits root growth irrespective of irradiation.

Discussion. – These results indicate that the irradiation treatments abolish the mechanism(s) by which galactose and mannose inhibit leaf growth and by which mannose inhibits coleoptile growth. By contrast, all actions of xylose, ribose, and arabinose on growth seemed independent of the irradiation. These results with xylose, ribose, and arabinose illustrate additional physiological similarities between gamma-plantlets and unirradiated controls.[8,9] The results with galactose and mannose provide the first examples we have found of physiological differences between gamma-plantlets and unirradiated controls other than those one would expect to result from radiation-induced inhibition of DNA synthesis, mitosis, and cell division.

[8] T. J. Long, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, p 177.

[9] A. H. Haber, W. L. Carrier, and D. E. Foard, *Am. J. Botany* **48**, 431 (1961).

PHOTOSYNTHESIS

W. A. Arnold W. F. Bertsch

We are attempting to understand the initial energy conversion steps of photosynthesis. The approach now being stressed involves measurement of the delayed light emission of living plants. This dim light is emitted from the first excited singlet state of chlorophyll, has decay characteristics of untrapping of electrons in a semiconducting crystal, and is intimately associated with photosynthesis. Since earlier work from this laboratory has shown that the photosynthetic apparatus, when dried, has other semiconducting properties (photoconduction, thermal conduction, and thermoluminescence), it seemed that the light emission of plants might make possible the direct physical measurement of electron transitions involved in photosynthetic energy conversion.

We have now studied the delayed light over seven orders of magnitude in time (6×10^{-4} to 6000 sec). Over this range, the emission falls into two categories according to the effect of temperature. At times longer than about 10^{-2} sec, raising the temperature from 9 to 40°C increases the intensity of light emission; while at times shorter than 10^{-2} sec, raising the temperature decreases the emission. An explanation for the two temperature effects is suggested by the old observation that the turnover time for photosynthesis is about 10^{-2} sec. It would appear that delayed light emission at times longer than 10^{-2} sec reflects a flow of electrons into a chlorophyll system due to back reactions of photosynthesis (increased enzymatic rates would increase the light emission). On the other hand, delayed light measurements at very short times must reflect a flow of electrons out of a chlorophyll system due to the forward reaction of photosynthesis (increased enzymatic rates would reduce the light emission).

Further evidence for the interaction of enzymes with a chlorophyll solid-state system has been obtained with the use of poisons which specifically attack the photosynthetic apparatus. By studying the light emission at times faster than 10^{-2} sec, it is possible to observe the effect of poisons on early electron steps of photosynthesis. The chemical herbicide, Monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea], is of particular interest. Monuron treatment resulted in a nearly continuous delayed light emission and in a reversal of the temperature effect. Monuron is known to inhibit an enzyme that is very close to the short wavelength photoreaction of photosynthesis, and its effect on the photosynthetic light emission is best explained in terms of a block of electron flow from chlorophyll during the forward reactions of photosynthesis. The almost continuous emission of Monuron-poisoned plants would then represent a very slow light-emitting decay of electrons that were blocked in the forward reactions of photosynthesis. It is interesting to note that the temperature-induced increase in light emission of Monuron-poisoned cells had a Q_{10} of 3.0, implying a 0.9-ev activation energy. Although this is far larger than would be expected of an enzyme system, it is about the right size to explain the 35% efficiency of photosynthesis. (Every quantum absorbed by chlorophyll has about 1.8 ev of energy, but only 0.8 ev of this energy is utilized in photosynthesis.) Such a large Q_{10} in the poisoned cells may imply a total trap depth (several trap depths may be involved) of 0.9 ev in a chlorophyll solid-state system. The fact that increased temperatures had an opposite effect in the unpoisoned cells would then reflect the increased enzymatic utilization of electrons from the 0.9-ev trap of the solid-state chlorophyll reaction center. This interpretation would also explain why the back reaction of photosynthesis is so small (it has been observed only by the extremely sensitive delayed light technique), since the 0.9-ev trap depth would essentially eliminate back reactions.

Our delayed light measurements in the millisecond region were made possible by the development of a fast phosphoriscope and utilization of appropriate electronics. This apparatus was built in collaboration with J. B. Davidson of the ORNL Instrumentation and Controls Division. The delayed light decay at 6×10^{-4} sec (our fastest measurement) clearly indicates that earlier electron processes could be observed by measuring the emission at still faster times. We are now attempting the construction of such an instrument.

Biophysics

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PHOTOREACTIVATION OF KILLING OF *STREPTOMYCES GRISEUS* CONIDIA BY FAR ULTRAVIOLET AND VACUUM ULTRAVIOLET RADIATION (1500–2600 A)

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Introduction. – Very little work has been done on the effects of radiation below 2200 A on living cells. The difficulties of working in this region are great and include (1) poor availability of proper radiation sources, (2) very low penetration of the radiation, and (3) for the shorter wavelengths, the necessity of placing the cells in a vacuum or in dry nitrogen. Our experiments were done with equipment less than ideal, in that (1) the source has a spectral distribution that makes dosimetry difficult, and it emits carbon particles that coat windows even during a single run, and (2) the spectrum had to be altered by inserting cutoff filters, instead of by using a monochromator. In spite of such drawbacks, we were able to obtain quantitative data, of considerable reproducibility, on ultraviolet (uv) killing of dry spores and on their subsequent photoreactivation (PR).

Methods. – The source is an experimental magnetically constrained dc carbon arc, $\frac{1}{4}$ in. in diam by 4 in. long, in argon at low pressure. This source emits strongly at 1548 A, moderately at 2296 A, and at a low level at other wavelengths. About 30% of the output is in the visible and infrared, and the output in the region 2300–3000 A is quite low. The arc was made available to us and operated by personnel of the Thermonuclear Division of ORNL. We used filters of calcium fluoride (CaF_2), which transmits the 1548-A line, Suprasil (a quartz), which cuts off the 1548-A line, and a Corning 7910 glass filter, which transmits the 2296-A line. Photoreactivation was effected by two G.E. 15-w Daylight fluorescent lamps (3500–7000 A).

Conidia of *Streptomyces griseus* were suspended in distilled water and filtered through cotton and Millipore filters, to eliminate clumps and debris. They were dried on Millipore filters in a vacuum desiccator, irradiated in vacuum on the filters, and photoreactivated on the filters at 23°C after placement on a nutrient agar surface. They were incubated in this state at 28°C for 2 days, after which microcolonies were counted.

Control experiments at 2537 A were done in an identical manner, except that the source was two G.E. 15-w germicidal lamps.

Results. – Data are shown in Fig. 45. The survival curve at 2537 A was similar to that for the 7910 filter. It is clear that the predominant mode of inactivation changes in the vicinity of 1900 A. Difficulties of dosimetry, as well as variation in

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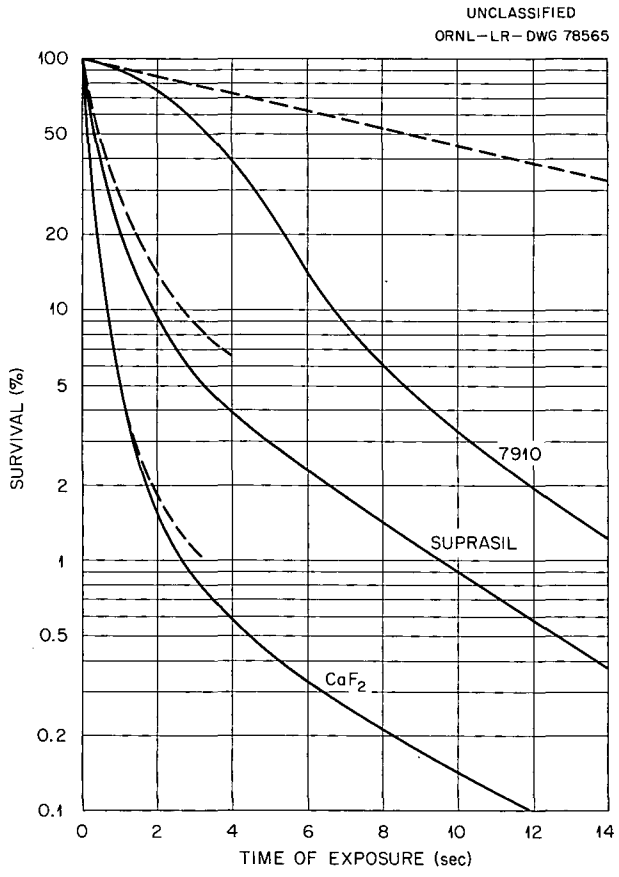

Fig. 45. Percentage Survival on a Logarithmic Scale vs Time of Exposure to uv for *S. griseus* Conidia. Broken lines are survival after maximum PR. The CaF_2 filter transmits above 1400 A, the Suprasil above 1600 A, and the 7910 above 2200 A. The intensity incident on each filter is constant, so that, for example, Suprasil transmits the same intensity and quality of radiation that 7910 transmits, plus additional radiation in the region 1600–2200 A.

curve shape, precluded the drawing of a complete action spectrum, although we can say that the efficiency is relatively constant in the vacuum uv (below 1900 A). Since both protein and nucleic acid have relatively flat absorption spectra in this region, it is not possible to conclude whether either is a vacuum-uv chromophore for killing.

The survival curve at 2537 A after maximum PR is similar to the curve after maximum PR for the 7910 filter, although less steep, having about one-half the slope. Constant dose reduction is not observed above 2200 A, since the survival curve after maximum PR is different in shape (exponential) from that without PR (sigmoid). From the curves, it is concluded that the photoreactivable sector (fractional decrease in effective uv dose, as a result of maximum PR) starts to drop in the vicinity of 2300 A, is low at 1900 A, and has fallen to zero at 1548 A. (At high doses, inactivation with CaF_2 and Suprasil is presumably only by longer wavelengths, since the final slopes are all parallel.) This is consistent with the PR lesion being associated primarily with the 2600-A absorption peak of nucleic acid, which would "tail" down to about 2000 A, and only very little with the 1900-A peak.

The amount of light required for maximum PR (not shown here) increases linearly with uv *dose* (regardless of shape of survival curve) at all wavelengths. Regardless of wavelength or uv dose, the amount of light required for maximum PR is proportional to the photoreactivable sector. Furthermore, the kinetics of PR are single-hit in all cases. These findings are consistent with the PR lesion being identical in all cases, and with the mechanism of PR being identical in all cases.

Conclusions. – Below about 1900 A, conidia of *S. griseus* are killed primarily by a different mechanism than above 1900 A.

Photoreactivation has fallen to zero at 1548 A, thus showing that not all of the nonionizing uv that could be absorbed in nucleic acid will lead to photoreactivable lesions in cells.

From kinetic data, it is concluded that, even though the relative amount of PR lesion decreases rapidly with decreasing wavelength, the nature of the PR lesion, as well as the mechanism of PR, is the same for all wavelengths below 2600 A at which PR occurs.

ANALYSIS OF GENETIC DAMAGE AS A FUNCTION OF LET

M. L. Randolph

Introduction. – Variation of radiosensitivity to different ionizing radiations has been examined to display the true variation of dose coefficients with linear energy transfer (LET or *L*), and therefrom to study biophysical mechanisms of chromosomal damage.[2] Each coefficient generally in-

[2]M. L. Randolph, *Ann. N.Y. Acad. Sci.* (in press).

volves fewer mechanisms than cause the overall observed biological effect.

Method. – Low levels of effect E are usually described as a power series of the dose D. Thus:

$$E = \sum_n R_n D^n , \qquad (1)$$

where the statistically determined coefficients R_n depend on the LET distribution and other parameters.

Ionizing radiations almost inevitably produce wide LET spectra, whereas for plotting and analysis one desires a single average value for each spectrum. We suggest an objectively chosen average. Letting $\phi(L)dL$ be the fraction of total dose delivered at spatial rates L to $L + dL$, the observed average coefficient is

$$\bar{R}_n[\phi(L)] = \int_{L_{min}}^{\infty} R_n(L)\phi(L)dL \equiv R_n(\bar{L}) , \qquad (2)$$

where $R_n(L)$ is the desired true variation of R_n with LET, and $\bar{L}$ is what we call the biophysical average LET, arising from both biological and physical considerations. For each biological system, one seeks functions $R_n(L)$ which come sufficiently close to satisfying Eq. (2) for all LET spectra used.

Results. – Analytically, one can show that the track average $\bar{L}_x$ applies if a plot of experimental $\bar{R}_n$ vs $1/\bar{L}_x$ is linear, and the energy average $\bar{L}_E$ if $\bar{R}_n$ vs $\bar{L}_E$ is linear. Other, less simple, analytic cases have also been solved.

We have applied this method to available, coherent data for *Tradescantia* chromatid aberrations for 6–8 LET distributions.[3,4] Our LET calculations allow for delta tracks,[5] recent low-energy stopping powers,[6] and inelastic collisions with 14-Mev neutrons.[7] An empirical fit for the linear dose coefficient for chromatid plus isochromatid deletions was found:

$$R_1(L) = 1.2L + 10^3 e^{-0.0027(L-L_0)^2} \text{ (aberrations/}10^9\text{ rads-cells)} , \qquad (3)$$

where $L_0 = 66$ kev/μ. Figure 46 shows this curve and the fit obtained. Although mathematical fits are not yet available, the data for both chromosome exchanges and chromosome deletions are similar.

Discussion. – Our empirical fit unrealistically predicts that R increases without limit as L increases. However, most striking is the strong peak of sensitivity for linear dose response at $L \cong 65$ kev/μ or roughly 10 A between primary ionizations along the tracks. This peak suggests that under these experimental conditions: (1) most aberrations result from multiple physical interactions, (2) these chromosomes are relatively stable against manifestation of deletions when successive primary ionizations are much more distant than 10 A, and (3) when successive primary ionizations are much closer than 10 A, the microscopically localized energy dissipation is excessive and the overall efficiency decreases because of shortened track length per unit dose.

[3]A. D. Conger *et al.*, *Radiation Res.* **9**, 525 (1958).

[4]J. P. Kotval and L. H. Gray, *J. Genetics* **48**, 135 (1947).

[5]P. R. J. Burch, *Brit. J. Radiol.* **30**, 524 (1957).

[6]J. Neufeld and W. S. Snyder, pp 35–44 in *Selected Topics in Radiation Dosimetry*, International Atomic Energy Agency, Vienna, 1961.

[7]M. L. Randolph, *Radiation Res.* **7**, 47 (1957).

THYMINE DIMERS IN UV-IRRADIATED DNA: THEIR IDENTIFICATION AND PHOTOREACTIVABILITY

R. B. Setlow W. L. Carrier
Amleto Castellani[8]

Previous photochemical and enzymatic studies indicated that there are nuclease-resistant sequences of the form pXpTpT in uv-irradiated P^{32}-labeled DNA.[9] The irradiation of DNA's containing P^{32} and H^3-thymidine has allowed us to make a more direct identification of thymine dimers as constituents of the nuclease-resistant sequences. Formic acid hydrolysis of the sequences yields an H^3-containing product that has the chromatographic mobility (in several solvent systems) of an authentic sample of thymine dimers. P^{32}- and H^3-labeled, irradiated DNA has been treated with a photoreactivating enzyme preparation from yeast. Thymine dimers are split en-

[8]Visiting investigator from abroad; now in Nucleic Acid Chemistry Group.

[9]F. J. Bollum and R. B. Setlow, *Federation Proc.* **21**, 374 (1962).

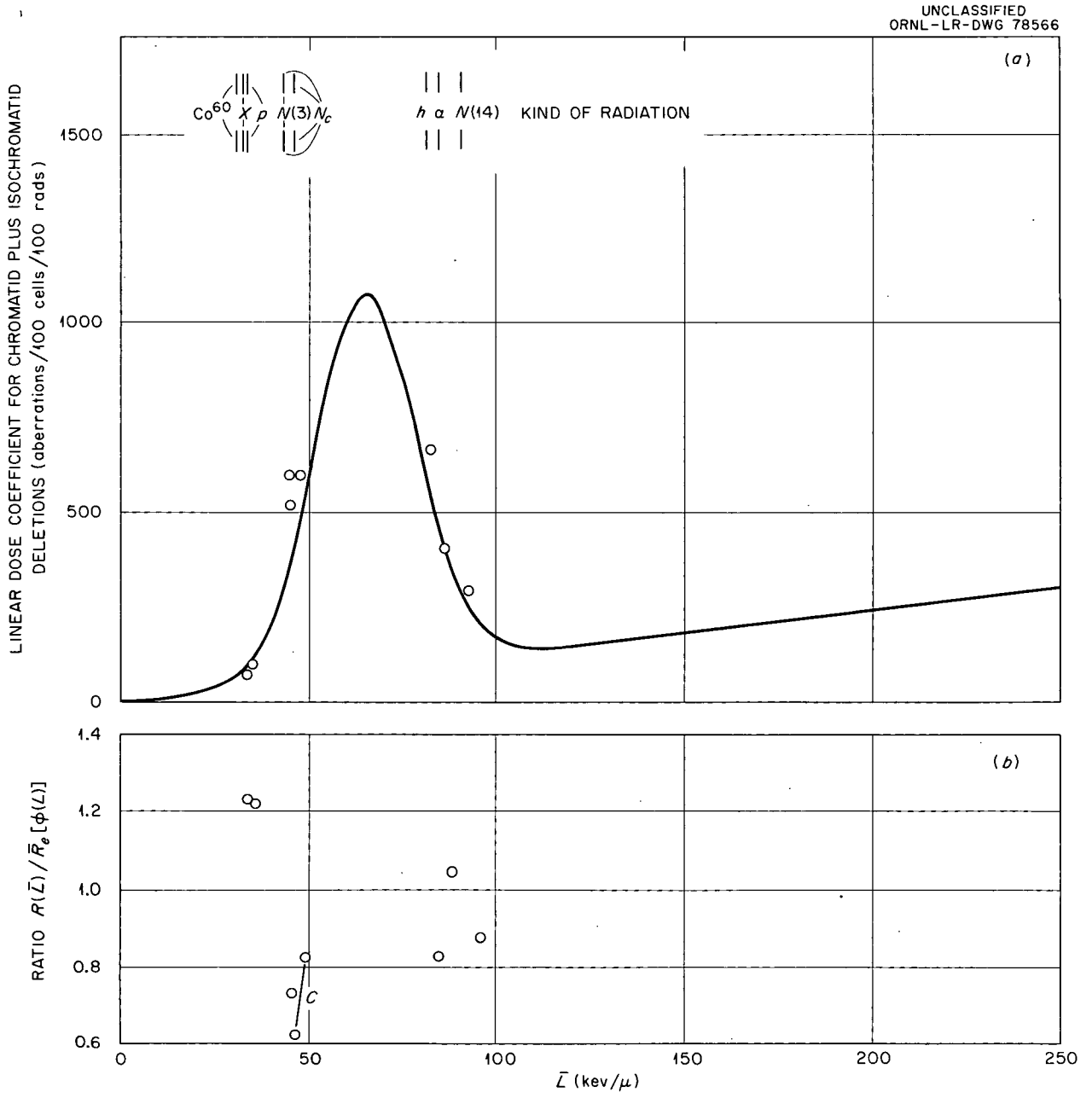

Fig. 46. (*a*) **Fit of Variation of Linear Dose Coefficient vs LET for Chromatid Deletions;** (*b*) **Plot of Theoretical to Experimental** R **Values vs Biophysical Average LET. Line is specified by Eq. (3).** The radiations were Co^{60} gamma rays (Co^{60}), 250 kvp x rays (X), 3-Mev neutrons [$N(3)$], cyclotron neutrons with average energy roughly 1 Mev (N_c) for which there are two estimates of LET spectra, radon alpha particles, 14-Mev neutrons ($N14$), and therefrom recoil protons of average initial energy 7-Mev (p), and heavy ions (h).

zymatically, and preliminary observations indicate, as suggested by previous biological data,[10,11] that (1) photoreactivation is more rapid with native than with denatured DNA and (2) photoproducts that do not contain thymine dimers are not photoreactivable.

The ability to attain high levels of H^3-thymidine incorporation into non-thymine-requiring strains of *Escherichia coli*[12] makes it possible to determine, at low uv doses, the production of dimers in radiation-resistant and -sensitive mutants and to find out if there are any dark-recovery processes that split thymine dimers.

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[10] R. B. Setlow and Jane K. Setlow, quoted in R. B. Setlow, *Brookhaven Symposia in Biol.* **14**, 1 (1961).

[11] Jane K. Setlow and R. B. Setlow, *Nature* (in press).

[12] R. P. Boyce and R. B. Setlow, *Biochim. Biophys. Acta* **61**, 618 (1962).

Tissue Culture

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SOMATIC CHROMOSOME ABERRATIONS IN HUMANS RECEIVING LOW WHOLE-BODY DOSES OF GAMMA IRRADIATION

M. A Bender P. Carolyn Gooch

Introduction. – We have previously studied the frequency of chromosome aberrations in the peripheral leukocytes of four men who received whole-body mixed gamma and fission neutron irradiation.[1] Recently, we have analyzed material from three additional normal, healthy men who were accidentally exposed to low whole-body doses of gamma radiation. The examination of such material will help to establish the correlation between aberration rates induced *in vivo* and those previously measured *in vitro*.[2]

Material. – Peripheral blood samples were obtained 3 days after the irradiation, and again approximately 4 and 14 weeks later. The dose estimates for these men were 55–58, 19–24, and 15–18 rads. Leukocyte cultures and chromosome preparations were made by methods previously described.[2] Visual scoring was performed to detect gross aberrations such as deletions, rings, and dicentrics. Karyotype analyses are being made to detect less obvious aberrations such as symmetrical translocations.

Results and Discussion. – Table 26 shows the results for visual scoring of the three sets of samples. Examination of large numbers of control cells from a number of donors has shown that the spontaneous chromosome-type deletion rate is not higher than 0.0025 per cell. We have never seen any dicentric chromosomes in cells from unirradiated people. Thus, the chromosome type aberration frequencies shown in Table 26, although not very high, may be regarded as highly significant. The marked drop in aberration frequency in the 4-week sample from individual "F," followed by a rise at 14 weeks, is unexplained. In contrast to the present material, the previously studied samples from men irradiated with mixed gamma rays and fission neutrons showed no evidence of a decrease in aberration frequency over the first 4 weeks,[1] although a decrease has since been seen in material obtained 6 weeks after irradiation. The effective integrated dose to the men may be estimated from the aberration yields using the coefficients of aberration production obtained earlier from our study of human blood irradiated *in vitro*.[2] Estimates may be made

[1]M. A Bender and P. Carolyn Gooch, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 195–97.

[2]M. A Bender and P. C. Gooch, *Proc. Natl. Acad. Sci. U.S.* **48**, 522 (1962).

Table 26. Chromosomal Aberrations in the Blood of Men Irradiated with Gamma Rays

One hundred cells scored for each of the samples.

Case	Estimated Dose (rads)	Time of Sampling	Chromatid Aberrations	Chromosome-Type Aberrations				
				$2n \neq 46$	Deletions	Dicentrics	Other[a]	Breaks[b] (%)
		3 days	3	16	11	2	0	15
K	55–58	4 weeks	3	9	0	2	1	4
		14 weeks	0	4	2	1	1	4
		3 days	1	13	4	0	1	4
P	19–24	4 weeks	1	1	0	1	4	2
		14 weeks	1	7	3	0	0	3
		3 days	2	11	10	2	0	14
F	15–18	4 weeks	4	9	1	0	1	1
		14 weeks	4	6	5	2	2	9

[a]Pericentric inversions, symmetrical translocations, etc.

[b]Deletions + twice the dicentrics; inversions, etc., are excluded because of the subjective nature of their determination.

Table 27. Dose Estimates for Irradiated Men, Based on the 3-Day Samples

Case	Physical Estimate (r)	Deletion Frequency	Dose Estimate[a] (rads)	Dicentric Frequency	Dose Estimate[b] (rads)
K	55–58	0.11	98	0.02	62
P	19–24	0.05	43	0.00	
F	15–18	0.10	89	0.02	62

[a]From $Y = 0.0025 + 0.0011D$.

[b]From $Y = 0.52 \times 10^{-5} D^2$.

independently from the deletion frequencies and from the dicentric frequencies. Dose estimates calculated from the first set of samples are shown in Table 27. Both the deletion and the dicentric data indicate that individuals "K" and "F" received similar doses of about 60 to 100 rads, while individual "P" appears to have received about half this dose. Although these estimates do not agree completely with the doses estimated from the men's film badges, neither are they in serious disagreement, particularly in view of the uncertainties involved in estimating the integrated whole-body dose from the physical dosimeter readings.

COMPARISON OF REPRODUCTIVE DEATH IN MAMMALIAN CELLS AFTER INCORPORATION OF H^3-LABELED THYMIDINE OR URIDINE

G. Marin M. A Bender

Introduction. – We have previously determined the survival kinetics of HeLa S3 cells allowed to incorporate various amounts of H^3-labeled thymidine into their DNA.[3] The beta radiation from the decay of the H^3 atom, which is responsible for

[3]G. Marin and M. A Bender, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1962*, ORNL-3352, pp 197–98.

cell killing, is largely limited to the nucleus when it is incorporated as labeled thymidine. If cells are allowed to incorporate H^3-labeled uridine into their RNA, however, the largest part of the dose is delivered to the cytoplasm. This difference may be used to determine the relative importance of nuclear and cytoplasmic irradiation in the radiation-induced death of mammalian cells.

Method. – Since RNA synthesis takes place over almost the whole cell cycle, while DNA synthesis is limited to a fraction of the interphase, it was necessary to pulse-label synchronized cell populations to achieve comparable doses. The technique of Terasima and Tolmach[4] was used to achieve synchrony of Chinese hamster cell cultures which were then exposed to constant concentrations of labeled precursor. Dose was varied by changing specific activity. Survival of plated cells was measured as the fraction of the cells plated which developed into macroscopic colonies after 12 days. To determine dose accurately, silver grain counts were made on liquid emulsion autoradiographs of aliquots of the cells used for plating. Relative autoradiographic efficiency was determined by liquid scintillation counting of aliquots of samples which were also used for autoradiographs and of suspensions of killed, unlabeled cells to which known amounts of labeled nucleoside were added.

Results and Discussion. – The uncorrected survival curves obtained for treatment with the two labeled nucleosides indicate that for equal labeling of the cells, as indicated by silver grain counts, H^3-thymidine is almost twice as effective as H^3-uridine. When appropriate corrections for autoradiographic efficiency are made and survival plotted against *nuclear* tritium, the survival curves shown in Fig. 47 result. These curves indicate that nuclear H^3-uridine is more efficient than H^3-thymidine. The efficiency of cytoplasmic irradiation is not as great as suggested by the curves, however, because of the relatively much larger dose to the cytoplasm than to the nucleus in H^3-uridine–labeled cells. It is concluded that radiation-induced cell killing of mammalian cells is largely due to nuclear damage, just as has been shown to be the case for invertebrate cells.

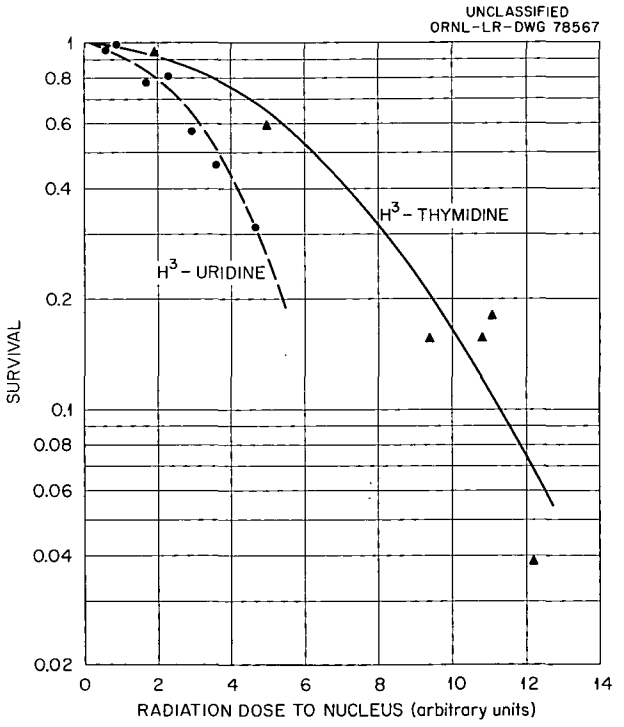

Fig. 47. Survival of Chinese Hamster Cells Pulse-Labeled with H^3-Thymidine or H^3-Uridine Plotted Against Corrected Relative Radiation Dose to the Nucleus.

[4]T. Terasima and L. J. Tolmach, *Nature* **190**, 1210 (1961).

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