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BIOLOGY DIVISION
SEMIANNUAL PROGRESS REPORT
For Period Ending February 15, 1962

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director

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Publications and Lectures

Publications. – A total of 337 papers, abstracts, and book reviews written by Biology Division members were published or are in press for this six-month period. This figure compares with 318 for the last reporting period, ending August 1961, and with 278 for the period ending February 1961.

The proceedings of the 1961 Gatlinburg Symposium, *Recovery of Cells from Injury*, were published as a supplement to the December 1961 *Journal of Cellular and Comparative Physiology*.

Listed below are the 102 papers and 3 book reviews that so far have been published. Not listed are 35 published abstracts. Also not shown are 141 papers, 53 abstracts, and 3 book reviews, all in press.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Adler, H. I., and M. S. Engel	Factors influencing the survival of bacteria after exposure to ionizing radiation	*J. Cellular Comp. Physiol.* 58 (Suppl. 1), 95–105 (1961)
Amy, R. L.	The embryology of *Habrobracon juglandis* (Ashmead)	*J. Morphol.* 109, 199–217 (1961)
Anderson, N. G., and Sr. Francis Clare Ladd	Separation of nucleotides, nucleosides, and bases from ribonucleic acid on Dowex 1-X8	*Biochim. et Biophys. Acta* 55, 275–76 (1962)
Bender, M. A, and P. Carolyn Gooch	Persistent chromosome aberrations in irradiated human subjects	*Radiation Research* 16, 44–53 (1962)
Bender, M. A, and P. Carolyn Gooch	Spontaneous and x-ray-induced somatic-chromosome aberrations in the Chinese hamster	*Intern. J. Radiation Biol.* 4, 175–84 (1961)
Bender, M. A, and P. Carolyn Gooch	An unusually long human Y chromosome	*The Lancet* 11, 463–64 (1961)
Bollum, F. J.	*The Chemistry of Nucleic Acids*, by D. O. Jordan. Butterworths, Washington, D.C., 1960, ix, 345 pp plus indices	*Arch. Biochem. Biophys.* 95, 185 (1961) (book review)
Bradford, R. H., Raymond Shapira, and D. G. Doherty	The intracellular distribution and binding of radiation-protective mercaptoalkylguanidines	*Intern. J. Radiation Biol.* 3, 595–608 (1961)
Carlson, J. G., Mary Esther Gaulden, and John Jagger	Mitotic effects of monochromatic ultraviolet irradiation of the nucleolus	In *Progress in Photobiology, Proceedings of 3rd International Congress on Photobiology* (ed. by B. Chr. Christensen and B. Buchmann), Elsevier, Amsterdam, 1961, pp 251–53
Celada, Franco, and Alicia Ramos	Passive cutaneous anaphylaxis in mice and chickens	*Proc. Soc. Exptl. Biol. Med.* 108, 129–33 (1961)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Clark, A. M.	Some effects of x rays on longevity in *Habrobracon* females	*Radiation Research* **15**, 515–19 (1961)
Clayton, R. K.	Ionization and semiconductor mechanisms in photosynthesis	In *Proceedings of the American Society of Plant Physiologists*, 1961, pp 66–81
Clayton, R. K.	Role of H_2O_2 in the photooxidative killing of blue-green mutants of *Rhodopseudomonas spheroides* (note)	*J. Bacteriol.* **82**, 314–15 (1961)
Clayton, R. K., and H. I. Adler	Protein synthesis and viability in x-irradiated *Rhodopseudomonas spheroides*	*Biochim. et Biophys. Acta* **56**, 257–67 (1952)
Cohn, W. E.	Chromatography of nucleic acids and related substances	In *Chromatography* (ed. by Erich Heftmann), Reinhold, New York, 1961, pp 554–83
Congdon, C. C., and Takashi Makinodan	Splenic white pulp alteration after antigen injection: relation to time of serum antibody production	*Am. J. Pathol.* **39**, 697–709 (1961)
Cosgrove, G. E.	The use of bone marrow cell injections in lethally irradiated mice	*Metropolitan Detroit Sci. Rev.* **23**, 33 (1962)
Cosgrove, G. E., and A. C. Upton	Some delayed effects of partial-body irradiation in mice of the RF strain	*Intern. J. Radiation Biol.* **4**, 97–103 (1961)
Crouse, Helen V.	Irradiation of condensed meiotic chromosomes in *Lilium longiflorum*	*Chromosoma* **12**, 190–214 (1961)
Cudkowicz, Gustavo	Evidence for immunization of F_1 hybrid mice against parental transplantation antigens	*Proc. Soc. Exptl. Biol. Med.* **107**, 968–72 (1961)
Cudkowicz, Gustavo	Relative inability of AET and APMT to protect immunologically competent cells against radiation injury	*Transplant. Bull.* **29**, 19–22 (1962)
Cudkowicz, Gustavo	Suppression of the foreign bone marrow reaction by preirradiation of donor mice	*Proc. Soc. Exptl. Biol. Med.* **107**, 821–24 (1961)
Davidson, Douglas	Mechanisms of reorganization and cell repopulation in meristems in roots of *Vicia faba* following irradiation and colchicine	*Chromosoma* **12**, 484–504 (1961)
DeHart, R. L., G. E. Cosgrove, and A. C. Upton	Modification of the foreign spleen reaction: amelioration of blood and tissue changes	*Lab. Invest.* **10**, 872–82 (1961)
Dent, J. N.	Cytological response of the newt pituitary gland to thyroidal depression	*General and Comparative Endocrinol.* **1**, 218–31 (1961)
Dent, J. N.	Seasonal and sexual variation in the pituitary gland of *Triturus viridescens*	*Anat. Record* **141**, 85–95 (1961)
de Serres, F. J.	Neurospora	*Science* **135**, 322–25 (1962)
de Serres, F. J., H. G. Kølmark, and H. E. Brockman	Factors influencing the survival of *Neurospora crassa* conidia in sorbose-sucrose media	*Nature* **193**, 556–57 (1962)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Dolin, M. I.	Activation of polyadenylate synthesis by basic polypeptides	*Biochem. Biophys. Research Communs.* 6, 11–15 (1961)
Dolin, M. I.	Cytochrome-independent electron transport enzymes of bacteria	In *The Bacteria*, vol. II (ed. by I. C. Gunsalus and R. Y. Stanier), Academic Press, New York, 1961, pp 425–59
Dolin, M. I.	Survey of microbial electron transport mechanisms	*Ibid.*, pp 319–63
Doria, Gino, Joan W. Goodman, Nazareth Gengozian, and C. C. Congdon	Immunologic study of antibody-forming cells in mouse radiation chimeras	*J. Immunol.* 88, 20–30 (1962)
Elias, C. A.	The x-ray sensitivity of *Escherichia coli* in the presence of β-mercaptoethylamine	*Radiation Research* 15, 632–40 (1961)
Engel, M. S., and H. I. Adler	Catalase activity, sensitivity to hydrogen peroxide, and radiation response in the genus *Escherichia*	*Radiation Research* 15, 269–75 (1961)
Garcia-Benitez, Carlos, and Sheldon Wolff	On the increase of sites for chromosome exchange formation after chromosome duplication	*Science* 135, 438–39 (1962)
Gart, J. J.	Modification of a sequential procedure for radiation protection studies with mice	*Radiation Research* 15, 616–24 (1961)
Gengozian, Nazareth, Rachel R. Carter, and W. J. Peterson	Antibody synthesis by transplanted heterologous chimera spleen cells in irradiated recipients	*J. Immunol.* 87, 209–17 (1961)
Grell, E. H.	The genetics and biochemistry of red fat cells in *Drosophila melanogaster*	*Genetics* 46, 925–33 (1961)
Grell, E. H.	The tetrasomic for chromosome 4 in *Drosophila melanogaster*	*Genetics* 46, 1177–83 (1961)
Grell, E. H.	Variation in preferential segregation of chromosome two in triploid females of *Drosophila melanogaster*	*Genetics* 46, 1267–71 (1961)
Grell, Rhoda F.	A new hypothesis on the nature and sequence of meiotic events in the female of *Drosophila melanogaster*	*Proc. Natl. Acad. Sci. U.S.* 48, 165–72 (1962)
Grell, Rhoda F.	The penetrance of sparkling-cataract	*Drosophila Info. Serv.* 35, 85 (1961)
Grimm, F. C., and D. G. Doherty	Properties of the two forms of malic dehydrogenase from beef heart	*J. Biol. Chem.* 236, 1980–85 (1961)
Haber, A. H.	Effects of indoleacetic acid on growth without mitosis & on mitotic activity in absence of growth by expansion	*Plant Physiol.* 37, 18–26 (1962)
Hastings, J. W., Lazarus Astrachan, and B. M. Sweeney	A persistent daily rhythm in photosynthesis	*J. Gen. Physiol.* 45, 69–76 (1961)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Hochman, Benjamin	On fourth chromosome lethals from a natural population of *Drosophila melanogaster*	*Am. Naturalist* **95**, 375–82 (1961)
Hochman, Benjamin	On the viability of the brown-variegated ^{1}/brown-variegated^{57e} heterozygote	*Drosophila Info. Serv.* **35**, 85–86 (1961)
Hollaender, Alexander	Finsen and basic research (and Introduction to the Proceedings)	In *Progress in Photobiology, Proceedings of 3rd International Congress on Photobiology* (ed. by B. Chr. Christensen and B. Buchmann), Elsevier, Amsterdam, 1961, pp 5–11 and v-vi
Hollaender, Alexander	*Studies on Quantitative Radiation Biology*, by K. G. Zimmer, translated by H. D. Griffith. Oliver and Boyd, Edinburgh, 1961, 124 pp, illustrations	*Science* **134**, 1233 (1961) (book review)
Hollaender, Alexander	Symposium on Recovery of Cells from Injury – Introduction	*J. Cellular Comp. Physiol.* **58** (Suppl. 1), 248 pages, December 1961
Hollaender, Alexander, and S. F. Carson	*Biology Division Semiannual Progress Report for Period Ending August 15, 1961*	ORNL-3201
Kenney, F. T., and R. M. Flora	Induction of tyrosine-α-ketoglutarate transaminase in rat liver. I. Hormonal nature	*J. Biol. Chem.* **236**, 2699–2702 (1961)
Kimball, R. F.	Postirradiation processes in the induction of recessive lethals by ionizing radiation	*J. Cellular Comp. Physiol.* **58** (Suppl. 1), 163–70 (1961)
Kirby-Smith, J. S., and M. L. Randolph	Modification of radiation-induced electron spin resonances in dry materials	*Ibid.*, pp 1–11
Kondo, Sohei	Responses of silver-activated phosphate glass to α, β, γ rays and neutrons	*Health Physics* **7**, 25–35 (1961)
Lion, M. B., J. S. Kirby-Smith, and M. L. Randolph	Electron-spin resonance signals from lyophilized bacterial cells exposed to oxygen	*Nature* **192**, 34–36 (1961)
Makinodan, Takashi, M. A. Kastenbaum, and W. J. Peterson	Radiosensitivity of spleen cells from normal and preimmunized mice and its significance to intact animals	*J. Immunol.* **88**, 31–37 (1962)
Makinodan, Takashi, and W. J. Peterson	Relative antibody-forming capacity of spleen cells as a function of age	*Proc. Natl. Acad. Sci. U.S.* **48**, 234–38 (1962)
Mazur, Peter	Manifestations of injury in yeast cells exposed to subzero temperatures. I. Morphological changes in freeze-substituted and in "frozen-thawed" cells	*J. Bacteriol.* **82**, 662–72 (1961)
Mazur, Peter	Manifestations of injury in yeast cells exposed to subzero temperatures. II. Changes in specific gravity and in the concentration and quantity of cell solids	*J. Bacteriol.* **82**, 673–74 (1961)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Monesi, Valerio	Relazione fra la sensibilitá ai raggi X ed il ciclo mitotico degli spermatogoni del topo (relationship of x-ray sensitivity to the mitotic cycle in spermatogonia of the mouse – publ. in Italian)	*Bol. zool.* 28, 227–34 (1961)
Novelli, G. D.	The biosynthesis of coenzyme A	In *Biochemists' Handbook* (ed. by Cyril Long), D. Van Nostrand, Princeton, N.J., 1961, pp 615–16
Novelli, G. D.	The use of radiation and radioisotopes to study biochemical processes	In *Applications of Radioisotopes and Radiation in the Life Sciences – Hearings Before the Subcommittee on Research, Development, and Radiation of the Joint Committee on Atomic Energy of the Congress of the United States, 87th Congress,* Chet Holifield, Chairman (Joint Committee), Melvin Price, Chairman (Subcommittee). GPO, Washington, D.C., 1961, pp 277–95
Novelli, G. D., Tadanori Kameyama, and J. M. Eisenstadt	The effect of ultraviolet light and x rays on an enzyme-forming system	*J. Cellular Comp. Physiol.* 58 (Suppl. 1), 225–44 (1961)
Novitski, E., and G. D. Hanks	Analysis of irradiated *Drosophila* populations for meiotic drive	*Nature* 190, 989–90 (1961)
Oakberg, E. F.	Population dynamics of irradiated spermatogonia (summary)	*Intern. J. Radiation Biol.* 4, 214 (1961)
Oakberg, E. F., and Evelyn Clark	Effect of dose and dose rate on radiation damage to mouse spermatogonia and oocytes as measured by cell survival	*J. Cellular Comp. Physiol.* 58 (Suppl. 1), 173–82 (1961)
Odell, T. T., Jr.	Platelet labeling with radioisotopes and *in vivo* platelet survival	In *Henry Ford Hospital International Symposium – Blood Platelets* (ed. by S. A. Johnson, R. W. Monto, J. W. Rebuck, and R. C. Horn), Little, Brown, Boston, 1961, pp 643–49
Odell, T. T., Jr., T. P. McDonald, and T. C. Detwiler	Stimulation of platelet production by serum of platelet-depleted rats	*Proc. Soc. Exptl. Biol. Med.*, 108, 428–31 (1961)
Odell, T. T., Jr., and A. C. Upton	Late effects of internally deposited radioisotopes	In *Radioactive Isotopes in Physiology, Diagnostics, and Therapy,* 2d ed. (ed. by H. Schwiegk and F. Turba), Springer-Verlag, Berlin, Gottingen, Heidelberg, 1961, pp 375–92

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Parsons, D. F.	A simple method for obtaining increased contrast in araldite sections by using postfixation staining of tissues with potassium permanganate	*J. Biophys. Biochem. Cytol.* **11**, 492–97 (1961)
Parsons, D. F., and E. B. Darden	Optimal conditions for methacrylate embedding of certain tissues and cells sensitive to polymerization damage	*Exptl. Cell Research* **24**, 466–83 (1961)
Peck, H. D., Jr.	Enzymatic basis for assimilatory and dissimilatory sulfate reduction	*J. Bacteriol.* **82**, 933–39 (1961)
Peck, H. D., Jr.	The role of adenosine-5′-phosphosulfate in the reduction of sulfate to sulfite by *Desulfovibrio desulfuricans*	*J. Biol. Chem.* **237**, 198–203 (1962)
Peck, H. D., Jr., and Earl Fisher, Jr.	The oxidation of thiosulfate and phosphorylation in extracts of *Thiobacillus thioparus*	*J. Biol. Chem.* **237**, 190–97 (1962)
Phan The Tran and M. A Bender	Preservation of viable bone marrow by freezing	In *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, pp 59–64
Popp, R. A.	Competence of retransplanted homologous marrow cells in relation to time after original transplantation into irradiated mice	*Intern. J. Radiation Biol.* **4**, 155–61 (1961)
Popp, R. A.	Repopulation of thymus by immunologically competent cells derived from donor marrow	*Proc. Soc. Exptl. Biol. Med.* **108**, 561–64 (1961)
Prescott, D. M., and M. A Bender	Preparation of mammalian metaphase chromosomes free of cytoplasm	*Exptl. Cell Research* **25**, 222–23 (1961)
Randolph, M. L.	Physical aspects of radiobiological damage	*Nuclear Safety* **3**, 12–15 (1961)
Randolph, M. L., and A. H. Haber	Production and decay of free radicals induced by x-irradiation of dry lettuce seeds	In *Effects of Ionizing Radiations on Seeds, Proceedings Series, IAEA*, Kaerntnerring, Vienna, Austria, 1961, pp 57–65
Russell, Liane B.	Radiation hazards during embryonic development	In *Transactions of the IXth International Congress of Radiology*, vol. II (ed. by B. Rajewsky), Georg Thieme, Stuttgart, Germany, 1961, pp 1212–19
Russell, Liane B., and Clyde Lee Saylors	Induction of paternal sex chromosome losses by irradiation of mouse spermatozoa	*Genetics* **47**, 7–10 (1962)
Russell, W. L.	Effect of radiation dose rate on mutation in mice	*J. Cellular Comp. Physiol.* **58** (Suppl. 1), 183–87 (1961)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Russell, W. L.	Recent findings in mammalian radiation genetics	In *Transactions of the IXth International Congress of Radiology*, vol. II (ed. by B. Rajewsky), Georg Thieme, Stuttgart, Germany, 1961, pp 1087–89
Setlow, Jane K., and R. B. Setlow	Ultraviolet action spectra of ordered and disordered DNA	*Proc. Natl. Acad. Sci. U.S.* **47**, 1619–27 (1961)
Setlow, R. B.	Macromolecular sensitivity	In *Brookhaven Symposia in Biol., No. 14: Fundamental Aspects of Radiosensitivity*, Brookhaven National Laboratory, Upton, New York, 1961, pp 1–17
Stuy, J. H.	Radiation inactivation of intracellular transforming deoxyribonucleic acid	Centrex Publishing Ltd., Eindhoven, The Netherlands, 1961, 86 pp
Swartzendruber, D. C., R. R. Bigelow, C. C. Congdon, and Takashi Makinodan	Effect of removal of lymphatic tissue on immune response in mice	*Am. J. Physiol.* **200**, 1272–76 (1961)
Upton, A. C.	Biological basis for routine exposure criteria	*Nuclear Safety* **2**, 1–4 (1961)
Upton, A. C.	Cancer in the world around us: radiations, natural and man-made	In *Fourth National Cancer Conference Proceedings*, Lippincott, Philadelphia, 1961, pp 153–60
Upton, A. C.	Changing concepts concerning cancer: radiation as an etiologic factor	*Ibid.*, pp 63–69
Upton, A. C.	*Radiation Biophysics*, by Howard L. Andrews. Prentice-Hall, Englewood Cliffs, New Jersey, 1961, 328 pp, 105 illus.	*Nuclear Science and Engineering* **11**, 348 (1961) (book review)
Upton, A. C., F. F. Wolff, and E. P. Sniffen	Leukemogenic effect of myleran on the mouse thymus	*Proc. Soc. Exptl. Biol. Med.* **108**, 464–67 (1961)
Volkin, Elliot	Synthesis and function of the DNA-like RNA	*Federation Proc.* **21**, 112–19 (1962)
von Borstel, R. C.	Induction of nuclear damage by ionizing and ultraviolet radiation	In *Progress in Photobiology, Proceedings of 3rd International Congress on Photobiology* (ed. by B. Chr. Christensen and B. Buchmann), Elsevier, Amsterdam, 1961, pp 243–50
von Borstel, R. C., and Margaret Fine	A Medium suitable for hatchability and eclosion tests	*Drosophila Info. Serv.* **35**, 107 (1961)
Wells, Carolyn	Evidence for micronuclear function during vegetative growth and reproduction of the ciliate, *Tetrahymena pyriformis*	*J. Protozool.* **8**, 284–90 (1961)
Welshons, W. J., B. H. Gibson, and B. J. Scandlyn	Slide processing for the examination of male mammalian meiotic chromosomes	*Stain Technol.* **37**, 1–5 (1962)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Wolff, Sheldon	Some aspects of radiation genetics (chapter 6)	In *Mechanisms in Radiobiology*, vol. I (ed. by M. Errera and A. Forssberg), Academic Press, New York, 1961, pp 419–75
Wolff, Sheldon	Some postirradiation phenomena that affect the induction of chromosome aberrations	*J. Cellular Comp. Physiol.* 58 (Suppl. 1), 151–62 (1961)
Wolff, Sheldon, and H. E. Luippold	On the apparent synergistic effect of far-red and x rays in the production of chromatid aberrations	In *Progress in Photobiology, Proceedings of 3rd International Congress on Photobiology* (ed. by B. Chr. Christensen and B. Buchmann), Elsevier, Amsterdam, 1961, pp 457–60
Wolff, Sheldon, and A. M. Sicard	Postirradiation storage and the growth of barley seedlings	In *Effects of Ionizing Radiations on Seeds, Proceedings Series, IAEA*, Kaerntnerring, Vienna, Austria, 1961, pp 171–79
Wust, C. J.	Coenzyme protection of tryptophan synthetase from antibody neutralization	*Biochim. et Biophys. Acta* 54, 172–78 (1961)
Wust, C. J., and D. M. Bonner	Apparent heterogeneity of tryptophan synthetase in *Neurospora crassa*	*Biochim. et Biophys. Acta* 54, 165–72 (1961)
(Members of the Biology Division)	*Summary of Reports of Bone Marrow Conferences Organized by Members of the Biology Division, ORNL*	Reprint No. 1982

Lectures. – During the reporting period August 1961 through February 1962, members of the Biology Division presented 135 lectures. This figure compared with 116 for the August–February period last year. Of the 135, a total of 36 was given under the Traveling Lecture program; 34 by invitation of universities, colleges, or research organizations; and 65 before professional societies, including 6 given in foreign countries – Chile, Czechoslovakia, Italy, and Mexico. Six Traveling Lectures, 3 invited lectures, and 3 speeches before a professional meeting were presented in Puerto Rico. In addition to the talks listed below, members of the Division served as chairmen, discussion leaders, and invited participants in meetings, symposia, and round-table discussions.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Adler, H. I.	Factors influencing bacterial survival after ionizing radiation	Research Laboratory of the General Electric Company, Schenectady, N.Y.
Adler, H. I.	Genetic aspects of radiation sensitivity in bacteria	1. University of Florida, Gainesville 2. Tuskegee Institute, Alabama 3. University of Kentucky, Lexington
Adler, H. I.	Physiological aspects of radiation sensitivity in bacteria	University of Florida, Gainesville
Adler, H. I. (and J. C. Copeland)	Genetic analysis of x-ray resistance in *Escherichia coli*	Kentucky-Tennessee Branch, Am. Soc. Microbiol., Lexington, Ky.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Anderson, N. G.	Isolation of liver cell components in the density-gradient ultracentrifuge	Biophysical Society, Washington, D.C.
Anderson, N. G.	The organization of living systems	Davidson College, Davidson, N.C.
Anderson, N. G. (and C. L. Burger)	Cell fractionation in the density-gradient ultracentrifuge	Am. Soc. Cell Biol., Chicago, Ill.
Arnold, W. A.	Solid state phenomena in photosynthesis	Research Laboratory of the General Electric Company, Schenectady, N.Y.
Astrachan, Lazarus	Resemblance of bacterial RNA to DNA	Purdue University, Lafayette, Ind.
Becker, R. R.	Properties of polypeptidyl-protein derivatives	Auburn University, Auburn, Ala.
Bender, M. A	Preservation of mouse marrow	Conference on Cryobiology and Methods of Preserving Living Tissues, Buffalo, N.Y.
Bender, M. A	Radiation-induced chromosome aberrations in human cells *in vitro* and *in vivo*	University of Texas M. D. Anderson Hospital and Tumor Institute, Houston
Bender, M. A (and P. Carolyn Gooch)	Chromosome aberrations in human blood irradiated *in vitro* [*Genetics* 46, 851 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Bender, M. A (and D. M. Prescott)	DNA, RNA, and protein synthesis, and the onset of mitosis in human leukocytes *in vitro*	Blood-Bone Marrow Tissue Culture and Cell Separation Conference, Bethesda, Md.
Bertsch, W. F.	The photoinhibition of etiolated stem segments	University of Puerto Rico, Rio Piedras
Bertsch, W. F.	Solid state phenomena in photosynthetic systems	Puerto Rico Nuclear Center, Mayaguez
Bollum, F. J.	Primers for DNA synthesis	Purdue University, Lafayette, Ind.
Bollum, F. J.	Studies on DNA primers: effect of ultraviolet and x radiation	University of Wisconsin, Madison
Bollum, F. J. (and R. B. Setlow)	Effect of monochromatic UV radiation on "primer" DNA	Biophysical Society, Washington, D.C.
Bond, H. E.	A sex-associated protein in liver tissues of the rat and its response to castration and hormone treatment [*Am. Zool.* 1, 344 (1961)]	AIBS, Am. Soc. Zool., Lafayette, Ind.
Carson, S. F.	1. Hydrogen transfer reactions and hydrogen balance in fermentation chemistry 2. Principles of isotope tracer experiments and interpretations in studies on microbial metabolism	University of Georgia, Athens
Celada, Franco (and Takashi Makinodan)	Homograft-rejecting vs antibody-synthesizing capacities of lymphoidal cells	IV International Congress of Allergology, New York, N.Y.
Celada, Franco (and Takashi Makinodan)	Radiosensitivity of homograft-rejecting and antibody-synthesizing capacities of isolated cells: its significance to tolerance [*Folia Biologica* 7, 417 (1961)]	Symposium on Mechanisms of Immunological Tolerance, Prague, Czechoslovakia

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Chu, E. H. Y.	Dose relations in the induction of human chromosome aberrations	Conference on Biophysical and Biochemical Mechanisms in the Production of Radiation-Induced Chromosome Aberrations, San Juan, Puerto Rico (sponsored by Natl. Acad. Sci.–Natl. Research Council)
Chu, E. H. Y. (J. H. Rubinstein, and Josef Warkany)	Chromosome studies in a family with four cases of atypical mongolism [*2nd Intern. Conf. Human Genetics, Rome 1961* (Abstracts) Excerpta Medica Foundation, Amsterdam, 1961, pp E96–E97]	2nd International Conference on Human Genetics, Rome, Italy
Clayton, R. K.	1. Induced synthesis of catalase in photosynthetic bacteria 2. Peroxide and catalase in the radiobiology of *Rhodopseudomonas spheroides* 3. Primary reactions in bacterial photosynthesis	University of California, Davis
Clayton, R. K.	Primary reactions in bacterial photosynthesis	1. Brookhaven National Laboratory, Upton, Long Island, N.Y. 2. Massachusetts Institute of Technology, Cambridge 3. Dartmouth College, Hanover, N.H.
Clayton, R. K.	Primary reactions in photosynthesis	Symposium on The Excited State and Photobiology, Biophysical Society, Washington, D.C.
Cohn, W. E.	Pseudouridine, structure and metabolism	1. Research Division of Charles Pfizer and Company, Terre Haute, Ind. 2. Argonne National Laboratory, Ill.
Cohn, W. E.	Some aspects of the metabolism of pseudouridine	Tufts Medical School, Boston, Mass.
Congdon, C. C.	Lymphatic tissues and the suppression of the immune mechanism	Bone Marrow Transplantation Conference on Chemical Suppression of the Immune Response (sponsored by the Biology Division, ORNL), New York, N.Y.
Congdon, C. C.	Modification of transplant immunity	29th Annual Scientific Assembly, the Medical Society of the District of Columbia, Washington, D.C.
Congdon, C. C. (and Joan W. Goodman)	Changes in lymphatic tissues during foreign tissue transplantation	International Symposium on Tissue Transplantation Problems, Santiago, Chile (sponsored by scientific societies and the University of Chile)
Copeland, J. C. (and H. I. Adler)	Transfer of UV (2537 A) resistance in mating strains of *Escherichia coli*	Kentucky-Tennessee Branch, Am. Soc. Microbiol., Lexington, Ky.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Cromroy, H. L.	Modification of ultraviolet-induced aberration frequencies in *Tradescantia* pollen [*Genetics* 46, 860–61 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Cudkowicz, Gustavo	Suppression of the homologous disease in mouse chimeras by preirradiation of donor cells	Bone Marrow Transplantation Conference on Chemical Suppression of the Immune Response (sponsored by the Biology Division, ORNL), New York, N.Y.
Doherty, D. G.	Protection against ionizing radiation by organic sulfur compounds	Fifth Organic Chemistry Conference on Organic Sulfur Compounds, Natick, Mass.
Doria, Gino	Development of transplantation antigenicity in mouse hemopoietic tissues	Fifth Tissue Homotransplantation Conference, New York, N.Y. (sponsored by the New York Academy of Sciences)
Doria, Gino	A quantitative bio-assay for homograft reaction [*Genetics* 46, 861–62 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Ehling, U. H. (and M. L. Randolph)	Skeletal abnormalities in offspring of irradiated male mice [*Genetics* 46, 863 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Eisenstadt, J. M. (and G. D. Novelli)	The role of gene-specific DNA in the cell-free synthesis of β-galactosidase	AAAS, Physiological and Biochemical Aspects of Human Genetics, Denver, Colo.
Finamore, F. J. (and Elliot Volkin)	Separation and characterization of amphibian egg nucleic acids [Abstr. of Papers, 140th Mtg., Am. Chem. Soc. (1961), p 53C]	140th Meeting, American Chemical Society, Chicago, Ill.
Gardiner, D. A.	Application of response surface techniques to the preservation of mammalian cells	Conference on Cryobiology and Methods of Preserving Living Tissues, Buffalo, N.Y.
Gooch, P. Carolyn	Induced and spontaneous chromosome aberrations in human leukocytes irradiated *in vivo* and *in vitro*	Blood-Bone Marrow Tissue Culture and Cell Separation Conference, Bethesda, Md.
Goodman, Joan W. (and G. S. Hodgson)	Evidence for stem cells in the peripheral blood of mice	Blood-Bone Marrow Tissue Culture and Cell Separation Conference, Bethesda, Md.
Haber, A. H.	Effects of indoleacetic acid on growth without mitosis and on mitotic activity in the absence of growth by expansion [*Plant Physiol.* 36 (Suppl), xl (1961)]	AIBS, Am. Soc. Plant Physiol., Lafayette, Ind.
Haber, A. H.	Morphogenesis and the cell theory	Southern Section, Am. Soc. Plant Physiol., Jacksonville, Fla.
Hollaender, Alexander	Comparison of biological research in Japan, Italy, and Russia	University of Tennessee, Knoxville

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Hollaender, Alexander	Introductory remarks	Bone Marrow Transplantation Conference on Chemical Suppression of the Immune Response (sponsored by the Biology Division, ORNL), New York, N.Y.
Hollaender, Alexander	Recent developments in the border fields of basic biology	International Symposium on Tissue Transplantation Problems, Santiago, Chile (sponsored by scientific societies and the University of Chile)
Kenney, F. T.	Hormonal control of enzyme formation	University of Georgia, Athens
Khym, J. X.	Addition of methylamine between the 2′ and 3′ carbon atoms of periodate-oxidized adenosine 5′-phosphate to form a cyclic tertiary amine [Abstr. of Papers, 140th Mtg., Am. Chem. Soc. (1961), p 4D]	140th Meeting, American Chemical Society, Chicago, Ill.
Kimball, R. F. (and Stella W. Perdue)	Autoradiographic studies of nucleic acid synthesis in *Paramecium aurelia*	Am. Soc. Cell Biol., Chicago, Ill.
Kimball, R. F. (and Stella W. Perdue)	Further studies on the refractory period for mutation induction in *Paramecium aurelia* [*Genetics* 46, 875 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Kirby-Smith, J. S.	Chromosome aberrations by combined x- and ultraviolet radiations	Conference on Biophysical and Biochemical Mechanisms in the Production of Radiation-Induced Chromosome Aberrations, San Juan, Puerto Rico (sponsored by Natl. Acad. Sci.–Natl. Research Council)
Krieg, D. R.	Ethyl methanesulphonate-induced phage reversions	McArdle Memorial Laboratory, University of Wisconsin, Madison
Lindsley, D. L.	Genetic effects of chromosome aberrations	Conference on Biophysical and Biochemical Mechanisms in the Production of Radiation-Induced Chromosome Aberrations, San Juan, Puerto Rico (sponsored by Natl. Acad. Sci.–Natl. Research Council)
Lindsley, D. L.	Resume of the Conference on Biophysical and Biochemical Mechanisms in the Production of Radiation-Induced Chromosome Aberrations	Puerto Rico Nuclear Center, Rio Piedras
Löbbecke, E. -A. (and R. C. von Borstel)	Differential sensitivity of *Habrobracon* oocytes in the first meiotic prophase and metaphase to chemical mutagens [*Genetics* 46, 879 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
McDonald, T. P.	Civil defense and acute effects of radiation	Rotary Club, Lenoir City, Tenn.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Makinodan, Takashi	Studies on immune reactions in *in vivo* cultures	International Symposium on Tissue Transplantation Problems, Santiago, Chile (sponsored by scientific societies and the University of Chile)
Makinodan, Takashi	Kinetics of antibody formation	University of California Medical Center, San Francisco
Mans, Rusty J.	Biosynthesis of protein by cell-free systems from plants	1. University of Florida, Gainesville 2. Florida State University, Tallahassee 3. Florida Southern College, Lakeland
Mans, Rusty J.	Factors involved in the cell-free incorporation of amino acids into maize protein [*Plant Physiol.*, Proc. Ann. Mtg. 36 (Suppl.), xvii-xviii (1961)]	AIBS, Am. Soc. Plant Physiol., Lafayette, Ind.
Mans, Rusty J.	Incorporation of amino acids into mazie protein	University of Florida, Gainesville
Mattingly, Sr. M. A.	Basic protein synthesis in the meristematic nucleus	AAAS, Botanical Sciences, Denver, Colo.
Mazur, Peter	Detection of intracellular ice formation in yeast cells by differential thermal analysis and conductometry	Biophysical Society, Washington, D.C.
Mazur, Peter	Physical mechanisms involved in cell death at subzero temperatures	Conference of Cryobiology and Methods of Preserving Living Tissues, Buffalo, N.Y.
Miller, O. L., Jr.	Chromosome and nucleolar structure in *Triturus* oocytes	Am. Soc. Cell Biol., Chicago, Ill.
Monesi, Valerio	Relation of x-ray sensitivity to mitotic cycle in spermatogonia of the mouse [*Genetics* 46, 884 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Monesi, Valerio	Study of DNA synthesis and the mitotic cycle in spermatogonia of the mouse, using tritium-labeled thymidine	AIBS, Am. Soc. Zool., Lafayette, Ind.
Novelli, G. D.	The biological and chemical uses of isotopes	Smith College, Northampton, Mass.
Novelli, G. D.	The effect of ultraviolet and x-irradiation on the induced synthesis of β-galactosidase in *E. coli* and the relationship to antibody synthesis	International Symposium on Effects of Ionizing Radiation on Immune Processes, Lawrence, Kan.
Novelli, G. D.	1. The effect of ultraviolet light on the synthesis of induced β-galactosidase in whole cells of *Escherichia coli*	University of Puerto Rico, Rio Piedras (series of 3 lectures)
	2. The incorporation of radioactive amino acids into protein by cell-free preparations	University of Puerto Rico, Mayaguez (series of 3 lectures)
	3. The role of gene-specific DNA in cell-free protein synthesis	

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Novelli, G. D.	Protein synthesis and antibody formation	Am. Acad. Allergy, Denver, Colo.
Novelli, G. D.	The synthesis of β-galactosidase in cell-free preparations	Vanderbilt University, Nashville, Tenn.
Oakberg, E. F.	Radiation damage to the gonads and its bearing on subsequent fertility and genetic effects	1. Annual Crittendon Lecture, Kansas City Gynecological Society, Kansas City, Mo. 2. University of Alabama, University, Ala. 3. Sigma Xi, University of Alabama Medical Center, Birmingham
Oakberg, E. F. (and Evelyn Clark)	Meiotic prophase stage and species differences in radiation response of the mammalian primary oocyte	AAAS, Radiation Biology, Denver, Colo.
Oakberg, E. F. (and Evelyn Clark)	Survival of spermatogonia of the mouse at different x- and gamma-ray dose rates [*Genetics* 46, 888 (1961]	AIBS, Genetics Society, Lafayette, Ind.
Odell, T. T., Jr.	Acute and late somatic effects of radiation in mammals	Hollins College, Hollins College, Va.
Odell, T. T., Jr. (T. P. McDonald, T. C. Detwiler, and F. L. Howsden)	Platelet response to natural and foreign agents	Am. Soc. Hematol., Los Angeles, Calif.
Peck, H. D., Jr.	The role of sulfur-containing nucleotides in the metabolism of inorganic sulfur compounds	Washington University, St. Louis, Mo.
Perkins, E. H.	Studies on radiation-protective and antibody-synthesizing capacities of hematopoietic cells	Auburn University, Auburn, Ala.
Peterson, W. J.	The effect of antigen dose on antibody formation as a function of time	Medical Section, Tennessee Academy of Science, Martin, Tenn.
Popp, R. A.	Inheritance of different serum esterase patterns among inbred strains of mice [*Genetics* 46, 890 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Popp, R. A.	The inheritance of different physico-chemical properties of mouse hemoglobins	The University of North Carolina, Chapel Hill
Prescott, D. M.	Protein of the chromosome	University of Texas, Austin
Prescott, D. M.	Tritiated precursors employed for the study of nuclear function and nuclear replication	Conference on Radioisotopes in Animal Biology and the Medical Sciences, Mexico City, Mexico (sponsored by IAEA)
Randolph, M. L.	Calculations of absorbed dose to fast neutron flux at above 10 Mev	Biophysical Society, Washington, D.C.
Roesel, Hilde A. (and A. H. Haber)	Gibberellin sensitivity, endogenous growth and maturity of wheat coleoptiles	Southern Section, Am. Soc. Plant Physiol., Jacksonville, Fla.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Russell, Liane B. (and C. L. Saylors)	Spontaneous and induced abnormal sex-chromosome number in the mouse [*Genetics* 46, 894 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Russell, W. L. (and E. M. Kelly)	Mutation frequency in mice exposed to radiation of intermediate dose rate [*Genetics* 46, 894 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Schwartz, Drew	Genetic studies on proteins	Cornell University, Ithaca, N.Y.
Setlow, Jane K.	A possible method for determining an altered base in T4 bacteriophage	Biophysical Society, Washington, D.C.
Setlow, R. B.	Molecular biophysics	1. University of Cincinnati, Cincinnati, Ohio 2. University of Kentucky, Lexington
Setlow, R. B.	The production of thymine dimers in DNA by ultraviolet irradiation	Biophysical Society, Washington, D.C.
Stevens, Jean	The fine structure of nucleolar formation during mitosis	Am. Soc. Cell. Biol., Chicago, Ill.
Thompson, P. E. (read by title)	The effect of asynapsis on mutability in the testis of *Drosophila* [*Genetics* 46, 904 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Upton, A. C.	Observations on the delayed somatic effects of ionizing radiation in the mouse	New England Deaconess Hospital, Boston
von Borstel, R. C.	Chromosome structure and function	1. University of Arkansas, Fayetteville 2. Spring Hill College, Mobile, Ala.
von Borstel, R. C.	The future of biology	College of the Ozarks, Clarksville, Ark.
von Borstel, R. C.	Genetic control of populations	North Carolina State College, Raleigh
von Borstel, R. C.	How radiation kills cells	1. Southern University, Baton Rouge, La. 2. Louisiana State University, Baton Rouge, La. 3. University of Arkansas, Fayetteville 4. Oklahoma State University, Stillwater 5. University of Oklahoma, Norman
von Borstel, R. C.	Mutational research on the wasp *Habrobracon*	North Carolina State College, Raleigh
von Borstel, R. C.	Physiological genetics in entomology	Entomological Society, Miami, Fla.
von Borstel, R. C.	Spontaneous and induced mutations	Sigma Xi, Louisiana State University, Baton Rouge
von Borstel, R. C. (and G. Magni)	Spontaneous mutation rates in vegetative and sporulating yeast [*Genetics* 46, 879–80 (1961)]	AIBS, Genetics Society, Lafayette, Ind.
Welshons, W. J.	The genetic nature of mutations at specific loci	1. Florida State University, Tallahassee 2. University of Florida, Gainesville
Welshons, W. J.	Sex determination in mammals	University of Alabama, Tuscaloosa

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Wolff, Sheldon	Biophysical aspects of chromosome production	Washington University, St. Louis, Mo.
Yamada, Tuneo	An approach to the problem of differentiation	The University of North Carolina, Chapel Hill

Visiting Lecturers. – During the six months preceding this report, 32 lectures were given on the Biology Seminar program by guest speakers from scientific institutions and universities at home and abroad. Visiting lecturers included scientists from Austria, England, Germany, Italy, Japan, The Netherlands, and Norway.

SPEAKER	AFFILIATION	SUBJECT
Richard Biebl	Pflanzenphysiologisches Institut der Universität Wien, Wien, Austria	Protection against the effect of short-wave UV-rays on plant cells
Victor P. Bond	Medical Research Center, Brookhaven National Laboratory, Upton, L.I., N.Y.	Kinetics of erythropoietic cells studied with tritiated thymidine
W. Elwood Briles	Blood Typing Laboratory, DeKalb Agricultural Assoc. Inc., DeKalb, Ill.	Blood group genetics of chickens
P. R. J. Burch	University of Leeds, Leeds, England (Speaker presently with Health Physics Division of ORNL)	A theory of natural carcinogenesis with reference to cytogenetic, immunological, and deletion factors
P. R. J. Burch	University of Leeds, Leeds, England (Speaker presently with Health Physics Division of ORNL)	The relation of radiation to natural carcinogenesis
Elof Carlson	Department of Zoology, University of California, Los Angeles, Calif.	Mutagenesis at the dumpy locus in *Drosophila melanogaster*
Ruggero Ceppellini	Instituto di Genetica Medica dell' Universita di Torino, Torino, Italy	Genetics and antibody specificity
Sidney P. Colowick	Department of Microbiology, School of Medicine, Vanderbilt University, Nashville, Tenn.	Some properties of yeast hexokinase
Renato Dulbecco	Division of Biology, California Institute of Technology, Pasadena, Calif.	Recent studies on transformation of mammalian cells by the polyoma virus
L. N. M. Duysens	Biophysical Laboratory of the State University, Leiden, The Netherlands	Function of two pigment systems in photosynthesis
Richard Epstein	Biology Division, California Institute of Technology, Pasadena, Calif.	Phage mutants with host-sensitive reproduction
G. Fankhauser	Department of Biology, Princeton University, Princeton, N.J.	Unbalanced chromosome complements and development
Joseph Gall	Department of Zoology, University of Minnesota, Minneapolis, Minn.	Studies on the organization of chromosomes
Edward Glassman	Department of Biochemistry and Nutrition, School of Medicine, University of North Carolina, Chapel Hill, N.C.	Eye-color pigments in *Drosophila*

SPEAKER	AFFILIATION	SUBJECT
Walter Herndon	Head, Botany Department, University of Tennessee, Knoxville, Tenn.	Factors related to the production of motile cells in green algae in cultures
Robert T. Hersh	Department of Biochemistry, University of Kansas, Lawrence, Kan.	Physical studies of ribosomes
George S. Hurst	Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.	Electron interactions in molecular gases
T. C. Hsu	M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston	Evolution of cell population
Yukiaki Kuroda	Department of Genetics, Faculty of Medicine, Osaka University, Osaka, Japan	Tissue culture of *Drosophila* in synthetic medium
L. F. Lamerton	Department of Physics, Institute of Cancer Research, Royal Cancer Hospital, Belmont, Sutton, Surrey, England	The response of certain tissues to continuous irradiation
Giovanni E. Magni	Instituto de Genetica, Universita di Pavia, Pavia, Italy	The number and the nature of recessive lethal mutations induced by x rays in yeast
Alexander Pihl	Norsk Hydro's Institute for Cancer Research, Norwegian Radium Hospital, Oslo, Norway	Studies on the chemical reactivity and radiosensitivity of enzyme sulfhydryl groups
Sam Price	Agricultural Research Service, Crops Research Division, U.S. Department of Agriculture, Beltsville, Md.	The cytology of sugar cane
Michel Rabinovitch	Department of Zoology, University of Wisconsin, Madison, Wis.	Studies on the cytoplasmic incorporation of tritiated thymidine in *Amoeba proteus*
Harald Rossi	Columbia University, New York City	The influence of LET on the RBE of ionizing radiations
A. Sibatani	Cytochemistry Laboratory, Yamaguti Medical School, Ube, Japan	Fractionation of RNA with phenol
Wilfried Stubbe	Botanisches Institut der Universität Köln, Köln-Lindenthal, Germany	Plastid inheritance oenothera
Millard Susman	Biology Division, California Institute of Technology, Pasadena, Calif.	Temperature-sensitive mutants
Hewson Swift	Department of Zoology, University of Chicago, Chicago, Ill.	Cell fine structure during protein synthesis
Yataro Tazima	National Institute of Genetics, Misima, Japan	Mutational dose rate dependence in the silk worm
Adolf Wacker	Institut für Therapeutic Biochemistry, Universität Frankfurt, Frankfurt am Main, Germany	Molecular mechanisms of radiation effects (UV, x-ray)
Rudolf K. Zahn	Institut für Vegetative Physiologie der Universität Frankfurt/Main-Süd, Germany	Biological actions of DNase

Speakers at Professional Meetings, Spring-Summer 1962. – During the period between this and the next semiannual report, 68 speakers on Biology Division research are scheduled to present papers or to participate in symposia at 15 professional society meetings. Several members of the Division will also serve as chairmen of sessions or symposia. It is anticipated that additional speeches will be scheduled later in the period. For the corresponding time last year, March 1961 through August 1961, 59 talks were originally listed; 92 were given. The society meetings during the coming period are given below in calendar order, followed by the names of the speakers, and coauthors when applicable, listed alphabetically.

Biophysical Society – N. G. Anderson, F. J. Bollum (coauthor, R. B. Setlow), R. P. Boyce (coauthor, R. B. Setlow), R. A. Deering (coauthor, R. B. Setlow), Peter Mazur, M. L. Randolph, Jane K. Setlow, R. B. Setlow (will also serve as chairman of one session).

Symposium – "The excited state and photobiology," Alexander Hollaender, chairman. R. K. Clayton.

Solar Energy Symposium – W. A. Arnold.

Sixth Southeastern Developmental Biology Conference – Tuneo Yamada.

141st National Meeting of the American Chemical Society – B. C. Pal (coauthor, W. E. Cohn).

Symposium on "Cellular basis and aetiology of late somatic effects of ionizing radiations" (Third of a series of *International Symposia on Selected Topics of Radiobiology*, sponsored by UNESCO and IAEA) – W. L. Russell, A. C. Upton (3).

Fifteenth Annual Biology Research Conference – Takashi Makinodan (coauthor, J. F. Albright), Tuneo Yamada. Chairman, Round-table discussion, D. M. Prescott.

Association of Southeastern Biologists – Martha R. Leonard.

53rd Annual Meeting of the American Association for Cancer Research – A. C. Upton (J. W. Conklin and A. C. Upton).

Federation of American Societies for Experimental Biology – J. F. Albright (coauthor, Rachel R. Carter), F. J. Bollum (coauthor, R. B. Setlow), E. E. Capalbo (coauthors, Franco Celada and Takashi Makinodan), R. K. Clayton, W. E. Cohn (coauthor, A. M. Michelson), C. C. Congdon, D. G. Doherty, J. M. Eisenstadt (coauthors, W. H. Spell and G. D. Novelli), Joan Wright Goodman, K. B. Jacobson (coauthors, Kazuo Nishio and A. C. Upton), F. T. Kenney, J. X. Khym (coauthor, Elliot Volkin), L. M. Krausz (coauthors, F. C. Scaduto, Fumio Sawada, and R. R. Becker), A. L. Kretchmar (coauthor, C. C. Congdon), C. G. Mead, E. H. Perkins (coauthor, Martha R. Leonard), R. A. Popp, Herbert Schuel (coauthor, N. G. Anderson), Paul Urso, C. J. Wust (coauthor, Lazarus Astrachan), Tuneo Yamada (coauthor, Shuichi Karasaki).

54th Annual Meeting of the American Society for Clinical Investigation – C. C. Congdon.

American Society of Microbiology – H. I. Adler (coauthor, J. C. Copeland), J. C. Copeland (coauthor, H. I. Adler), G. E. Stapleton (coauthors, M. S. Engel and L. V. Orce), A. K. Williams (coauthor, G. D. Novelli), J. P. Williams (coauthor, H. D. Peck).

Argonne National Laboratory Symposium on "Implications of organic peroxides in radiobiology" – H. I. Adler.

Radiation Research Society – G. E. Cosgrove (coauthor, C. C. Congdon), L. H. Smith (coauthor, Otto Vos), H. E. Walburg, Jr.

36th National Colloid Symposium – N. G. Anderson.

Second International Congress of Radiation Research – H. I. Adler (coauthor, J. C. Copeland), M. A Bender (coauthor, P. Carolyn Gooch), E. E. Capalbo (coauthors, Martha R. Leonard and Takashi Makinodan), Gustavo Cudkowicz, Gino Doria, Jurgen Fränz (coauthor, M. L. Randolph), Joan Wright Goodman, R. F. Kimball, D. L. Lindsley (coauthors, Cloé Camba and Mary Warters), R. A. Popp, M. L. Randolph, G. E. Stapleton (coauthors, M. S. Engel and L. V. Orce), A. C. Upton (coauthors, M. L. Randolph and J. W. Conklin), R. C. von Borstel (coauthor, E. -A. Löbbecke), and Sheldon Wolff.

Foreign Travel. – In early September 1961, Alexander Hollaender visited Chile and Peru. He attended the International Symposium on Tissue Transplantation held at the Universidad de Chile, Santiago, and presented a paper on "Recent developments in the border fields of basic biology." He also consulted with investigators at the university. In Lima, Peru, he visited scientific personnel at the University of Peru, the Laboratorio de Investigacion de Cancer, and the Universidad Nacional Mayor de San Marcos.

C. C. Congdon, Takashi Makinodan, and E. E. Capalbo also attended the Symposium. Congdon presented a paper entitled "Changes in lymphatic tissues during foreign tissue transplantation," coauthored by Joan Wright Goodman. After the Symposium, Congdon consulted with scientific investigators at institutions in Chile and Peru. Before attending the Symposium, Makinodan conferred with scientific personnel at the following institutions in Lima, Peru: the National University of San Marcos, the Institute of Andean Biology, and the Laboratory of Cancer Investigation.. Makinodan spoke on "Studies on the immune reaction in *in vivo* cultures" at the Symposium. Capalbo visited personnel at the Argentine Research Council of Scientific and Technical Research and at the Comision Nacional de Energie Atomica, Buenos Aires.

The same month, E. H. Y. Chu presented a paper entitled "Chromosome studies in a family with four cases of atypical mongolism" at the Second International Conference on Human Genetics in Rome, Italy. He later visited scientific institutions and universities in France, England, and Scotland.

Franco Celada attended the Symposium on Mechanisms of Immunological Tolerance in Prague, Czechoslovakia, November 8–10, and spoke on "Radiosensitivity of homograft-rejecting and antibody-synthesizing capacities of isolated cells: its significance to tolerance."

In November, D. M. Prescott traveled to Mexico City, Mexico, to attend the Conference on the Use of Radioisotopes in Animal Biology and the Medical Sciences and to present a paper entitled "Tritiated precursors in the study of nuclear function and replication."

In addition to travel to foreign countries, Dr. Hollaender visited outside the continental United States, attending the Conference on Biophysical and Biochemical Mechanisms in the Production of Radiation-Induced Chromosome Aberrations held on November 16–18 at San Juan, Puerto Rico. He also consulted with the staff of the University of Puerto Rico, the Puerto Rico Nuclear Center, and the Atomic Energy Commission.

The Puerto Rico Conference was arranged by Sheldon Wolff, and was sponsored by the National Academy of Sciences–National Research Council. Drs. Chu, Kirby-Smith, and Lindsley also attended the Conference, presenting papers entitled "Dose relations in the induction of human chromosome aberrations," "Chromosome aberrations by combined x and ultraviolet radiations," and "Genetic effects of chromosome aberrations," respectively. After the Conference, Lindsley gave a résumé of the proceedings at the Puerto Rico Nuclear Center, Rio Piedras.

In December, W. F. Bertsch visited the Agriculture Experiment Station in Rio Piedras. While in Puerto Rico, Bertsch spoke on "The photoinhibition of etiolated stem segments" at the University of Puerto Rico, and on "Solid state phenomena in photosynthetic systems" at the Puerto Rico Nuclear Center. G. D. Novelli presented a series of three lectures, under the Traveling Lecture Program, at the University of Puerto Rico in January. The series was given first at Mayaguez then at Rio Piedras.

Fifteenth Annual Biology Research Conference. – The 1962 Spring Conference, "Specificity of Cell Differentiation and Interaction," will be held April 9–12 at the Mountain View Hotel in Gatlinburg, Tenn. The Conference is supported by the Atomic Energy Commission. This year's invited speakers and participants will discuss a central problem in modern biology, the specificity (individuality) of cell differentiation and interaction. Immunologic, genetic, cytologic, and biochemical aspects of these phenomena will be considered. The organizing committee for the conference was C. C. Congdon (chairman), Takashi Makinodan, R. A. Popp, D. M. Prescott, and W. L. Russell. The program is as follows:

FIFTEENTH ANNUAL BIOLOGY RESEARCH CONFERENCE

Monday, April 9

Morning

Opening Remarks – Alexander Hollaender

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

Introduction – C. C. Congdon, Chairman, Organizing Committee

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

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

Nuclear transplantation and problems of specificity in developing embryos – John A. Moore, Departments of Zoology, Barnard College and Columbia University, New York, N.Y.

Afternoon

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

Interactive processes in cytodifferentiation – Clifford Grobstein, Department of Biological Sciences, Stanford University, Stanford, Calif.

The inductive phenomenon as a tool for understanding the basic mechanism of differentiation – Tuneo Yamada, Biology Division, Oak Ridge National Laboratory

Evening

Smoker – Host: Union Carbide Corporation

Tuesday, April 10

Morning

Chairman – P. A. Weiss, The Rockefeller Institute, New York, N.Y.

Analysis of cell recombinations in experimental synthesis of tissues in vitro – A. A. Moscona, Department of Zoology, University of Chicago, Chicago, Ill.

Specificity in neurogenesis – Viktor Hamburger, Department of Zoology, Washington University, St. Louis, Mo.

Phylogeny and ontogeny of the immune mechanism – Milan Hašek, Biological Institute, Czechoslovak Academy of Sciences, Praha, Czechoslovakia (tentatively scheduled)

Afternoon

(Free)

Evening

Reception and dinner

Wednesday, April 11

Morning

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

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

Phenotypic expression of mouse isoantigens – Göran Möller and Erna Möller, Department of Tumor Biology, Karolinska Institutet, Stockholm 60, Sweden

Afternoon

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

Cellular variation during immune response: one possible model of cellular differentiation – Takashi Makinodan and J. F. Albright, Biology Division, Oak Ridge National Laboratory

Genetics of somatic cells in relation to the H-2 locus (tentative title) – Leonard A. Herzenberg, Department of Genetics, School of Medicine, Stanford University Medical Center, Palo Alto, Calif.

Evening

Round-table discussion: Embryological Development of the Immune System

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

Participants: S. J. Singer, University of California, San Diego (La Jolla)
Tuneo Yamada, Oak Ridge National Laboratory
Robert Auerbach, University of Wisconsin, Madison
C. C. Congdon, Oak Ridge National Laboratory

Thursday, April 12

Morning

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

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

Tolerance induction during development – R. E. Billingham and W. K. Silvers, The Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

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

Annual Information Meeting. – The annual meeting of the ORNL Advisory Committee for Biology was held November 8, 9, and 10, 1961. Division scientists discussed areas of research in progress on the first day. The last two days were spent in visits to laboratories and informal talks with individual scientists. Present were: Erwin Chargaff, Columbia University; Curt Stern, University of California, Berkeley; and Maxwell M. Wintrobe, University of Utah. E. L. Tatum, Rockefeller Institute, and Paul C. Zamecnik, Massachusetts General Hospital, were unable to attend. On Wednesday evening, November 8, Committee

members and Division staff were guests of Union Carbide Corporation at a dinner at the Oak Ridge Country Club. Clarence E. Larson, Vice President of Union Carbide Nuclear Company and General Manager of Oak Ridge Operations, spoke on "Biological Characteristics of Certain Gases."

AET-Bone Marrow Conferences. – Interest has steadily increased in these conferences since they were first started five years ago in Oak Ridge. An *International Symposium on Tissue Transplantation* was held August 30–September 2, 1961, in Santiago, Chile, in which Biology Division members took active part.

Other meetings held during this report period were:

Conference on Cryobiology and Methods of Preserving Living Tissues, August 18–19, 1961, University of Buffalo and the Linde Company, Buffalo and Tonawanda, N.Y.

Blood-Bone Marrow Tissue Culture and Cell Separation, October 20–21, 1961, National Institutes of Health, Bethesda, Md.

Chemical Suppression of Immune Mechanism, February 7, 1962, New York City.

A list of meetings being planned for the coming months and for 1963 follows:

April 15, 1962, Atlantic City, N.J. Round-table discussion. Dr. L. M. Tocantins, Jefferson Medical College, Philadelphia, Pa.

June 1–2, 1962, Long Beach California. Bone Marrow Transplantation and Chemical Protection in Large Animals and Man. Dr. N. B. Kurnick, University of California, Veterans Administration Hospital, Long Beach.

December 1–3, 1962, New York City. Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection. Dr. L. D. Hamilton, Sloan-Kettering Institute, New York City.

Winter, 1963, Brooks Air Force Base, Texas. Topic to be decided (tentative – Chemical Protection Against Radiation Damage). Col. Gerrit Hekhuis, Brooks Air Force Base, Texas.

Summer, 1963, Bethesda, Maryland. Bone Marrow Preservation. Dr. R. B. Gresham, Tissue Bank Department, U.S. Naval Medical School, Bethesda.

1963, Oak Ridge. Biochemical and Metabolic Changes in Secondary Disease and other Immunologic Disorders. Dr. A. L. Kretchmar, Oak Ridge Institute of Nuclear Studies, Oak Ridge.

Drosophila Genetics Conference. – A Research Conference of Drosophila Geneticists was held November 4–6 in the Biology Division. About 50 of these research workers from throughout the country discussed their latest work informally. This conference has led to interest in an annual meeting among investigators in the field. D. L. Lindsley and members of the Drosophila Genetics Group organized the conference.

Education. – In addition to participating in the Traveling Lecture program, Biology Division members have lectured in local schools and throughout the country. They have also taken part in training programs set up by the Oak Ridge Institute of Nuclear Studies or organized within the Division for the benefit of its personnel.

A. C. Upton lectured on "Biological Effects of Radiation for the ORINS Special Training Course" and on "Pathological Effects of Radiation" for the ORINS Medical Division seminar for pathologists.

C. C. Congdon and G. E. Cosgrove contributed to the second-quarter program of the Naval Research Company 6-3, given at Oak Ridge High School. Dr. Congdon's subject was "Experimental Treatment of Biological Radiation Injury." Dr. Cosgrove spoke on "Biomedical Effects of Radiation."

Two courses and a workshop were held within the Division during this period. M. L. Randolph presented a 10-session course on "Radiological Physics," and R. B. Setlow gave a 6-lecture course on "Hit and Target Theory." D. A. Fuccillo conducted a six-session workshop on technical communication.

A series of lectures will be given on background work of the speakers at the Annual Spring Conference. Division members presenting these lectures will be Drs. von Borstel, Yamada, Marin, Albright, Popp, Congdon, Haber, and Smith; thus, other staff members have an opportunity to "preview" work to be given at the meeting.

In connection with the cooperative program between the Biology Division and the University of Georgia, S. F. Carson presented two lectures on chemical microbiology before the Bacteriology Department, and F. T. Kenney lectured before the Biochemistry Department at the University at Athens, Georgia.

In December, W. D. Gude spoke on "Biological Effects of Radiation" before the Tennessee Academy of Science–Tennessee Department of Education Short-Term Institute for Science and Mathematics.

Trips being planned as part of the Small College program include T. T. Odell, to a group of six schools in central New York; W. D. Gude, to Tennessee Polytechnic Institute; F. J. de Serres, to Incarnate Word College in San Antonio, Texas, and other colleges in that area; E. H. Y. Chu, to colleges in Maryland; John Jagger, to Virginia and the Carolinas; D. G. Doherty, to colleges in Louisiana and Mississippi; and H. I. Adler, to Alabama and Louisiana.

Applications for the Summer Student Trainee program have been reviewed, and 20 students have been selected to participate in 1962.

Cytology and Genetics

Paramecium

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

Tradescantia, Vicia, Hordeum

Sheldon Wolff
H. E. Luippold
Sister Augustine Mattingly[1]

Maize and Phage

Drew Schwartz
D. R. Krieg
Jane K. Setlow[1]
Rea M. Fulkerson
C. B. Kincaid
Kathrine H. McGrath
Claire J. Witten

Neurospora

F. J. de Serres
W. E. Barnett[1]
H. E. Brockman[2]
Giorgio Morpurgo[3]
B. B. Webber[4]
Ida R. Cox
Arlee P. Teasley
Rosilie S. Osterbind[5]

Cell Growth and Reproduction

D. M. Prescott
R. C. von Borstel
Tuneo Yamada
E. G. Calef[3]
J. G. Carlson[4]
Mary E. Gaulden[4]
Shuichi Karasaki[3]
E. A. Löbbecke[3]
O. L. Miller, Jr.[6]
G. E. Stone[1]
Gladys Van Pelt[4]
Anna R. Whiting[4]
Martha J. Bond
Romance F. Carrier
P. E. Eide
Lola M. Kyte
Jackie A. Barnes[7]

Drosophila

D. L. Lindsley, Jr.
E. H. Grell
Rhoda F. Grell
W. J. Welshons
D. T. Suzuki[6]
J. E. Petty
Guthrie T. Pratt
Bobbie J. Scandlyn
Elizabeth S. Von Halle[4]
Mary Warters[4]
Ruby D. Wilkerson

CYTOGENETIC EFFECTS OF RADIATION

EFFECTS OF DOSE RATE AND DOSE FRACTIONATION ON THE INDUCTION BY X RAYS OF RECESSIVE LETHAL AND SLOW GROWTH MUTATIONS IN PARAMECIUM

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

Introduction. – Previous studies[8] had suggested that the amount of mutation induced in *Paramecium aurelia* by ionizing radiation was the same whether the dose was given in a single exposure lasting a few minutes or in two or more exposures separated by one or more cell generations. It seemed desirable, how-

[1]USPHS Fellow.
[2]NAS–NRC Fellow.
[3]Visiting investigator from abroad.
[4]Consultant.
[5]Student trainee.
[6]Research associate.
[7]Loanee.
[8]R. F. Kimball, *Genetics* 34, 210–22 (1949), and unpublished data.

ever, to check the effect of decreasing the dose rate, especially since dose-rate effects have been reported for mutation induction in other organisms.[9] Somewhat to our surprise, fewer mutations were induced when the x rays were given at 30 r/min than at 1000 r/min. We have undertaken a careful analysis of this phenomenon because dose-rate effects on mutation are not well understood and the Paramecium system offers a favorable opportunity for analysis.

Methods. – The methods of detecting mutations in Paramecium and of obtaining specimens in known stages of the cell cycle have been described in several of our previous reports. Mutation was measured by a quantity, *M*, that has been found to increase linearly with dose over the range used.

The General Electric Maxitron 250 was the source of 250-kvp x rays. The dose rate was varied by changing the tube current from about 30 ma to 0.8 ma.

In most experiments the x-ray beam was monitored during irradiation with a Radocon chamber next to the container of paramecia, and with a Victoreen 250-r thimble chamber some distance below the container. In addition, the dose rate was measured by readings before and after the irradiation with the thimble chamber substituted for the container of paramecia. The doses calculated from these three kinds of measurements were averaged to give the final estimates.

Each comparison was made using three groups of paramecia. One was given a single exposure at high dose rate at the beginning of the experiment. The second was given either a single exposure at low rate or two equal exposures at high rate, one at the beginning and one at the end of the experiment. The third group was given a single exposure at high rate at the end of the experiment. The three groups were given the same total dose within 2 or 3%, and it was assumed that the amount of mutation in the second group should equal the mean of the amount in the first and third since *M* increases linearly with dose over the range of doses used.

Results. – The results are summarized in Table 1. There was significantly less mutation in the low dose-rate group when log-phase paramecia were irradiated in preduplication interphase (G1), but there was no clear effect of dose rate when stationary-phase paramecia were irradiated and kept without food for one day afterward. The low amount of mutation in the stationary-phase group is the result of repair of a large fraction of all premutational damage when the paramecia are held for a day without food.[10] No statistically significant differences were detected with either schedule of fractionation, though the small differences are in the direction of less mutation for the divided than for the single exposures.

Discussion. – The experiments establish that a dose-rate effect exists for mutation induction in *Paramecium.* They show furthermore that the interaction between radiation events responsible for this effect must be essentially complete within the minute or so required to give a half dose at high rate; otherwise, fractionation effects should have been found. Moreover, they show that the rate effect must be on the premutational damage that is reparable for some time after irradiation. Were this not so, the stationary-phase paramecia should have shown a greater not a lesser effect, since rapidly formed mutations, if they exist,

[9] W. L. Russell, *J. Cell. Comp. Physiol.* 58 (suppl 1), 183–87 (1961).

[10] R. F. Kimball, N. Gaither, and S. M. Wilson, *Proc. Natl. Acad. Sci. U.S.* 45, 833–39 (1959).

make up a greater fraction of the total yield in stationary than in log phase. Specifically, the results exclude a dose-rate effect by rapid reunion of chromosome breaks, since two-break aberrations, once formed, should be irreparable. These experiments do not establish the precise way in which the dose rate influences reparable damage, but the techniques developed for *Paramecium* make this question attackable.

Table 1. Effects of Dose Rate and Dose Fractionation on Mutation in Paramecium

Stage of Growth	Exposure Schedule	Dose Rate (r/min)	Total Dose (r)	Mutation (M ± standard error)
Log phase	Single in G1	33	3051	0.81 ±0.03
	Single in G1	1137	3054	0.97 ±0.02
Stationary phase	Single	31	3408	0.48 ±0.03
	Single	1007	3327	0.51 ±0.03
Log phase	Two exposures in same G1	1229	3371	1.09 ±0.04
	Single in G1	1226	3343	1.11 ±0.03
Log phase	Two exposures in successive G1's	1613	4598	1.46 ±0.05
	Single in G1	1613	4598	1.47 ±0.04

ON THE TRANSITORY NATURE OF THE FAR-RED EFFECT

Sheldon Wolff H. E. Luippold

Introduction. – Treatment with far-red radiation, either before or after a dose of x rays, can increase the yield of radiation-induced chromosome aberrations. This effect is reduced by exposure to red radiation after the far-red treatment. One of the more puzzling aspects of these studies has been the transitory nature of the effect. Some experiments show a marked far-red enhancement of aberration induction, whereas others show no effect at all.

Studies by Borthwick and Hendricks[11] on the effect of red–far-red interactions in morphogenesis have demonstrated the existence of a pigment that is in equilibrium between a red- and a far-red-adsorbing form. When radiation of one type is administered, the pigment shifts to the form that will absorb the opposite type. They showed that the transition of the pigment from its far-red-adsorbing form to its red-absorbing form will occur not only when far-red radiation is administered but also at a somewhat slower rate in the dark. Since in studies on the effects of far-red radiation on aberration production, the far red has to be administered for long periods of time, that is, up to 3 hr, and also since the usual control, against which

[11]H. A. Borthwick and S. B. Hendricks, *Science* 132, 1223–28 (1960).

the radiated material is compared, is kept in the dark, it was decided to see whether or not the transitory nature of the effect results from the dark-treated controls being the equivalent of the far-red-treated material.

Methods. – Lateral roots of *Vicia faba* were grown by suspending the germinated seeds in aerated spring water. They were immersed in water and placed in a light-tight box that had a Corning filter No. 7-69 for a top. This filter transmits light from around 700 to 1100 mμ with a rather sharp peak at 800 mμ. The box was then placed under a photoflood incandescent lamp for 3 hr. The intensity of the far red as measured with the thermopile was about 37,000 $\mu w\ mm^{-2}\ sec^{-1}$. Either just before or just after the treatment with far red, the roots were given 150 r of 250-kvp x rays at 100 r/min. Twenty-one, 24, and 27 hr after x irradiation, roots were treated with colchicine for 2 hr and then fixed. Feulgen-stained smear preparations were made and metaphase figures analyzed for each point. These times were picked because it had been shown that at these times after radiation a positive far-red effect could occur.[12] The control experiments were either treated in light-tight boxes with a light-tight top instead of the Corning filter for a top (i.e., dark controls) or were left out in the room under fluorescent lamps for an ordinary-light control or were placed on the window sill as a daylight control.

Results. – The results, as presented in Table 2, show that there is a marked far-red enhancement of radiation-induced chromosome aberrations as measured against either of the light controls but either no effect or only a slight one when measured against the dark controls.

Table 2. Effect of Far Red on Radiation-Induced Chromatid Aberrations

Treatment	Chromatid Aberrations per Cell at Specified Time After X-Irradiation		
	21 hr	24 hr	27 hr
150 r in light	0.627	0.423	0.346
150 r in window	0.563	0.350	0.266
150 r + 3 hr dark	0.767	0.583	0.586
150 r + 3 hr far red	0.793	0.727	0.726
3 hr dark + 150 r	0.883	0.743	0.486
3 hr far red + 150 r	0.712[a]	0.740	0.553

[a]250 cells scored. All other points, 300 cells scored.

Conclusions. – Here in a single experiment one can find that treatment with far red either (1) gave an increased aberration yield over that expected from x rays alone or (2) gave no increased aberration yield or only a slight one, depending upon the control roots with which the far-red treated material is compared

[12]S. Wolff, pp 457–60 in *Proceedings of the 3d International Congress on Photobiology*, ed. by B. Christensen and B. Buchmann. Elsevier, Amsterdam, 1961.

and the time after x radiation that the comparison is made. It seems that 3 hr in the dark can on occasion bring about the same result as a 3-hr treatment with far-red radiation.

If this experiment were done in the ordinary way with only a dark control, it would have appeared in most pickings to have been a negative experiment, however, when compared to the light control, the far-red effect became apparent. This result is consistent with the idea that the far red-absorbing pigment has changed to the red-absorbing form not only when far red is shone on root tips but to a lesser extent even in the dark.

THE EFFECTS OF ACTINOMYCIN-D ON ROOT GROWTH

Sister Augustine Mattingly

Introduction. – Previous papers discussing the effects of the antibiotic Actinomycin-D reported that that the drug principally inhibits the synthesis of RNA while the synthesis of DNA is not affected.[13] Furthermore, use of the drug with mammalian cells in tissue culture resulted in the disappearance of nucleoli.[14]

Those results indicated that the use of this drug could prove of interest in the study of nuclear protein metabolism, which is in progress in this laboratory. However, the effects of Actinomycin-D on higher plants and, in particular, the extent of inhibition of either DNA or RNA synthesis, had not been reported. Therefore, experiments have been initiated to determine the effect of the drug on mitotic index, cell elongation and expansion, and production of chromosome aberrations. Lateral roots of *Vicia faba* were used for all experiments, and analyses of mitotic index and autoradiographic data were derived from root-tip smears of formalin-fixed material.

Results and Discussion. – The concentrations of Actinomycin-D reported to be effective when used with animal cells in tissue culture, that is, up to 1 μg/ml, were found ineffective in the production of any discernible abnormalities in *Vicia faba*. The present experiments, therefore, were performed with the higher concentration of 50 μg of Actinomycin-D per milliliter of 2.5% ethyl alcohol. Since the alcohol was necessary to dissolve these high concentrations of the antibiotic, alcohol controls were run for all experiments.

After an 8-hr treatment with Actinomycin-D, there is an obvious decrease in the mitotic index when compared with the alcohol controls. While no chromosome aberrations are evident at this time, the metaphase chromosomes appear highly contracted. By the end of 24 hr, the mitotic block is virtually complete. In a series of ten root preparations, only one metaphase configuration was found, and, in this figure, the chromosomes are extremely contracted and abnormal in appearance. The interphase nuclei still stain well at this time, however. After a 48-hr treatment, on the other hand, the mitotic block is still complete, and DNA staining with Feulgen reagent is poor.

[13] E. Reich *et al.*, *Science* 134, 556–57 (1961).

[14] D. E. Rounds, Y. H. Nakanishi, and C. M. Pomerat, *Antibiotics and Chemotherapy* 10, 597 (1960).

Although it has been reported that the nucleoli of tissue culture cells are destroyed by Actinomycin-D, this effect has not been seen in *Vicia faba*. While the nucleoli may decrease slightly in size, there is no evidence of the complete disappearance of the organelle from any of the cells, even after extended periods of treatment.

Preliminary observations from thymidine-incorporation studies are recorded in Table 3. It is evident that RNA synthesis in *Vicia faba* is completely inhibited by extended treatment with Actinomycin-D, and the damage is irreparable. On the other hand, in the cases in which DNA synthesis had not completely ceased, recovery is possible.

Since the data presented above are only preliminary, it is not possible to draw any firm conclusions about the nature of the metabolic effects of Actinomycin-D on plants. It is certainly of interest that the nucleolus, which has a high concentration of RNA, is not greatly diminished in size even after extensive periods during which it is improbable that RNA synthesis occurred. Experiments utilizing tritiated precursors of RNA and protein are in progress in order to help determine the relation of the mitotic block and inhibition of DNA synthesis to other metabolic processes in the cell.

Table 3. Incorporation of Tritiated Thymidine

The roots were placed in Hoagland's solution for periods of posttreatment recovery. In all experiments, the roots were exposed to tritiated thymidine for 30 min immediately after the treatment. Degree of thymidine incorporation indicated by: slight (+), moderate (++), intense (+++), and no incorporation (–).

Hours of Treatment	Hours of Posttreatment	Degree of Thymidine Incorporation	
		Actinomycin-D	Control
8	0	+	+++
24	0	–	++
48	0	–	+
8	16	+	+++
8	40	++	+++
24	24	–	+++

MODE OF SYNTHESIS OF THE HYBRID ESTERASE IN MAIZE

Drew Schwartz Kathrine H. McGrath

F_1 plants heterozygous for two different alleles of the *E* gene contain a new (hybrid) esterase enzyme which is not synthesized by either of the homozygous parental plants.[15] Five heterozygous combinations have been tested involving five different alleles, and in each case the hybrid enzymes show migration rates intermediate between the enzymes specified by the parental homozygotes.

[15] D. Schwartz and K. H. McGrath, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 43–44.

Investigations carried out in the past six months on the particle-bound enzymes have established that the new enzyme of the heterozygote is synthesized as a hybrid and does not result from dimerization of previously synthesized monomers. By treatment with deoxycholate we have been able to liberate the particle-bound esterases from washed particles of immature kernel and young seedling tissue. In homozygotes only one esterase type is found associated with the particle, while in the heterozygotes three particle-bound esterase types are found – the two parental types and the hybrid. It thus appears that the hybrid enzyme is synthesized as such on the ribosomes in heterozygotes. We have been able to eliminate the possibility that the particle-bound esterases are not newly formed enzymes but represent nonspecific adsorption of previously synthesized enzymes to the particles. Particles from plants which synthesize one esterase type were homogenized in supernatant from plants carrying a second type of esterase. Before washing and treatment with the deoxycholate, the enzymes liberated from these particles showed no contamination by the supernatant enzymes. Also, no esterase was liberated from particles obtained from seedlings in which synthesis of this enzyme had ceased even though there was still a high concentration of the enzyme in the supernatant.

The possibility of dimerization occurring immediately upon release of the enzymes from the particles by the deozycholate was eliminated by mixing experiments. No hybrid enzyme is found after the deoxycholate treatment of a mixture of particles from homozygous plants of different genotypes. The hybrid enzymes probably result from an interaction of two messenger RNA molecules specified by different alleles, each of which contributes part of the information to the synthesis of the enzyme.

SOME PROPERTIES OF INDUCED GENE REVERTANTS

D. R. Krieg Claire J. Witten

The analysis of ethyl methanesulfonate-induced reversion of *r*II mutants of phage T4 has been further extended.[16,17] Some additional mutants have been found to be revertible by this agent, and more information has been obtained on some of those previously shown to be revertible.

Induced revertants from some *r*II mutants are readily distinguishable by their phenotype on the bacterial strain used for their selection and hence have been referred to as partial revertants rather than true back mutants.[16] Cases have now been found in which revertants, indistinguishable from wild type of that strain, can be distinguished by the appearance of their plaques on another bacterial strain. Induced revertants previously described as "apparent wild types" are being further investigated. While further illustrating the possibility of "false positives" in inferences of true back mutation, indicating the need for further work and caution in evaluation of hypotheses of specific mutagenesis based on that inference, partial revertants may be useful in the investigation of gene-protein relations.

[16] D. R. Krieg and Rea M. Fulkerson, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 28–29.

[17] D. R. Krieg and Rea M. Fulkerson, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 32–35.

The test we have routinely applied to measure the extent of induced revertants does not impose selective conditions until after the reversions have had an opportunity to occur. Another test, which exposes the phage to selective growth conditions directly after their treatment with the mutagen, has been applied to determine the fraction of the potential revertants recoverable under these conditions. Our finding that two mutants differ markedly in the fraction of induced revertants recovered[17] has been extended to other revertible mutants, and a wide range in this fraction has been observed. At least three different values of this fraction have been found for induced revertants, all of which appear to be true wild-type revertants by the criteria so far employed. These findings indicate that differences in mutational mechanisms and/or activity of newly induced revertants may exist, but they also emphasize the importance of the technique we have used routinely for the measurement of the extent of induced revertants.

PHYSIOLOGICAL CONTROL OF EXTRAPOLATION NUMBER IN X-RAY SURVIVAL CURVES

Jane K. Setlow

Introduction. – The x-ray survival of *Escherichia coli* strain B/r grown aerobically in nutrient broth or peptone-phosphate is of a simple exponential type, but the survival curve has an extrapolation number between 10 and 100 in the case of cells grown in these media with added glucose,[18,19] although the final slope may be similar. One possible explanation of the difference in shape of these survival curves is that for low doses the glucose-grown cell has an enhanced ability to repair damage to the genome. If this explanation is correct, then the genome of bacteriophage might also be expected to show more repair after irradiation inside glucose-grown cells.

It has been shown that the cells which show survival curves with high extrapolation numbers contain twice as much DNA as the others.[20] In an attempt to correlate increase in the DNA with the extrapolation number of survival curves, use was made of *E. coli* strain 15 $T^-A^-U^-$, which has requirements for thymine, arginine and uracil. This organism, when transferred to a synthetic medium containing T but lacking A and U, shows an increase in total DNA per cell.[21]

Results and Discussion. – The x-ray survival curves of complexes of T4 with glucose-grown and non-glucose-grown cells were exponential and identical. It is concluded that an enhanced repair mechanism is not the likely explanation for the shape of the survival curve of the glucose-grown cell.

The x-ray survival curve of *E. coli* 15 $T^-A^-U^-$ grown in synthetic medium supplied with T, A, and U is exponential. When the cells are growing logarithmically in this medium and then transferred to a medium lacking A and U for 90 min, there is an increase in extrapolation number of the survival curve comparable

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

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

[20] M. S. Engel and G. E. Stapleton, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 61–62.

[21] O. Maaløe and P. C. Hanawalt, *J. Mol. Biol.* **3**, 144 (1961).

to that of the glucose-grown B/r. It is concluded that the change in shape of the survival curve in this case is very probably related to the increase in DNA per cell. A relatively small increase in DNA per cell could result in a large extrapolation number, with the following type of model: Assume that there are n radiation-sensitive units in the cell. A hit in any of these units inactivates a cell, with a resulting exponential survival curve, but if some or all of these units occur in duplicate in the cell, then a hit in each of a pair of units is necessary to inactivate a cell. This can result in a survival curve of the form $N/N_0 = [1 - (1 - e^{-CD})^2]^n$, where N/N_0 is the surviving fraction, C is a constant, D is the dose, and n is the number of units. It is of interest that the survival data appear to fit the theoretical curve when $n = 4$, since this is the same number of units which must be postulated to explain the distribution of radioactive label among progeny of tritium-labeled *E. coli*,[22] and also the number of mutable units in this organism postulated to account for the pattern of increase of spontaneous and induced mutants in a population.[23,24] The genetic evidence suggests that the four units are replicas, and it must be assumed that damage in any one of them results in inactivation, in accordance with the observed exponential survival.

EVIDENCE FOR A PARTICULAR BASE ALTERATION IN TWO T4 BACTERIOPHAGE MUTANTS

Jane K. Setlow

Introduction. – An ultraviolet action spectrum for reversion of a point mutant for the *r*II region of T4 phage should reflect the absorption spectrum of the affected nucleotide, provided that the DNA is irradiated in the disordered condition, such as at 7 min after infection,[25] when there is little energy transfer from one base to another.[26,27] In accordance with this prediction, it has been found that action spectra for reversion of two such mutants irradiated during the disorganized period of the viral DNA do indeed suggest a particular nucleotide change.

Results and Discussion. – The dose relation for reversion of the two 2-aminopurine-induced mutants studied appears to be linear for all wavelengths of ultraviolet radiation tested. The action spectra for reversion of these mutants differ markedly from the absorption of hydroxymethylcytosine and adenosine, differ somewhat from guanosine absorption and the action spectrum for killing 7-min complexes, but bear more resemblance to the thymidine absorption spectrum. The hypothesis is suggested that these two mutants revert to apparent wild type as a result of a base-sequence alteration

[22] R. P. van Tubergen and R. B. Setlow, *Biophys. J.* 1, 589 (1961).

[23] F. J. Ryan and L. K. Wainwright, *J. Gen. Microbiol.* 11, 364 (1954).

[24] F. J. Ryan, P. Fried and M. Schwartz, *J. Gen. Microbiol.* 11, 380 (1954).

[25] J. K. Setlow, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 35–36.

[26] R. Setlow and R. Boyce, *Biophys. J.* 1, 29 (1960).

[27] J. K. Setlow and R. B. Setlow, *Proc. Natl. Acad. Sci. U.S.* 47, 1619 (1961).

at the site of an ultraviolet-affected thymine in phage DNA. The ultraviolet action spectra provide direct support for the tentative conclusion drawn indirectly from chemical mutagenesis studies of these mutants[28] that the mutant sites contain an adenine-thymine pair.

ESTIMATION OF THE RELATIVE FREQUENCIES OF X-RAY-INDUCED VIABLE AND RECESSIVE LETHAL MUTATIONS IN THE *ad-3* REGION OF *NEUROSPORA*

F. J. de Serres Rosalie S. Osterbind Ida R. Cox

Introduction. – A direct method[29] has been developed to estimate forward-mutation rates at specific loci in *Neurospora* and to obtain essentially unselected samples of mutants with either complete or partial biochemical requirements. However, since *Neurospora* is a haploid organism, the samples of mutants from treatment of wild-type strains would not include any mutations that are homozygous-lethal. Even if such mutations made purple colonies and were recovered as heterokaryons, in subsequent platings we would not expect to recover viable homokaryons.

The specific locus method in mice[30] or *Drosophila*,[31] however, has shown that a substantial fraction of x-ray-induced mutations are lethal. Furthermore, a detailed analysis[32] of mutations in the dilute-short-ear (*d se*) region in mice has shown that the percentage of lethal mutations at a given locus can vary quite markedly.

Since apparent differences in the *Neurospora* system could be attributed to such differences in the relative frequencies of viable and lethal mutation, we tried to develop a method to recover mutations of both types. Atwood[33] has shown that recessive lethal mutations can be recovered and maintained in balanced heterokaryons between strains with the appropriate genetic markers. We used a variation of this technique to try to recover any recessive lethal mutations that might occur spontaneously or after mutagenic treatment.

Tests of such a system are given in this report along with preliminary data on the relative incidence of x-ray-induced viable and recessive lethal mutation from such studies on mutation in the *ad-3* region.

Results. – In two separate experiments, a heterokaryon of the following constitution was used:

1. A *hist-2* *ad-3A* *ad-3B* *nic-2* + ; + ; *inos* ; +
2. A + + + + *al-2* ; *cot* ; + ; *pan-2*

[28] E. Freese, *Proc. Vth Internat. Congr. Biochem., Moscow, 10–16 Aug., 1961*, Pergamon Press, Oxford (in press).

[29] F. J. de Serres and H. G. Kølmark, *Nature* **182**, 1249 (1958).

[30] W. L. Russell and L. B. Russell, *Radiation Research*, (suppl 1) 296 (1959).

[31] C. L. Ward and M. L. Alexander, *Genetics* **42**, 42 (1957).

[32] L. B. Russell and W. L. Russell, *J. Cell. Comp. Physiol.* **56** (suppl 1) 169 (1960).

[33] K. C. Atwood, *Proc. Natl. Acad. Sci. U.S.* **39**, 1027 (1953).

Conidia from a culture grown on minimal medium were incubated as described previously,[29] either untreated, or after irradiation with a dose of 30 kr; no spontaneous and a total of 29 x-ray-induced purple colonies were recovered. Heterokaryon tests on the adenine-requiring heterokaryons recovered from these colonies showed that in this sample there were 8 *ad-3A*, 12 *ad-3B* and 9 *ad-3A ad-3B* double mutants. All heterokaryons were then plated on minimal medium supplemented with adenine and pantothenic acid, at 37°C, to determine whether the induced mutants were homozygous-viable or -lethal. The data from these tests are given in Table 4. In the present samples, 76% (22/29) of all of the x-ray-induced mutations in the *ad-3* region are homozygous lethal. Furthermore, there is no difference in the percentage of lethal *ad-3A* or *ad-3B* mutations. Mutations that affect both loci, however, are always lethal.

Table 4. The incidence of Viable and Recessive Lethal Mutations Among *ad-3* Mutants Induced in a Balanced Heterokaryon After X Irradiation (30 kr)

Mutant Type	Total Number	Homozygous Viable		Homozygous Lethal	
		Number	Percent	Number	Percent
ad-3A	8	3	37.5	5	62.5
ad-3B	12	4	33.3	8	66.7
ad-3A ad-3B	9	0	0	9	100
Total	29	7	24.1	22	75.9

Discussion. – The data from the present experiments on a heterokaryon of *Neurospora* are in excellent agreement with those obtained with the specific locus method in mice or *Drosophila*.[30,31] Lethal mutations obtained in these three organisms following x irradiation may be contrasted as follows: *Neurospora*, 76% (22/29); mice, 77% (71/92); *Drosophila*, 52% (33/64). Since such a large proportion of the *ad-3* mutants actually induced are lethal, it is evident that our previous estimates[29] of forward-mutation rates are at least fourfold too low. It is of considerable interest that 9/29 x-ray-induced mutations are *ad-3A ad-3B* mutants and that all mutations of this type are lethal. The data are in perfect agreement with the analysis of true *d se* mutations in mice which are 100% homozygous lethal.[32] If *ad-3A ad-3B* double mutants are always lethal, and arise in the main part, or entirely, by gross chromosome deletion, then this provides an explanation for the absence of such mutations in large samples of x-ray-induced mutations from similar forward-mutation experiments[29] on wild type.

MODIFICATION OF EMS-INDUCED REVERSION FREQUENCIES IN *NEUROSPORA* WITH ENRICHED PLATING MEDIA

W. E. Barnett F. J. de Serres

Introduction. – It is well known that ultraviolet induced mutation frequencies may be modified by various posttreatment nutritional conditions. Maling[34] found that the nitrous acid-induced reversion frequency

[34] B. Maling, personal communication.

of certain *ad-3 Neurospora* mutants may be enhanced by plating on medium supplemented with low levels of adenine. It was considered of importance, therefore, to ascertain the effects of several enriched plating media on the recovery of reverse mutations following exposure to ethyl methanesulfonate (EMS).

Results. – Conidia from an *ad-3* mutant of *Neurospora* were uniformly treated with EMS and plated on minimal medium supplemented with various levels of adenine, and minimal medium containing casamino acids supplemented with various levels of adenine.

Conclusions. – From the results obtained, we may draw the following conclusions:

1. Following EMS exposure, plating conidia on minimal medium containing casamino acids enhances the observed mutation frequency twofold over that seen on minimal medium.

2. The addition of low levels of adenine to minimal medium also increases the frequency of recovered mutations by a factor of two.

3. The addition of low levels of adenine to minimal medium supplemented with casamino acids has very little or no effect.

These results establish that the recovery of EMS-induced revertants may be modified quantitatively by plating the conidia immediately after treatment on enriched media. While superficially these findings are similar to those obtained in bacteria, at this time we cannot conclude that similar mechanisms are involved.

INDUCED PHENOTYPIC REVERSION BY 8-AZAGUANINE AND 5-FLUOROURACIL

W. E. Barnett H. E. Brockman

Introduction. – When cells are grown in the presence of certain RNA base analogues, the analogues are incorporated into RNA and result in the production of altered enzymes.[35–37] If, as these authors have suggested, analogue incorporation causes errors in information translation by RNA, one might predict that many mutant systems, differing from normal by subtle changes in information content, would respond to RNA analogues. Benzer and Champe[38] have shown that certain *r*II mutants of phage T4 are capable of multiplication in *Escherichia coli* strain KB in the presence but not in the absence of 5-fluorouracil (5FU). This report shows that approximately 50% of the nitrous acid induced, leaky, *ad-3* mutants of *Neurospora crassa* exhibit partial phenotypic reversion when grown in the presence of either 5FU or 8-azaguanine (8AG).

Results and Discussion. – The *ad-3* mutants used were derived from nitrous acid-treated conidia of wild-type strain 74A-OR by F. J. de Serres and H. G. Kølmark. Growth assays were performed in Westergaard's basal medium containing 1.0% glucose and solidified with 1.5% agar for growth-tube assays of linear growth rate. A series of test tubes containing 3 ml of medium was supplemented with 10, 1, 10^{-1},

[35] S. Naono and F. Gros, *C. R. Acad. Sci. Paris* **250**, 3889 (1960).

[36] H. Chantrenne, p 281 in *Biological Structure and Function*, ed. by T. W. Goodwin and O. Lindberg, Academic Press, New York, 1961.

[37] R. Hamers and C. Hamers-Casterman, *J. Mol. Biol.* **3**, 166 (1961).

[38] S. Benzer and S. P. Champe, *Proc. Natl. Acad. Sci. U.S.* **47**, 1025 (1961).

10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 0 μg/ml of 5FU or 8AG and inoculated with 0.1 ml of conidial suspension. All concentrations were in duplicate, incubated at 30°C, and scored over a 21-day period. A positive response was recorded when the amount of growth in duplicate tubes containing some level of analogue was at least twofold greater than that in the tubes containing no analogue.

Forty-eight mutants were tested. Growth of 14 of 24 strains in the first group was markedly stimulated in the presence of 8AG. The results obtained with the second group are shown in Table 5. To test the possibility that the observed positive responses were due to back mutation, conidia were harvested from eight of the strains growing on 8AG and from six on 5FU, washed by centrifugation and then retested. All strains were clearly mutant and were again stimulated in the presence of analogue. These results demonstrate that analogue-induced growth is a result of phenotypic rather than genotypic change.

Growth tubes containing various concentrations of 8AG were inoculated with one of the strains to obtain a quantitative measure of the stimulatory effect (Table 6). The maximal growth rate achieved in this mutant in the presence of 8AG is approximately 75% of that in the wild type. The level of analogue required for maximal stimulation differs among the strains, usually ranging from 10^{-3} to 1 μg/ml, with higher levels being inhibitory. Some strains do, however, give maximum response at 10 μg/ml.

Table 5. Effect of 8AG and 5FU on Phenotype of *ad-3* Mutants

Number of Mutants Tested	Number of Mutants Showing Response to:			
	8AG and 5FU	8AG	5FU	Neither
24	10	3	0	11

Table 6. Growth Rate of an *ad-3* Mutant on 8AG

Concentration of 8AG (μg/ml)	Linear Growth Rate (mm/hr)
0	0.44
10^{-3}	0.63
10^{-2}	2.63
10^{-1}	3.13
1	3.13
10	2.88

Guanosine and uridine eliminate the responses to 8AG and 5FU, respectively, when present in tenfold excess over the analogue. Furthermore, none of the mutants respond to the normal bases at concentrations that are stimulatory in the case of the analogue.

A number of *ad-3* mutants of *Neurospora* have been shown to revert in phenotype but not in genotype when exposed to either 8AG or 5FU. The properties of 8AG and 5FU may be such that their substitution

for the normal purine or pyrimidine in a coding sequence of bases in RNA changes the amino acid this code imparts to a peptide sequence in a protein molecule. If the analogue-induced change happens to be the reverse of the change resulting from the mutational phenomenon, an enzyme with normal function would be produced. Such a mechanism could account for the results reported here.

CELL GROWTH AND REPRODUCTION

BEHAVIOR OF PROTEINS IN THE NUCLEUS OF AMOEBA DURING GROWTH AND DIVISION

D. M. Prescott Romance F. Carrier

Introduction. – The nucleus synthesizes proteins, but the function of these proteins is unknown. We have begun autoradiographic studies on nuclear proteins by labeling with tritiated amino acids and observing the behavior of the labeled proteins during growth and cell division.

Methods. – *Amoeba proteus* were incubated for 12 to 24 hr in a mixture of six tritiated amino acids, all of which are known to occur in mammalian histone (including especially lysine). After the labeling period, the amoebae were washed and grown on nonradioactive food at 18°C. Approximately once every 36 hr half of the cytoplasm was cut off the amoebae. The operations had two desired effects – the amoebae were prevented from reaching mitosis, and the original cytoplasm containing radioactive proteins was replaced. After ten successive operations, for example, approximately 99.9% of the original cytoplasm had been replaced by cytoplasm synthesized in the absence of radioactive precursors and without the occurrence of cell division. A few amoebae were fixed and autoradiographed after each operation up to 12 cuts and after 12 cuts plus one, two, or three cell divisions.

Results. – In amoebae labeled with amino acids but not subjected to cutting operations, both the nucleus and cytoplasm were heavily radioactive, and the nucleus was more radioactive than the cytoplasm. With each operation, both the nuclear and cytoplasmic radioactivity decreased, but cytoplasmic activity declined more rapidly than nuclear. After 12 operations, the nucleus was still moderately radioactive but the radioactivity in the cytoplasm was only slightly (but significantly) above background. Groups of amoebae were also allowed to go through one, two, or three mitoses following the twelfth operation. In these experiments, the radioactivity per nucleus was reduced by approximately half with each division.

Discussion and Conclusions. – At least two protein fractions can be identified in the amoeba nucleus in these experiments. One fraction (which may, in fact, consist of several subfractions) leaves the nucleus and is eliminated from the cell by means of the cutting operations. The second fraction is retained in the nucleus during interphase and mitosis. The latter protein may be a part of chromosome structure. The experiments are being extended to determine whether there is a nuclear protein fraction which is conserved as efficiently as DNA over a very long period of growth and division.

SEGREGATION OF LABELED DNA DURING MITOSIS IN MAMMALIAN TISSUE CULTURE CELLS

D. M. Prescott M. A Bender

Introduction. – Taylor's[39] important autoradiographic studies on the chromatid segregation of labeled DNA in plant root cells are consistent with the proposition that the unduplicated chromosome is single-stranded, that is, it contains one double helix and undergoes replication by a semiconservative process. Although Taylor's work is generally accepted, it does not agree with the findings reported by La Cour and Pelc[40] and by Plaut and Mazia.[41] We have studied the behavior of DNA in chromatids in mammalian tissue-culture cells under conditions that allow accurate timing of the number of divisions undergone by each cell after the DNA has been labeled with tritiated thymidine and in a manner that completely eliminates the absorption of beta particles by cytoplasm lying between the chromosome and the photosensitive emulsion during autoradiographic exposure.

Method. – Chinese hamster tissue-culture cells or human leukocytes in vitro[42] were labeled with tritiated thymidine and autoradiographs of chromosomes in the first, second, third, and fourth metaphases after labeling were prepared. Metaphases were accumulated with colchicine treatment. In preparing the Chinese hamster cells for autoradiography, the chromosomes were isolated free of all cytoplasm.

Results and Discussion. – For both cell types at the first metaphase after labeling, both chromatids of each chromosome were radioactive. At the second metaphase after DNA labeling, only one chromatid length of each chromosome was labeled. Sister "chromatid exchanges" were observed, but the frequency was much lower than reported by Taylor for plant material. At the third metaphase only about half of the chromosomes contained a length of labeled chromatid, and at the fourth metaphase only about a quarter of the chromosomes contained a labeled chromatid. The numbers of labeled chromosomes at third and fourth metaphase were slightly higher than one-half and one-quarter because of sister "chromatid exchanges." Our results demonstrate semiconservative *segregation* of labeled DNA in mammalian chromosomes and are in agreement with the work of Taylor.

DNA SYNTHESIS IN THE MACRO- AND MICRONUCLEI OF *EUPLOTES* AND THE DISTRIBUTION OF MACRONUCLEAR DNA DURING AMITOSIS

D. M. Prescott R. F. Kimball Romance F. Carrier

DNA synthesis in the macronucleus of the protozoon, *Euplotes*, occurs in microscopically visible bands that start at the two ends of the elongated nucleus and proceed to the middle.[43] Initiation of

[39] J. H. Taylor, *Exptl. Cell Research* 15, 350 (1958).

[40] L. F. La Cour and S. R. Pelc, *Nature* 182, 506 (1958).

[41] W. Plaut and D. Mazia, *Texas Reports Biol. and Med.* 15, 181 (1957).

[42] M. A. Bender and D. M. Prescott, *Exptl. Cell Research* (in press).

[43] J. G. Gall, *J. Biophys. Biochem. Cytol.* 5, 295 (1959); D. M. Prescott and R. F. Kimball, *Proc. Natl. Acad. Sci. U.S.* 47, 686 (1961).

synthesis at the two ends of the nucleus and even in the two macronuclei of double cells is synchronous, demonstrating some cytoplasmic control over initiation.

DNA synthesis in the macronucleus occupies the last three quarters of the interdivision interval, but micronuclear doubling, as shown by microspectrophotometric measurements and H^3-thymidine incorporation, takes place immediately after telophase while cytokinesis is in progress. The synthesis periods for the two nuclei, therefore, do not overlap. This temporal separation of the synthesis periods for two unlike nuclei in the same cytoplasm demonstrates that conditions within the nucleus as well as in the cytoplasm dictate the position of synthesis within the cell cycle. The importance of nuclear conditions is also shown by the fact that initiation of synthesis normally occurs only at the two ends of the macronucleus and only rarely elsewhere.

Labeling experiments have given direct cytological support for the hypothesis, used in some genetic studies, of random segregation of macronuclear material during amitosis of the highly polyploid macronucleus of ciliates. Short sections of DNA in the macronucleus of *Euplotes* were labeled by brief exposure to H^3-thymidine. Prior to amitosis the labeled DNA was restricted to short sections of the macronucleus, but during amitosis it becomes evenly distributed throughout.

A CHAMBER FOR EXPOSURE OF BIOLOGICAL SPECIMENS TO HIGH FLUXES OF THERMAL NEUTRONS. I. RADIATION DOSIMETRY

R. C. von Borstel E. G. Calef A. Colomb

It has been suggested that thermal neutrons could perform a type of molecular surgery on biological specimens because of the very large difference in the capture cross section of N^{15} and N^{14} isotopes. As a first approach to this kind of experimentation, organisms such as bacteriophage can be grown on media containing either of the two isotopes, and the thermal neutrons, if the proper conditions of experiment were found, should cause differential killing.[44]

The first prerequisite of such differential killing appears to be a thermal neutron flux of very high intensity, comparatively free of gamma radiation. Thermal neutron flux with an intensity of 1.4×10^{12} neutrons cm^{-2} sec^{-1} has been made available by the use of the HB-2 hole in the LITR reactor at ORNL. A device which can keep phage samples in controlled conditions of humidity and temperature for several hours has been built; it has a usable space of 0.6 cm^3.

While the dosimetry of thermal neutrons can be accurately performed with relatively simple devices, it became clear that by the commonly available methods no dosimetry of the gamma radiation was possible. The calculable amount of gamma in our experimental setup was of the order of 2000 r/hr; the amount measured by common devices being roughly 10^6 r/hr. By the use of a standard suspension of phage exposed to a Co^{60} gamma source, we have made reference death-rate curves which, by comparison with those obtained in the reactor, gave an estimated amount of gamma lower than 2.16×10^5 r/hr in the experimental setup

[44] K. C. Atwood and F. Mukai, *Am. Naturalist* 58, 841, 295 (1954).

(Fig. 1). Killing by beta radiation due to neutron activation of the exposure chamber has been detected but not yet measured; it is likely that this killing will subtract a sizable fraction from the previous biological estimate of the gamma radiation.

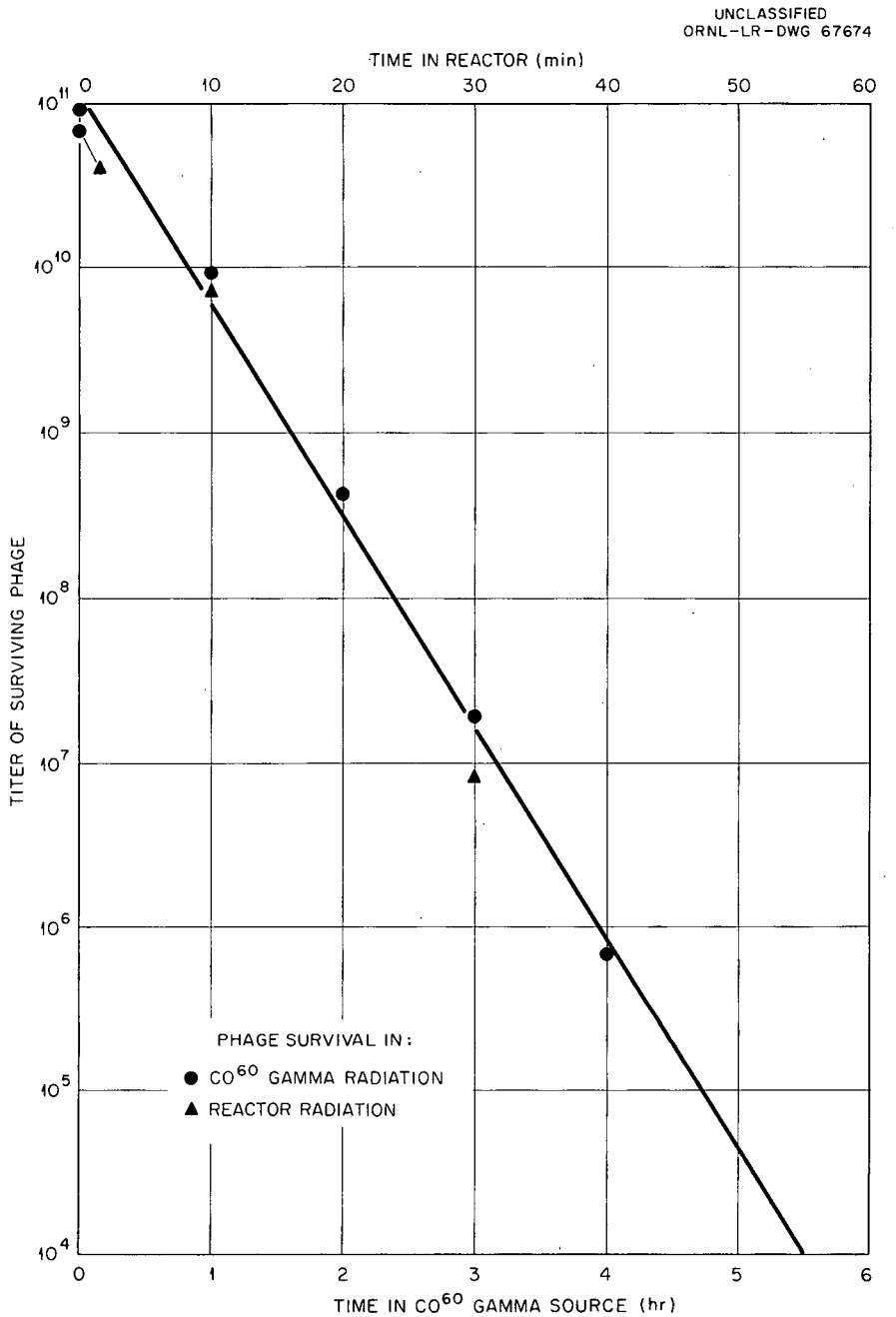

Fig. 1. Comparative Dose-Effect Curve for Exposure of Biological Specimens to Co^{60} Gamma Radiation and Mixed Radiations from the Reactor. The T4 bacteriophage was made of either N^{14} or N^{15}. A 1:1 mixture of the two phages was used in the reactor experiments. The N^{14} phage was genetically marked with the Ac^{+} gene and the N^{15} phage with the Ac^{-} gene. The Co^{60} gamma source delivered 3.6×10^{4} r/hr.

NUCLEAR RNA SYNTHESIS IN LENS REGENERATION IN THE NEWT

Tuneo Yamada Shuichi Karasaki D. M. Prescott

Introduction. – It is well known that a lens removed from the eye of the adult or larval newt is perfectly regenerated. Detailed morphological observations and experimental investigation of the regeneration process strongly favor the interpretation that the new lens is formed by the cells directly derived from iris cells originally present at the dorsal margin of the iris. This is one of rare cases in which the origin of cells participating in regeneration is clarified. According to the hypothesis that specificity of cell differentiation depends on the production of a specific set of RNA molecules necessary for the production of proteins which characterize the type of differentiation, a profound change in RNA synthesis should occur in the iris cells after removal of the lens. As a first approach to this problem, an autoradiographic study has been made of H^3-cytidine uptake in RNA of iris cells after removal of the lens but before appearance of the lens body in the adult eye of *Triturus viridescens*.

Results and Discussion. – The lens was surgically removed from the left eye, and H^3-cytidine was injected into the individual after a time interval ranging from one to seven days. Autoradiography demonstrated that incorporated radioactivity was preferentially localized in the nucleus. The radioactivity was removable to a large extent (about 90%) by treating the sections with ribonuclease prior to applying the emulsion. Counts of grains of iris cell nuclei showed the existence of a large difference between the iris cells of an operated eye and those of the nonoperated eye of the same individual. This difference increased during the period covered by the experiment. Since the morphological process of regeneration becomes apparent ten days after the operation, the enhancement of nuclear RNA synthesis is most likely a preparatory phase of regeneration. The increase in the grain count per nucleus due to lens removal is striking in the marginal areas of the iris but not clear in the nonmarginal area. However, no significant difference has been so far detected between the dorsal marginal area of the iris, which participates in the regenerative process, and the ventral marginal area, which does not.

When we take into account the well-founded possibility of transfer into the cytoplasm of RNA synthesized in the nucleus, the data fit in well with the earlier observation of Takata[45] that during the regeneration of the lens a large amount of RNA is present in the cytoplasm of cells that are being transformed into the lens tissue.

THE ULTRASTRUCTURE OF YOLK PLATELETS IN THE AMPHIBIAN EGG

Shuichi Karasaki

Yolk platelets of the amphibian egg have generally been designated as merely inert stores of raw materials for development. Recently, however, electron microscope observations[46,47] have suggested

[45] K. Takata, *Experientia* 8, 217 (1952).

[46] S. Karasaki, *Embryologia* 4, 273 (1959).

[47] G. Lanzaveccnia, *IV Intern. Conf. on Electron Microscopy* 2, 270 (1960).

that the main body of the yolk platelet consists of a crystalline lattice of lipoprotein and may be converted to cytoplasmic membranous structures during development. The major purpose of the present work, therefore, has been to provide a description of the ultrastructure of the yolk platelet which would serve as a basis for studies on their utilization and role during the process of differentiation.

The yolk platelets of young embryos and ripe oocytes of all amphibian species studied (*Rana pipiens, Triturus pyrrhogaster, Triturus torosus, Rana nigromaculata* and *Bufo vulgaris*) contain a superficial layer of fine particles of about 50 A diameter and a central main body having a crystalline lattice structure and are enclosed in a single membrane about 60 A thick. In some sections the main body displays a hexagonal, close-packing pattern of particles. It appears that main units in the crystal are approximately spheroidal and isometric in shape. They have a diameter of about 50 A and a center-to-center distance of about 70 A. In other sections, a regular arrangement of the interference fringes corresponding to a crystalline plane can be observed.

The type of fringe systems may be classified as follows: hexagonal net (average spacing of 62 A); cubic net (average spacing of 70 A); parallel lines (spacing from 35 to 100 A, but most frequent at about 70 A). These images suggest that the units are probably arranged close packed in a face-centered cubic lattice. However,the possible existence of subunits in each macromolecule rendersthis conclusion somewhat uncertain.

During oogenesis the main body is formed by a progressive accumulation and crystallization of 50-A particles within membranous systems which may be derived in part from mitochondria.

After the disappearance of the superficial layer of the yolk platelet during embryogenesis, loose stacks of membranes, each 60 A thick, appear around the main body. Some of these membranes reveal a triple-layered pattern of substructures, which is similar to the substructure of the plasma, mitochondrial, and rough-surfaced endoplasmic membrane systems. In *Rana* embryos (as opposed to urodele embryos) the crystalline structures are frequently observed within the interspaces of mitochondria. It is not improbable that these membranes represent an intermediate step in the formation of intracellular membranes at the expense of yolk platelets.

NITROGEN MUSTARD-INDUCED REVERTANTS OF MUTANTS IN THE rII REGION OF T4 BACTERIOPHAGE

E. A. Löbbecke D. R. Krieg

Introduction. – In previous experiments on *Habrobracon*, where the effects of nitrogen mustard (HN2), ethyl methanesulfonate (EMS) and x rays were compared, HN2 and EMS induced recessive lethals, with little or no difference in the frequency between treated metaphase and prophase oocytes. Both chemicals differ in this respect from the effect of x rays, which shows a twentyfold stage sensitivity difference. For dominant lethality, the effect of the chemicals was like that of radiation. It is believed that recessive lethals are genic. It is possible that HN2 induces recessive lethal mutations by the same action as EMS, for which the mutagenic action is better understood; both chemicals act, when their reaction with DNA was

tested (HN2,[48] EMS[49]), preferentially on guanine by forming 7-ethylguanine. In experiments on free phage, where the EMS-induced revertant frequency was measured,[50] it is thought that EMS induces mutations by means of the transition GC-AT. Nitrogen mustard, first used by Silvestri[51] (in a test for forward mutations), failed to act as a mutagen on free phage. Because of the general interest in mutagenesis correlated with the question of the structure of the gene, it seemed worthwhile to test HN2 again on free phage and to use for this experiment the same genetic method for inducing revertants described by Green and Krieg.[52]

Results. – Most of this study was done on the 2-aminopurine-induced mutant AP 72. A 20-min treatment of free phage with HN2 (0.016% concentration) was followed by a 1- or 2-hr treatment with 5% thiosulfate buffer, which in itself is ineffective, to stop the reaction. The phages were then absorbed to host bacteria and the progeny tested for induced revertants. After this treatment 1 to 3% survival was obtained. The results always showed a fivefold increase of the revertant frequency when compared with controls.

In some experiments dose response was measured. Samples were taken at various times after treatment with the same HN2 concentration. At doses where 75, 45, and 16% survival was obtained, the frequency of induced revertants was smaller, but a high variability was observed among tested growth bottles. However the data still suggest that a direct dose-effect relation holds for mutations. Higher doses will be tested in the future. Some other experiments were concerned with multiplicity reactivation, which is observed after UV- or x-radiation treatment. In the case of HN2 treatment, no multiplicity reactivation was observed when induced revertants were scored.

Three mutants have been tested. AP 72 and AP 275, both highly revertible with EMS (50- to 100-fold), show a three- to fivefold increase, whereas AP 156, responding only slightly to EMS, also shows only a very slight, if any, response to HN2.

Discussion. – Nitrogen mustard, known as a very potent mutagen in higher organisms, is also effective in bacteriophage when reversion rate is scored in progeny tests. Qualitatively, HN2 appears to act like EMS. The results can be explained in the same way, as mainly consisting of transition mutations of the type GC-AT. The revertant frequency of induced mutations is significantly lower for HN2 than for EMS. This may be due to the rapid induction of cross-linking of the DNA strands by HN2 which has two reactive groups per molecule. The cross-linking phenomenon will be tested in the future.

STUDIES ON THE ULTRASTRUCTURE AND METABOLISM OF NUCLEOLI IN AMPHIBIAN OOCYTES

O. L. Miller, Jr.

Introduction. – All amphibian genera have multinucleolar oocytes, the mature egg in some species containing over 1000 such bodies positioned peripherally in the nucleus close to the nuclear envelope,

[48] P. Brookes and P. D. Lawley, *Biochem. J.* 77, 478–84 (1960).

[49] B. C. Pal, unpublished data.

[50] D. R. Krieg, unpublished data.

[51] L. Silvestri, *Bolletino dell' Instituto Sieroterapico Milanese* 28, 193–99 (1943).

[52] D. M. Green and D. R. Krieg, *Proc. Natl. Acad. Sci. U.S.* 47, 64–72 (1961).

with no structural association with the oocyte chromosomes. Although there is no conclusive proof, as yet, that these nucleoli have a chromosomal origin, the marked similarity in fine structure between these nucleoli and those found in many other cell types strongly suggest this to be so. The amphibian oocyte thus provides a system in which changes in nucleolar structure and metabolism during growth can be studied without the interference of any concurrent synthesis by chromosomal nucleolar-organizer loci.

Results. – Oocytes of two amphibian species, *Triturus viridescens* and *Rana* sp., were studied by phase and electron microscopy. In both species, the number of nucleoli increases rapidly in the very young oocyte, reaching a maximum when the eggs are only a fourth to a third of their largest diameter. At all stages of growth, the large majority of nucleoli exhibit two distinct morphological components, one relatively dense and fibrous and the other a less compact association of granules and fibers. In all *Rana* oocytes and in the larger *Triturus* eggs, the granular component surrounds a fibrous core. In young *Triturus* oocytes, the granular component is internal.

When *Triturus* nucleoli are isolated in neutral veronal buffer, the fibrous core (3 to 6 μ in diameter) and granular cortex (5 to 10 μ in diameter) can be clearly separated. The relative sizes of the two components vary with the oocyte age: The core portion decreases in average size as the oocyte matures, while the cortex increases.

Autoradiographic studies on isolated nucleoli components after in vivo H^3-cytidine incorporation into RNA, and with whole oocyte sections after in vitro H^3-lysine incorporation into protein have been carried out in *Triturus*. In the RNA study, cortex material from eggs of various sizes was consistently "hotter" than the cores. Grain location and staining characteristics suggest, in fact, that the "core emissions" may be derived primarily from still-adhering cortex material rather than from the core itself. The protein study with whole oocytes showed significant and approximately equal lysine incorporation into both nucleus and cytoplasm even in 1-min samples. Younger oocytes exhibited much higher incorporation than larger ones. Incorporation levels in nucleoli were, at most, only equivalent to nuclear or cytoplasmic levels and, in some samples, appeared to be less.

Discussion. – Microbeam irradiation experiments[53] have shown that a large part of both nuclear and cytoplasmic RNA precursor incorporation is nucleolar-dependent. In addition, both staining[54] and H^3-lysine incorporation data[55] indicate that basic protein synthesis in nucleoli occurs in cell types in which the nucleolus is attached to an organizer locus. In view of these findings, and of the relative size changes in the two components of the oocyte nucleoli, our results are consistent with the suggestions that the nucleolar core is an RNA-basic protein product synthesized repetitively by the chromosomal nucleolus organizer locus early in oocyte history, while the cortex consists of future ribosomal material continuously being derived from other chromosomal loci and core material.

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[53] R. P. Perry, A. Hell and M. Errera, *Biochim. et Biophys. Acta* **49**, 47–57 (1961).

[54] P. G. Martin, *Nature* **190**, 1078–79 (1960).

[55] A. Mattingly, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 31–32.

EFFECT OF AMINO ACID DEPRIVATION ON GENERATION TIME IN *TETRAHYMENA PYRIFORMIS*

G. E. Stone

Introduction. – Separation of the processes leading to growth of a cell and those leading to division is indicated by a number of investigations. However, little information exists on the relative role that protein metabolism plays in the two processes. A previous report[56] demonstrates that if a population of *T. pyriformis* is deprived of an essential amino acid, arginine, about 20% of the cells continue through the next division. These considerations have led to a desire to analyze more critically the effect on generation time of cells deprived of essential amino acids.

Methods. – At known times after division, cells were transferred to a synthetic medium lacking tryptophan and histidine, and the generation times were determined. As controls, we determined the generation times of cells transferred to a complete synthetic medium (with tryptophan and histidine) and cells which were grown in medium supplemented with proteose peptone (stock culture medium).

Results and Discussion. – The results of three experiments are averaged in Table 7. Cells deprived of these amino acids within 1 hr after division do not reach the next division. About 15% of the cells deprived of the amino acids 1.5 hr postdivision continue through the next division with an increase in generation time of about 60 min over the cells grown in complete synthetic medium. All cells deprived of tryptophan and histidine after 1.5 hr postdivision continue through the next division. The cells transferred to the incomplete medium at 2 and 2.5 hr after division show approximately the same lag in generation time as did those cells of the 1.5-hr group which divided. However, of those cells transferred to the incomplete medium at 3 and 3.25 hr postdivision, 80 to 90% exhibit a generation time which approximates growth in supplemented medium.

These results indicate three different phases in the interdivision cycle in response to amino acid deprivation: (1) an early phase during which amino acid deprivation completely inhibits further division, (2) an intermediate phase during which amino acid limitation only delays division, and (3) a late phase during which amino acid deprivation apparently has no effect.

[56] D. M. Prescott, *Exptl. Cell Research* 19, 228 (1960).

Table 7. Effect of Deprivation of Tryptophan and Histidine on Generation Time

	Control A	Control B	Postdivision Age of Cells when Transferred to Medium Deficient in Amino Acids (hr)						
			0	1	1.5	2	2.5	3	3.25
Average generation	225	276			341	328	325	220	231
Time, min								350[a]	325[a]

[a] 10 to 15% of cells in these groups showed a longer generation time. Control A, supplemented medium; Control B, completely synthetic medium.

DROSOPHILA

DISJUNCTION OF THE FOURTH CHROMOSOME OF *DROSOPHILA* IN TRANSLOCATION HETEROZYGOTES

E. H. Grell

The heterozygotes of four 3;4 translocations were analyzed for the nondisjunction of the free fourth chromosome. The chromosomes were marked in ways so that euploids and aneuploids for fourth-chromosome material could be classified. The four types of gametes that were identified are: (a) T(3;4), (b) free 3 ; free 4, (c) T(3;4)/free 4, (d) free 3. Type (c) gives rise to a triplo-4 zygote, and type (d) results in a haplo-4 zygote. Since the haplo-4's have reduced and variable viability, the calculations for fourth chromosome nondisjunction are based on the number of triplo-4's. The frequency of nondisjunction is taken as

$$F = \frac{2T}{D + 2T},$$

where T is the number of triplo-4's and D is the number of diplo-4's.

In Table 8 are presented the genotypes of the flies, the sex, the positions of the break points of the translocations on the salivary-gland map and the frequency of nondisjunction of the free fourth chromosome.

Only with T(3;4)89E/+; ci^D is the frequency of nondisjunction for males and females nearly equal. With the other translocations there is more nondisjunction of the 4 in the female than in the male.

The amount of nondisjunction also varies between translocations. In the female it appears to correlate with the position of the break point in the third chromosome. The break point of T(3;4)e is at 79E, which is near to the centromere. T(3;4)86D, T(3;4)88B and T(3;4)89E have break points that are progressively away from the centromere and toward the tip of the right arm of the third chromosome. The amount of nondisjunction is greatest when the break is near the centromere and becomes less as the break points are toward the tip of the chromosome.

In the male the correlation is not so clear, and nondisjunction does not appear to be related to the break point in 3. A better correlation seems to be the break point in 4. The two translocations with break points at 101F (near the centromere) have more nondisjunction than the two with breaks at 102F (near the tip of the chromosome).

In the last two tests in Table 8 the free third chromosome contained a rearrangement which interferes with pairing and greatly reduces the part of the translocation that consists of the right part of the third chromosome from the break point at 86D to the tip of the chromosome. The presence of the rearrangement in the third chromosome causes the nondisjunction of the free fourth chromosome to be greatly reduced in the female. In the male the frequency of nondisjunction is not altered by the rearrangement.

Discussion. – In the female the dependence of fourth chromosome nondisjunction in (3;4) translocation heterozygotes appears to be on the break point in the third chromosome. The closer the break point is to the centromere, and consequently the longer the translocated arm of the chromosome 3, the greater the amount of chromosome 4 nondisjunction. In accordance with the work of Grell,[57] the importance of the

[57] R. F. Grell, this report.

Table 8. Nature of the Translocation and Frequency of Nondisjunction

Genotype	Sex	Position of Break in 3	Position of Break in 4	Frequency of Nondisjunction
T(3;4)e / +;ci^D	♀	79E	102F	0.41
T(3;4)e / +;ci^D	♂	79E	102F	0.04
T(3;4)86D / +;ci^D	♀	86D	101F	0.36
T(3;4)86D / +;ci^D	♂	86D	101F	0.07
T(3;4)88B / +;ci^D	♀	88B	102F	0.21
T(3;4)88B / +;ci^D	♂	88B	102F	0.008
T(3;4)89E / +;ci^D	♀	89E	101F	0.09
T(3;4)89E / +;ci^D	♂	89E	101F	0.11
T(3;4)86D / Tp(3R)Vno;ci^D	♀	86D	101F	0.013
T(3;4)86D / Tp(3R)Vno;ci^D	♂	86D	101F	0.06

break point in 3 would seem to be that when the translocated arm is longer there are more crossovers between it and the homologous region in the normal third chromosome. Those translocated arms that are involved in an exchange are not available for pairing with chromosome 4, and in exchange tetrads the fourth chromosome segregates at random. When a rearrangement like Tp(3R)Vno is present which inhibits crossing over between the translocated arm of T(3;4)86D and the free chromosome 3, the translocated arm is then available for pairing with chromosome 4.

The nondisjunction in the male is usually at a fairly low level. There is no crossing over in the males, and, consequently, the length of the translocated arm is not a large factor. Even though there is

variation, the general stability of disjunction in the male reflects fundamental differences in the meiotic processes between males and females.

A NEW MODEL FOR MEIOSIS IN THE FEMALE OF *DROSOPHILA MELANOGASTER*

R. F. Grell

Although crossing over and disjunction each imply an association between chromosomes, existing meiotic models invoke a single pairing for both events. At the same time, a number of well-established observations are incompatible within the framework of these models. This investigation proposed to examine the relation of crossing over in chromosome 2 to nonhomologous association between one of the second chromosomes and a Y in order to obtain information that might better order the sequence of events during meiosis. Specifically, answers to the following questions were desired:

1. Are chromosomes participating in nonhomologous associations solely noncrossovers?
2. Do nonhomologous associations, involving one of a pair of homologues, reduce crossing over between those homologues?

Results. – The assortment of the Y chromosome with the crossovers and the noncrossovers for the second chromosomes is given in Table 9. Among the crossovers, approximate equality of the YT(2;3), T(2;3), YGla and Gla classes is observed, indicating no association between the Y and either second chromosome. Among the nonrecombinants, the Y and the Gla second chromosome are recovered apart about 75%, indicating YGla association at least 50% of the time. These data clearly demonstrate that nonhomologous associations involve only noncrossovers.

In the event of a single pairing, association between Y and Gla must occur at the expense of association between Gla and its homologue and should reduce crossing over accordingly, or about 50%. Table 10 gives the crossing-over values for chromosome 2 in the presence and absence of nonhomologous association between Y and Gla. No reduction in crossing over is observed as a consequence of such association.

Table 9. Assortment of the Y with Crossovers and Noncrossovers for Chromosome 2

		YT(2;3)[a]	T(2;3)	YGla	Gla	a[b] (%)
Exp. 1	Crossover	104	129	113	124	53.2
	Noncrossover	791	203	184	1042	
Exp. 2	Crossover	48	65	53	53	52.6
	Noncrossover	387	109	153	734	
Exp. 3	Crossover	24	56	63	62	49.6
	Noncrossover	317	95	91	500	

[a]YT(2;3) class shows reduced viability.
[b]a = nonhomologous association.

Table 10. Effect of Nonhomologous Association on Crossing Over Between Homologues

		Totals	% Crossing Over		
			Region 1	Region 2	Totals
Exp. 1	Association absent	4848			15.20 ± 0.52
	Association present	2690			17.47 ± 0.73
Exp. 2	Association absent	858	9.21 ± 0.99	4.55 ± 0.71	13.76 ± 1.18
	Association present	1602	9.18 ± 0.72	4.49 ± 0.52	13.67 ± 0.86
Exp. 3	Association absent	1521	11.37 ± 0.81	4.34 ± 0.52	15.71 ± 0.93
	Association present	1208	11.92 ± 0.93	5.05 ± 0.63	16.97 ± 1.08

Discussion. – Since associations between nonhomologues do not affect total exchange frequency, yet involve only noncrossovers, it appears that they do not precede exchange. Rather, these results are interpreted to mean that such associations occur after exchange and between elements not participating in exchange. Studies in progress on secondary nondisjunction of the X chromosomes indicate XXY associations that lead to secondary exceptions (and thus that are known to involve only noncrossovers for the X's) also do not affect total X crossover frequencies and must occur subsequent to exchange.

As a consequence of these findings, the following model for meiosis in the female is proposed. *Exchange pairing* between specific homologous loci precedes exchange. In the event of exchange, the pattern for distributive pairing is established, and regular disjunction follows. In the absence of exchange, *distributive pairing* occurs between pairs of noncrossover chromosomes and leads to their disjunction from one another. Under normal conditions, the noncrossovers available for distributive pairing are almost exclusively homologues, so that, in terms of segregation, the consequences of exchange and distributive pairing are indistinguishable. Under abnormal conditions, the supply of noncrossover chromosomes may be increased and varied so that association between nonhomologues and between heteromorphs as well as between homologues occurs. In such cases, the frequency of nonrandom assortment of nonhomologues, of secondary exceptions, or of dominant lethals, is a measure of the amount of distributive pairing between the nonhomologues or heteromorphs concerned.

A NON-NOTCH LETHAL AT THE NOTCH LOCUS OF *DROSOPHILA*

W. J. Welshons E. S. Von Halle

Introduction. – Pseudoallelism of sex-linked recessive lethals has been previously demonstrated at the Notch locus. All the recessive lethals used could be alternatively classified as dominant visible mutations since the *N*/+ heterozygotes display notched wings, thickened wing veins, and minor bristle abnormalities. Comparisons of the Notch locus, where all recessive lethals can be classified as dominant visibles, having the dumpy (*dp*) locus, where none of the recessive lethals can be alternatively classified, led

to the conclusion that the latter type of lethal should also occur at Notch. Quite accidentally, a lethal of this type was detected on a chromosome which already carried an *N*.

Results. – From the cross $y\ w^a\ N^{264\text{-}107}$+/+ + *spl rb* ♀♀ by $w^a\ fa^{no}$ *spl rb* ♂♂, four $y\ w^a$ + + *rb* recombinant chromosomes were recovered, indicating that $N^{264\text{-}107}$ occupied a position a short distance to the right of the *spl* pseudoallele. Three of the four recombinant chromosomes also carried a recessive lethal mutation which, unlike *N*, was phenotypically normal in the heterozygous condition. Initially, it was assumed that the recessive lethal was not associated with the Notch locus, but subsequent tests have indicated that it is a pseudoallele. Thus, the non-Notch lethal is localized between its two pseudoalleles *spl* and $N^{264\text{-}107}$; that is, the parental genotype from which the recombinants were recovered can be represented as $y\ w^a$ + *1* $N^{264\text{-}107}$+/+ + *spl* + + *rb*.

Conclusions. – The discovery of a non-*N* recessive lethal pseudoallele of *N* supports the view that the Notch locus is not atypical. Consequently, comparisons of *N* with other pseudoallelic loci of *Drosophila* gain validity and support the suggestion already made on the basis of such comparisons that pseudoallelic recessive lethals in *Drosophila* afford the best cross reference when comparing complex loci of macro- and microorganisms.

INTERCHROMOSOMAL EFFECTS OF MULTIPLY REARRANGED CHROMOSOMES

D. T. Suzuki

Introduction. – With the discovery that inversion heterozygosis in one pair of chromosomes increases crossing over in a nonhomologous pair in *Drosophila melanogaster*, a variety of chromosome aberrations has been found to have such interchromosomal effects on crossing over. Earlier, it was shown[58] that the formation of a ring or synaptic loop in the X chromosome is a sufficient, but not necessary, condition for increasing crossing over in chromosome 3. However, there are combinations which increase crossing over in this chromosome but which do not form rings or loops upon synapsis. This group includes: (1) the reversed acrocentric compound X chromosome (which synapses like two rods) with or without a Y chromosome; (2) the Bar Stone duplication, either as a free duplication or attached to a rod X as a second arm; (3) the reversed metacentric compound X with a free Y chromosome; and (4) two rod X's with a free Y. A feature common to these four constitutions is a definite frequency of mechanical interference with synapsis of the X chromosomes.

Results and Discussion. – To determine whether asynapsis *per se* is sufficient to increase autosomal crossover frequencies, two multiply rearranged chromosomes were tested for their effects on crossing over on chromosome 3. The assumption was made that the frequency of asynapsis of these chromosomes is high owing to mechanical difficulties of pairing (see Thompson[59]). The chromosomes tested were FM-6; an X chromosome having eight break points; and SM-5, a second chromosome bearing 13 break points.

[58] D. T. Suzuki, *Genetics* 47 (in press).

[59] P. E. Thompson, *Genetics* 47 (in press).

Crossing over in chromosome 3 was measured in females heterozygous for FM-6 or SM-5 and in control females which were homozygous for nonrearranged chromosomes. The results are listed in Table 11. All the chromosomes in the FM-6 bearing females were replaced with control chromosomes in the first test (lines 1 and 2) but were not controlled in the second test (lines 3 and 4). SM-5 chromosomes from two different stocks (lines 5 and 7) were tested at the same time as the controls (line 6).

It can be seen that both FM-6 and SM-5 increase crossing over greatly and that the pattern of increase is similar for both chromosomes. The large increases in the four regions on either side of the centromere region ($st - p^p$) are not typical of inversion-heterozygote effects and may reflect a different pattern of effects due to asynapsis. Since there are regions several crossover units in length in both FM-6 and SM-5 chromosomes that remain undisrupted by breakage, some regional pairing of the rearranged chromosomes with their homologues undoubtedly takes place. It can be concluded, nevertheless, that the formation of large inversion loops is not necessary for interchromosomal effects on crossing over and that asynapsis may be a sufficient condition for causing such effects.

Table 11. Crossover Values on Chromosome 3

Parental Genotype	Total Progeny	Crossover Values in Each Region of Chromosome 3					Map Length
		$ru - h$	$h - st$	$st - p^p$	$p^p - ss$	$ss - e^s$	
FM-6/+	808	25.5	28.7	8.2	19.3	14.4[a]	96.1
+/+	894	21.9	21.4	4.9	13.5	12.3	74.0
FM-6/+	1449	21.3[a]	27.3	6.7	15.7	17.1	88.1
+/+	1002	20.5	18.5	4.3	11.0	10.9	65.2
SM-5/+	1305	31.9	24.6	4.4[a]	12.3	16.2	89.4
+/+	1778	22.8	18.6	3.3	8.5	11.5	64.7
SM-5/+	2220	27.7	23.5	5.2	13.6	14.6	84.6

[a]Value is not significantly different from control.

THE PRODUCTION AND CHARACTERIZATION OF X;AUTOSOME TRANSLOCATIONS

Mary Warters D. L. Lindsley

A method has been perfected for recovering translocations from the daughters of irradiated males. The results from the preliminary trial of this method have already been presented,[60] but more recently a much larger experiment was performed. From 2188 adequately tested genomes derived from sperm that had received 4000 r, the following translocations were recovered: 63 T(X;2)'s, 59 T(X;3)'s, 28 T(X;2;3)'s, and

[60]M. Warters, D. L. Lindsley, and M. L. Rekemeyer, *Biol. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 68–70.

124 T(2;3)'s. (The procedure does not yet contain an adequate screen for fourth-chromosome translocations.) With very few exceptions, the above X-chromosome translocations are currently being carried in balanced stocks.

The method of recovery is such that each X;autosome translocation can be scored for male viability and, where translocation-bearing males survive the fertility of such males, may be tested easily. The characterization of a sample of the translocations recovered as well as those recovered in the trial run of the method is presented in Table 12. Approximately 20% of the translocations fail to survive as males; that is, they are associated with sex-linked recessive lethals. About three-fourths of the translocations that survive as males sterilize the male that carries them; one of the major questions to be answered by this investigation pertains to the causal relation between X;autosome translocation and male sterility. Fifteen of the male fertile translocations have been examined for homozygous viability; eight survived and seven were inviable. This inviability is presumably caused by a recessive lethal on the translocated autosome, and the frequency of such lethals agrees with similar observations made by Patterson *et al.*[61] (60/128 T(X;2)'s and T(X;3)'s viable as homozygotes). It appears that the probability that a T(X;A) will carry an autosomal recessive lethal is about twice the probability that it will carry a sex-linked recessive lethal.

Table 12. Genetic Analysis of X;Autosome Translocations

	Male Lethal	Male Viable		Σ
		Male Sterile	Male Fertile	
T(X;2)	14	46	16	76
T(X;3)	11	42	12	65
T(X;2;3)	12	16	5	33
Σ	37	104	33	174

This is what is expected for lethals that are induced independently of the translocation, since the lengths of the X and an autosome also stand in a ratio of approximately 1 to 2. The observed frequencies of lethality are far too high to be explained by independently induced mutation; consequently, there must be a good deal of lethality associated with the translocations themselves. Furthermore, the method of balancing the translocations has been such as to keep independently induced sex-linked mutations and to lose independently induced autosomal mutations. Therefore, the residue of independently induced lethals remaining after a number of generations in stock should exhibit a ratio of autosomal to sex-linked lethals of considerably less than 2. It would seem that lethals that are causally related to the translocation are twice as likely to be autosomal as they are to be sex-linked, yet there is no obvious reason why the autosomal break of a translocation should be more subject to lethality than the X chromosome break point.

[61] J. T. Patterson *et al.*, *Am. Naturalist* 68, 359–69 (1934).

Since sex-linked lethality can be measured on the entire sample of translocations recovered, whereas autosomal lethality can be tested on only that subset of translocations that are male viable and fertile, a negative correlation between the break combinations that lead to sex-linked lethality and/or sterility and those that lead to autosomal lethality could account for the observed excess of autosomal lethals among male fertile T(X;A)'s.

Finally, preliminary tests on the viability of T(X;A)/0 vs viability of T(X;A)/Y have been carried out, and a number of translocations have been found that survive as T(X;A)/Y males but succumb as T(X;A)/0 males; these translocations probably carry variegated-type, position-effect lethals, which have been described in detail elsewhere.[62] It might be argued that the low ratio of T(X;A)/Y lethals to T(X;A)/T(X;A) lethals described in the previous paragraph is attributable to suppression by the Y chromosome of sex-linked position-effect lethals in the first genotype. This is not in accord with our experience, however, since it has been observed that position-effect lethals survive as X/Y males and as females but die as X/0 males.

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

Mammalian Genetics and Development

Section Chief – W. L. Russell

Genetic Effects of Radiation in Mice

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Effects of Radiation on Mammalian Gametogenesis

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Mammalian Cytogenetics

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MUTATION FREQUENCY AND RADIATION DOSE RATE

W. L. Russell

Elizabeth M. Kelly

In an earlier report[2] the specific locus mutation frequency in mice exposed at the intermediate dose rate of 0.8 r/min for a total dose of 600 r was found to be significantly below the frequencies obtained from exposures at the high dose rates of 90 r/min and 24 r/min, and not significantly different from the

[1]Consultant.

[2]W. L. Russell and E. M. Kelly, *Biol. Div. Semiann. Progr. Rept., Aug. 15, 1960*, ORNL-2997, p 77.

frequencies obtained at the low dose rates of 0.009 r/min and 0.001 r/min. Another experiment in this series has now produced data sufficient for the drawing of preliminary conclusions.

In this new experiment, the specific locus mutation frequency at 7 loci is being measured for spermatogonia of mice irradiated at a dose rate of 9 r/min for a total dose of 600 r (250-kvp x rays). In a total of 28,339 offspring of the irradiated males so far observed, 14 mutations have been found. This mutation frequency is approximately half of that observed earlier with 90 r/min irradiation, and the difference is statistically significant ($P = 0.014$). Since the 90 r/min experiments also used x rays, with identical conditions, we now have, for the first time, completely excluded any possible effect of radiation quality. The point estimate of the mutation frequency in the new experiment at 9 r/min is intermediate between the frequencies obtained earlier at 24 r/min and 0.8 r/min. However, neither the difference between the 9 r/min and the 24 r/min experiments nor that between the 9 r/min and the 0.8 r/min experiments is, as yet, statistically significant.

The important point to emphasize at the present time is the statistically significant reduction in mutation rate that occurs when dose rate is lowered from 90 r/min to 9 r/min, a mere tenfold drop. If the present estimate is confirmed by additional data, it would indicate that lowering the dose rate from 90 r/min to 9 r/min in medical exposures would reduce the genetic risk to approximately one-half.

RADIATION RESPONSE OF OOCYTES IN MICE AND GUINEA PIGS

E. F. Oakberg Evelyn Clark

Continuation of experiments comparing oocytes in mice and guinea pigs confirmed the observations reported earlier that arrest of oocyte development occurs at different meiotic prophase stages in the guinea pig and mouse, and that guinea pig oocytes are more resistant to radiation.[3]

Serial sections of both ovaries were prepared for all animals, and all sections were scored for the mouse. In the guinea pig, oocytes with only a few follicle cells or a single layer of flattened follicle cells were counted in every twentieth section; more advanced stages were scored in every section. In both mice and guinea pigs, only those oocytes with the nucleolus appearing in the section were counted. For smaller oocytes, this ensures counting a cell only once. However, as the oocyte enlarges, the number of nucleoli increases, and they often appear in more than one section. Therefore, for follicles ranging from a single layer of cuboidal cells to those with large antra, adjacent sections were examined to avoid counting the same oocyte twice.

From the data of Table 13, it is clear that oocytes in early follicles (a few cells to a single layer of flattened follicular cells) are much more resistant in the guinea pig than in the mouse, with 30% survival 14 days after 200 r, compared with about 1% survival in comparable follicle stages 2 to 10 days after 50 r in the mouse. Follicles with an antrum, however, show a different response, with no decrease until 7 days after 500 r in the mouse. The decrease that does occur can be accounted for by ovulation and spontaneous

[3] E. F. Oakberg and E. Clark, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 57.

Table 13. Effect of Radiation on Oocytes in Early and Late Follicle Stages of Mice and Guinea Pigs

Time After Irradiation (days)	Early Follicles				Late Follicles			
	Mouse (50r)		Guinea Pig (200 r)		Mouse (500 r)		Guinea Pig (200 r)	
	No. Cells[a]	Exptl. / Control	No. Cells[b]	Exptl. / Control	No. Cells[a]	Exptl. / Control	No. Cells[b]	Exptl. / Control
Control	3549		37,710		71		25	
1	959	0.270	37,780	1.002	66	0.930	15	0.600
2	27	0.008			70	0.986		
3	35	0.010	17,580	0.466	64	0.901	0	0.000
7			15,420	0.409	47	0.662	2	0.080
10	24	0.007	13,300	0.353	39	0.549	0	0.000
14			11,560	0.307	25	0.352	41	1.640

[a]Mean of 3 mice for each treatment.
[b]Two control guinea pigs; one for each time after irradiation.

atresia. Only two normal follicles with antra were observed in the three guinea pigs killed at 3, 7, and 10 days. By 14 days a new group of large follicles had developed from surviving earlier stages. Our data still are meager, but the greater sensitivity of older follicles in the guinea pig probably results from destruction of follicular cells rather than high sensitivity of the oocyte.

The above results are further confirmation of large species differences in radiation response of the mammalian ovary. Our data demonstrate that both stage of the follicle and developmental stage of the oocyte are important variables and suggest that the relative importance of these factors may be species specific.

CHROMOSOME LOSS AND NONDISJUNCTION IN MOUSE SPERMATOCYTES

Liane B. Russell C. L. Saylors

Introduction. – Comparison of the spontaneous frequencies of the two types of sex chromosome abnormality that can be formed from nondisjunction in the first meiotic division of the male shows the frequency of $X^M/0$ to be much greater than that of $X^M/X^P/Y$. In an analysis of matings where the two types could be scored simultaneously,[4] the ratio between these frequencies was about 40 to 1. On the basis of a number of considerations, the hypothesis was advanced that a large portion of the excess of spontaneous $X^M/0$ over $X^M/X^P/Y$ was the result of events occurring after sperm entry into the vitellus. Irradiation administered at that stage (pronuclear) can greatly increase the frequency of sex chromosome loss.[4] Sex

[4]L. B. Russell, *Science* 133, 1795–1803 (1961).

chromosome loss can also be induced, though with considerably smaller frequency, by irradiation of sperm prior to its entry into the vitellus.[5,6] The present experiment was undertaken to determine (1) whether nondisjunction in meiosis of the male mouse could be induced by radiation and (2) the relative frequency of sex chromosome loss in comparison with that induced at other stages.

Method. – Adult (101 × C3H)F_1 males were irradiated with 200-r acute x rays and held unmated for 2 weeks. During the third week after irradiation, each was mated to eight miscellaneous females, and all young conceived were discarded without examination. The purpose of this mating was to exhaust any stored spermatozoa. Oakberg[7,8] has determined that the progeny of cells irradiated as primary spermatocytes can reach the ejaculate starting at day 22 after irradiation. Therefore, starting 21 days after irradiation, each male was placed with three females carrying sex-linked markers. At 28 days, he was transferred to a pen containing four fresh females; at 35 days, this procedure was repeated. Females used were either *Ta/+*, *Blo/+*, or *Ta+/+ Blo* (c.o frequency between *Ta* and *Blo* is about 4%). Irradiated groups and the contemporary control were matched as closely as possible for distribution of the various genotypes as well as for age of mates. Young were observed and sexed at birth, classified for phenotype at about 7 days, and classified again at 27 to 36 days. All phenotypically exceptional animals were saved for further verification (1) by test-mating and (2) by cytological study from tail or ear biopsies. These results will be reported later.

Results. – A summary of the results from 6143 animals classified to date is presented in Table 14. No breakdown by sex or phenotype is shown at this time since further numbers are still being collected.

[5] L. B. Russell and C. L. Saylors, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 58.

[6] L. B. Russell and C. L. Saylors, *Genetics* 47, 7–10 (1962).

[7] E. F. Oakberg, *Nature* 180, 1497 (1957).

[8] E. F. Oakberg and R. L. DiMinno, *Intern. J. Rad. Biol.* 2, 196–209 (1960).

Table 14. Frequency of Paternal Sex Chromosome Loss or Nondisjunction Following Irradiation of Mouse Spermatocytes

Treatment and Mating Schedule	Total Born	Total Classified[a]	X0	XXY	Adjusted % Exceptionals[b]
Mated 22–28 days postirradiation	1147	1099	3	0	0.51
Mated 29–35 days postirradiation	1750	1675	6 + 2?[c]	1	0.79–1.02
Mated 36–42 days postirradiation	1192	1142	2	0	0.35
All irradiated groups	4089	3916	11 + 2?[c]	1	0.59–0.69
Controls	2323	2227	1	0	0.09

[a] Includes exceptionals. The difference between number born and classified in general represents animals dying before 7 days of age.

[b] Estimated frequency of occurrence calculated by taking account of detectability, which, in the case of *Ta/+* or *Blo/+* mates, is 50%; in the case of *Ta+/+ Blo* mates, 100%.

[c] Classified by phenotype only (died before test).

A total of 13 to 15 exceptional animals was observed, one of these being XXY, the rest X0. It is obvious from a comparison of the sum of irradiated groups that the incidence of exceptional animals is considerably higher than in the control. There is an indication that the 29- to 35-day mating period may have the highest incidence of exceptionals, but the data are not yet conclusive in this respect.

Discussion. – Because spontaneous frequency of XXY males is known to be extremely low, the single XXY in the irradiated group suggests – but of course does not prove – that meiotic nondisjunction is being induced by radiation of spermatocytes. More such animals will have to be obtained to be able to calculate a frequency. The X0 animals resulting from radiation could be formed by a number of mechanisms: nondisjunction in the strictest sense at the first division (with X0 being formed complementary to XXY), nondisjunction in a broader sense (e.g., failure of the X-Y bivalent to get on the spindle), nondisjunction at the second division, or chromosome breakage. As it stands, the frequency of X0 induced in this experiment is similar to that induced in spermatozoa[6] (after making allowance for different doses used). Since all sperm-induced X0's, but presumably only a portion of the spermatocyte-induced X0's, result from chromosome breakage, it may be that spermatocytes have a lower sensitivity to breakage than do spermatozoa. On the other hand, it must be kept in mind that the spermatocytes sampled constitute a selected population, since, with the dose used, from 13 to 48%, depending on stage, are killed.[8]

PHOTOREACTIVATION AND ACTION SPECTRUM OF ULTRAVIOLET-INDUCED MAMMALIAN CHROMOSOME ABERRATIONS

E. H. Y. Chu

Introduction. – Earlier work[9] demonstrated the induction of chromosome aberrations in Chinese hamster cells in vitro exposed to monochromatic wavelengths of ultraviolet (UV) radiations. Recent experiments extended the quantitative data and more precisely established the temporal distribution of aberration types after irradiation. Attenuation of the UV light by the hamster cells and the UV action spectrum for chromosome aberration production have been studied. Furthermore, UV-induced chromosome aberrations were found to be photoreactivable – a phenomenon hitherto unknown for chromosome aberrations, except possibly in growing *Tradescantia* pollen tube cultures[10] – thus opening new avenues for studies of chromosome structure and the rejoining mechanisms of broken chromosomes.

Results. – A knowledge of cell size and shape is important for the estimation of UV absorption. Direct microscopic and microhematocrit measurements of living cells yielded an average cell volume of about 1200 μ^3. The rounded-up cells in suspension have an average diameter of 13 μ, but the cells grown in monolayer for irradiation are less than 3 μ thick. When the cells are suspended in phosphate-buffered

[9] E. H. Y. Chu and E. R. Monesi, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 61–62.

[10] J. S. Kirby-Smith, B. Nicoletti, and M. L. Gwyn, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 197–98.

saline (using a standard inoculum concentration of 2×10^5 cells/ml), crude measurements show that there is only a low degree of light absorption plus scattering (e.g., 37% at 2200 A, 26% at 2400 A, 21% at 2600 A, and 17% at 2800 A). The cells in monolayer must absorb even less.

The chromosome aberrations induced by various UV wavelengths reach a peak frequency at 2652 A (Table 15). At 2804 A, although the scorable aberrations show a slightly lower frequency than at 2652 A, there are additional chromosomal abnormalities such as fuzziness and extreme fragmentation and shattering, which are difficult to quantitate. The total aberration production may well be the highest at this wavelength.

When a posttreatment of visible light (at 3300 to 4400 A) is given to the cells immediately after irradiation, the final appearance of aberrations is significantly reduced (Table 15).

Table 15. Ultraviolet Induction and Photoreactivation of Chromosome Aberrations at Various Wavelengths

Wavelength (A)	Number of Cells Scored	Chromatid Aberrations			Aberrations Per Cell
		cd	iso	exch	
2250[a]	100	18	4	5	0.27
2378	100	23	9	4	0.36
2445	100	15	7	21	0.43
2652	350	105	51	109	0.76
2804	355	125	32	91	0.69
3130	100	2	1	0	0.03
3650	100	2	1	0	0.03
2652 + PR[b]	200	48	9	34	0.46
2804 + PR	200	26	8	11	0.23

[a]An incident surface exposure of 100 ergs/mm^2 was given for each wavelength series.
[b]Photoreactivating wavelengths in the range 3300 to 4400 A for 20 min at 37°C.

Discussion. – Light absorbance measurements show that the cells, under the cultural conditions, are highly transparent to UV and that the total light absorbed varies only slightly within the wavelength range most efficient for induction of chromosome aberrations. Thus, the high frequencies of chromosome aberrations induced at these wavelengths may be due to a selective absorption of UV energy by different constituents of the chromosome.

The action spectrum data for chromosome aberrations further suggest that UV produces at least two kinds of effects on chromosomes. First, it damages both the structural nucleic acid and protein moieties of the chromosomes. The nucleic acids are damaged because the action spectrum for aberration production approximately parallels the action spectrum for UV absorption by nucleic acids. The structural protein is also affected because UV at both 2378 and 2804 A induces chromosome fuzziness. Moreover, it was noted earlier[9] that chemical inhibition of protein synthesis, when combined with UV-irradiation at 2652 A, also induces chromosome fuzziness and shattering, although neither treatment alone produces these effects.

Second, UV impairs the repair mechanisms of chromosome breaks. It is known that UV at a wavelength of 2804 A also is absorbed by nucleic acids, but it further may damage the enzyme(s) necessary for chromosome rejoining.[11] The fact that UV-induced chromosome aberrations are partly photoreactivable probably means that, for a certain proportion of aberrations, DNA is the site of primary damage and that this damage is repairable by photoreactivation of enzyme(s) already present in the cell – a situation similar to that in the bacterial system.[12]

[11]Sheldon Wolff, *Radiation Research* (suppl 1), pp 453–62 (1959).

[12]C. S. Rupert, *J. Cellular Comp. Physiol.* 58 (suppl 1), 57–68 (1961).

Radiation Microbiology

Alexander Hollaender

Mutagen Mechanisms – Bacterial Metabolism

G. E. Stapleton Annie S. Angel

Bacterial Genetics

H. I. Adler J. C. Copeland

EFFECT OF CULTURAL CONDITIONS ON RADIOSENSITIVITY OF MICROORGANISMS

G. E. Stapleton

The radioresistance, as well as the resistance to inactivation by other damaging agents in *Escherichia coli*, is determined to a large extent by the cultural conditions before exposure of the cell populations to the deleterious agent. The outstanding features of the development of radioresistance are: (1) the presence of an oxidizable energy source such as glucose, (2) the attendant pH change resulting from acid production, (3) the presence of certain amino acids, and (4) a simultaneous increase in cell volume and parallel increase in the concentration of macromolecular components.

It is clear from our data that development of resistance in *E. coli* cultures is not the result of selection for a normally existing resistant organism in populations of this species.

The predominant effect of the development of radioresistance is reflected in a remarkable increase in the shoulder rather than a decrease in the slope of the survival curve. In this regard, it is important to understand the role played by the excess DNA or other macromolecular components. The "extrapolation number" of the survival curves is not simply related to the increased concentration of the DNA but may be represented as some power function of it. If this is the case, it is tempting to postulate an efficient recombination phenomenon to explain the data.

A number of experiments are planned to examine the activity of the DNA in the determination of radioresistance. It is clear that the DNA increase *per se* does not control the resistance since (1) the DNA content per cell doubles in the culture several hours before any increased radioresistance is measurable, (2) radioresistance develops late in the stationary phase of the culture cycle without any further increase in DNA, and (3) the expression of radioresistance is influenced dramatically by the presence of amino acids in the postirradiation plating medium.

These findings, in general, emphasize the importance of physiological processes in the expression of this development of radioresistance, which is assumed to be genetically determined.

SENSITIZATION OF *ESCHERICHIA COLI* TO X RAYS BY IODOACETIC ACID

G. E. Stapleton

In an attempt to assess the possible importance of reduced sulfhydryl groups in determining radiosensitivity of *Escherichia coli* we examined the effect of iodoacetic acid on the x-ray inactivation kinetics of resistant and sensitive *E. coli* B/r CSH.

Incubation of sensitive or resistant cells with up to 10^{-2} *M* iodoacetate, neutralized with NaOH, for 30 min at 37°C resulted in no measurable toxicity. However, if the cells so treated were cooled to ice-bath temperature and irradiated, they were sensitized to the inactivation by x rays.

About five times the concentration of iodoacetate is required to reduce the sensitivity of "resistant" cells to that of "sensitive" cells. Although this finding is suggestive of a possible involvement of sulfhydryl groups in radioresistance the data can be interpreted in several ways.

The survival curves suggest that the apparent "sensitization" is more likely due to reaction with a radiation-produced, short-lived product of iodoacetate. Attempts to look for such a toxic product and its reaction on irradiated or unirradiated cells have been unsuccessful. Moreover, it is necessary that iodoacetate be present during irradiation to measure any "sensitization."

GENETIC ANALYSIS OF RADIATION RESPONSE IN *ESCHERICHIA COLI*

H. I. Adler J. C. Copeland

In an earlier report[1] we demonstrated that genetic elements effecting the radiation resistance of *Escherichia coli* K12 could be passed from a donor to a recipient strain during conjugation. The data available at that time also indicated that the radiation response of these bacteria depended on a multigenic system. More complete data support this suggestion and allow us to indicate where these genes are located. Many recombinants of intermediate radiation sensitivity have been observed. The frequency with which they occur in classes selected for biochemical markers of known chromosomal locations allow us to suggest that a group of genes, located between proline and histidine on the *E. coli* K12 linkage map, can influence radiation sensitivity. The group of genes involves at least four distinct loci in this region. Since it is known that this region of the linkage map also contains several inducible prophages, and it has been suggested that induction may be a mechanism by which radiations produce their lethal effects, we have attempted to establish whether the genes influencing radiation resistance are identical with or closely linked to the inducible prophage sites. To date, it appears unlikely that these are identical or closely linked genes.

[1]H. I. Adler and J. C. Copeland, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 64.

These studies, originally investigating only survival after x irradiation, have been expanded to include ultraviolet (2537 A) radiation sensitivity. Similar results have been obtained, and the data indicates that the group of genes affecting x-ray sensitivity also affects ultraviolet sensitivity.

Mammalian Recovery

Hematopoietic Recovery

C. C. Congdon
R. R. Bigelow[1]
Gino Doria[2]
W. H. McArthur[1]
Stanfield Rogers[1]
Patricia G. Delker

Bone Marrow Physiology

L. H. Smith
Joan W. Goodman
G. S. Hodgson[2]
Wanda R. Carter
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Tissue Culture

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ANATOMIC STRUCTURE OF LYMPHATIC TISSUES AND IMMUNE FUNCTION

C. C. Congdon

For many years, attempts have been made to relate histologic changes in lymphatic tissues to measurements of immune mechanism functions, such as antibody production or foreign-tissue rejection. The first task of demonstrating that immune function resided in lymphatic tissues was finished a number of years ago and has been repeatedly confirmed. Immune function outside the lymphatic tissues we can reasonably well attribute to the disbursement of mobile cells from these tissues. New histologic studies seem to indicate clearly that immune function begins in the cortex of lymph nodes and in the white pulp of the spleen. Changes in the medulla of lymph nodes and in the red pulp of the spleen depend on the use of certain kinds of antigens and follow the primary changes in cortical and white-pulp areas of the lymphatic tissues. The primary changes are qualitatively the same after all kinds of antigenic stimulation and have been observed in several different mammalian species. For this reason we can suggest that the primary

[1]Consultant.

[2]Visiting investigator from abroad.

histologic changes constitute a potential method for diagnosing the presence or absence of immunologic function. It is necessary to account for changes after antigenic stimulation in marginal zone cells, lymphocytes, germinal centers, tingible bodies, and stromal elements.

The present work indicates that the germinal center is the major histologic structure associated with immune function. The tingible body is also a key anatomic structure whose function we do not understand. Perhaps it is concerned with the recognition of the antigenic material through phagocytosis of lymphocytes and granulocytes. The present work also requires a precursor cell for the germinal center. The two possibilities seem to be a fixed reticulum cell or the lymphocyte. The ultimate precursor cell may be the thymus. All questions of immune function and disease will need eventually to deal with these several anatomic structures.

INCREASED FREE ASPARTIC ACID IN LIVER OF MICE GIVEN HOMOLOGOUS BONE MARROW

A. L. Kretchmar[3]

It was previously reported[4] that increased free aspartic acid concentration occurs in liver of bone marrow chimeras. In the present work it was shown that the increase begins about 3 days after irradiation and administration of 40×10^6 homologous bone marrow cells and persists for at least 35 days after treatment. The highest levels are attained by about the seventh day after treatment and are somewhat lower on the 12th, 21st, and 35th days. Irradiated animals given 40×10^6 bone marrow cells from mice of the same strain (isologous cells) do not show this change. Mice that were not irradiated but were given daily injections of homologous cells for 11 days and then killed on the 12th day did not have an increased concentration of free aspartic acid in the liver. It is suggested that an actively proliferating graft of foreign bone marrow cells acts through a humoral intermediary to disrupt the balance between synthesis and utilization of aspartic acid in the liver of the host animal.

DEVELOPMENT OF TRANSPLANTATION ANTIGENICITY IN MOUSE HEMOPOIETIC TISSUES

Gino Doria

A quantitative study is being made concerning the capability of hemopoietic tissues to induce homotransplantation immunity as a function of age. The tissues studied are C3H fetal ($12\frac{1}{2}$ days and older) and neonatal liver, and adult (12-week-old) spleen.

The method is based on the observation that lethally irradiated mice are able to reject homologous bone marrow if they have been preimmunized to tissues of the same donor strain. The rejection is estimated by measuring the Fe^{59} uptake in erythrocytes. The methodology was developed by G. S. Hodgson. LAF_1 mice, 9 days after exposure to 800 r, are injected with 0.2 μc of Fe^{59}. Radioactivity is measured

[3]Medical Division, Oak Ridge Institute of Nuclear Studies. In collaboration with C. C. Congdon.

[4]A. L. Kretchmar and C. C. Congdon, *Federation Proc.* 20, 402 (1961).

in a blood sample taken 24 hr after injection. The iron uptake is less than 1% of the Fe^{59} dose. If the recipients are injected intravenously within 2 hr after irradiation with C3H bone marrow, the iron uptake increases linearly with the bone marrow dose (from 6×10^4 to 5×10^5 cells) up to 30%. A given dose of cells from the tissue to be studied is injected intravenously into LAF_1 mice before irradiation; these mice also received, after irradiation, a challenge dose of 5×10^5 C3H bone marrow cells. If immunization occurs, the iron uptake is less than in the nonpreimmunized controls. Hence, for any given immunization, it is possible to estimate by the calibration curve (iron uptake–bone marrow dose) the percentage of erythropoietically competent cells that have been rejected. The radiation is given 24 hr after the immunizing injection in order to stop any further growth of the cells and to be able to relate with good approximation the antigenicity to the age of the tissue studied.

Results show that neonatal liver and adult spleen are both antigenic; the former is three times less effective than the latter. Fetal liver, however, at $12\frac{1}{2}$ days gestation showed no antigenicity by the same technique. Older fetuses had intermediate degrees of antigenicity but less than that of the newborn.

NITROGEN BALANCE STUDIES IN RADIATION CHIMERAS

W. H. McArthur[5]

An investigation of nitrogen balance in bone marrow chimeras was undertaken to determine whether impaired utilization of dietary nitrogen is present in homologous disease. In addition, further information about the nitrogen intake in irradiated bone marrow treated mice would also be obtained in the balance study.

Several experiments have been carried to 30 days after irradiation. In each of these experiments LAF_1 mice were x-irradiated recipients. The homologous bone marrow donors were $(101 \times C3H)F_1$ mice. The recipients were exposed to 900 r and then given 40×10^6 isologous (IBM) or homologous (HBM) bone marrow cells. There were two IBM, two HBM animals, one normal and one x-irradiated untreated control in each experiment.

Table 16 shows the nitrogen intake data for one of these experiments. There was no significant difference in the nitrogen intake of HBM mice as compared with IBM animals. Despite the similar nitrogen intake, the HBM animals averaged a 1.75-g loss in body weight, while the IBM animals averaged a 2.15-g weight gain. Both of the bone marrow treated, irradiated pairs ingested less nitrogen than did the normal control.

There was no significant difference in the nitrogen balance between the bone marrow treated, irradiated pairs. In both, the mice were consistently in moderate positive nitrogen balance after the fourth posttreatment day.

It is concluded that dietary nitrogen is stored in mice treated with homologous bone marrow differently from in mice given isologous marrow after irradiation. The 4-g average difference in body weight between

[5]In collaboration with A. L. Kretchmar and C. C. Congdon.

Table 16. Average Daily Intake of Nitrogen by Bone Marrow Chimeras

Days After Treatment	Uptake (mg)			
	IBM-Treated Mice	HBM-Treated Mice	X-Irradiated Only	Normal Mice
1–10	90, 91	99, 71	84	118
11–20	129, 94	113, 97	32[a]	122
21–30	100, 100	90, 105		130
Final body weight, g	23.3, 25.5	19.5, 18.0	15	25.5
Initial body weight, g	23, 21.5	20, 21	23	24.0

[a]Died day 15.

HBM- and IBM-treated, irradiated mice could be accounted for if 115 mg of dietary nitrogen were stored as antibody-antigen complex in the HBM animal. Most of this storage would presumably occur during the last 20 days of the experiment.

FETAL THYMUS TRANSPLANTATION IN HOMOLOGOUS BONE MARROW CHIMERAS

P. G. Delker[6]

Miller[7] has recently shown that mice thymectomized at birth will not reject foreign skin grafts in the usual manner when the grafts are placed on the operated animals several months later. This work supports other evidence for the immunological function of the thymus[8] and, in addition, indicates that the thymus is the embryological source of the immune mechanism.

A study suggested by such experiments is using fetal thymus cells to help the foreign bone marrow chimera develop a tolerant immune mechanism and reduce the incidence of "secondary disease."

Table 17 shows the interim results of an experiment in which lethally irradiated LAF_1/J (C57/J ♀ × A/HeJ ♂) mice received homologous A•CA bone marrow followed a day later by an injection of thymus cells from fetal or adult mice of the same strain. The fetal isologous thymus did not alter the incidence of secondary disease as measured by the mortality at 60 days (67% vs 68%); however, it did not kill the irradiated recipients as did the adult isologous thymus cells. From other studies[8] we know the later result is caused by the thymus cells rejecting the foreign marrow graft.

In another set of experiments in which the fetal thymus was from homologous donors of the same type as the foreign marrow, $1C3F_1$ (101/Cum ♀ × C3H/Anf Cum ♂), the mortality results at 30 and 60 days do not show a beneficial effect of the fetal thymus injection.

[6]Work carried out under the direction of C. C. Congdon, J. W. Goodman, and J. Ferrebee (Cooperstown, N.Y.).

[7]J. F. A. P. Miller, *Lancet* 1961, 2, pp 748–49.

[8]C. C. Congdon and D. B. Duda, *Arch. Pathol.* 71, 311–23 (1961).

Table 17. The Effect of Isologous and Homologous Fetal Thymus on the Survival of Homologous Bone Marrow Chimeras

Treatment	Cell Dosage	Mice (No. and Sex)	Ratio and Percentage Mortality at	
			30 Days	60 Days
900 r + homologous (A•CA) bone marrow (i.v.)	10×10^6 marrow cells	49 ♂ 30 ♀	10/79 12.6%	53/79 67%
900 r + homologous (A•CA) bone marrow day 0 and isologous fetal thymus day 1	10×10^6 marrow cells and $5-17 \times 10^6$ fetal thymus cells	50 ♂ 34 ♀	7/84 8%	57/84 68%
900 r + homologous (A•CA) bone marrow day 0 and adult isologous thymus day 1	10×10^6 marrow cells and $5-17 \times 10^6$ adult thymus cells	45 ♂	All dead by day 17	
900 r + homologous ($1C3F_1$) bone marrow (i.v.)	10×10^6 marrow cells	100 ♂	12/100 12%	37/100 37%
900 r + homologous ($1C3F_1$) bone marrow day 0 and homologous ($1C3F_1$) fetal thymus day 1	10×10^6 marrow cells and $5-17 \times 10^6$ fetal thymus cells	103 ♂	9/103 9%	43/103 42%

IN VITRO SENSITIVITY AND PROTECTION OF IRRADIATED MOUSE BONE MARROW CELLS

L. H. Smith

Introduction. – Data from a series of experiments[9] designed to measure *in vitro* sensitivity and protection of x-irradiated mouse bone marrow cells have been tabulated and analyzed statistically.

Results and Discussion. – Thirty-day survival of lethally x-irradiated mice is a function of the number of normal isologous bone marrow cells injected. For C57BL/Rij female mice exposed to 798 r of 200-kv x rays, injection of about 0.1×10^6 normal nucleated cells resulted in 50% 30-day survival. Marrow cells were x irradiated *in vitro* with 100 to 400 r. By comparing the number of normal cells required to give 50% 30-day survival with the number of irradiated cells to give equivalent survival, a curve relating the percentage of surviving marrow cells to *in vitro* x-ray dose was constructed (Fig. 2). From the exponential portion of this curve the D_{37} was 84 r, with 95% confidence limits of 65 to 104 r. The extrapolation number is 2.5.

[9] Experiments were performed in collaboration with O. Vos at the Medical Biological Laboratories of the National Defense Research Council, T.N.O., Rijswijk (Z.H.), The Netherlands.

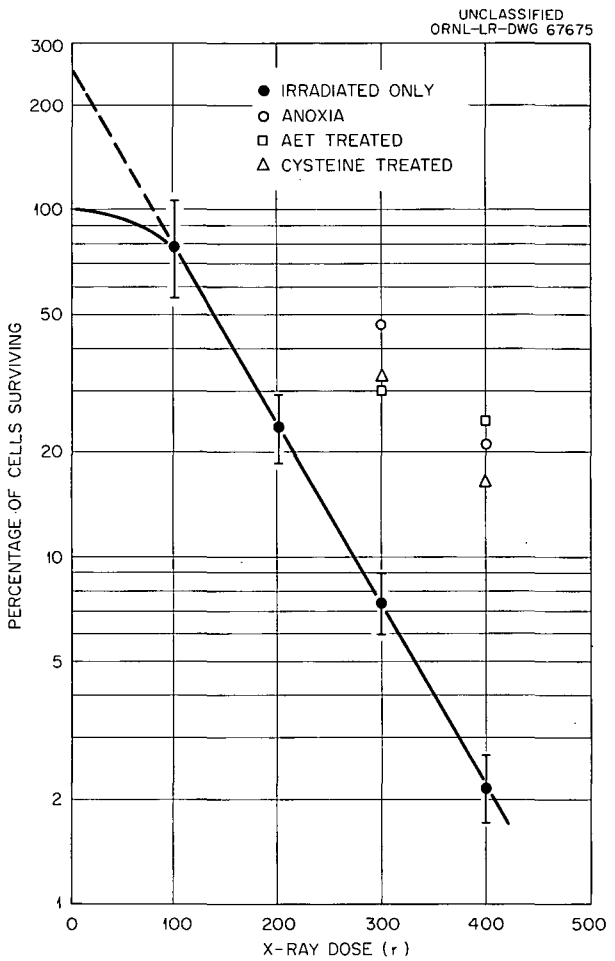

Fig. 2. The Effect of in vitro X Irradiation on Survival of Mouse Bone Marrow Cells. Vertical bars indicate 95% confidence intervals.

AET or cysteine, at a concentration of 4 m*M* or anoxia (nitrogen gassing), gave good protection to the cells irradiated *in vitro*. For these agents, dose-reduction factors ranged from 1.7 to 2.1. Noradrenaline at 4 m*M*, however, failed to protect the cells. Although the *in vitro* marrow system is not suitable for rapid, large-scale screening of protective agents, it does have the advantage of measuring the protection of normal cells in terms of their ability to proliferate *in vivo*. The system might be useful, therefore, as a means of further *in vitro* testing of those agents which, from results with more artificial *in vitro* mammalian systems, indicate protective potential.

BLOOD FORMING CAPACITY OF PERIPHERAL LEUKOCYTES

Joan W. Goodman[10]

Introduction and Methods. – The presence of immunologically competent cells in peripheral blood is well established for mice, rats, and rabbits, and there is suggestive evidence from radiation and trans-

[10]In collaboration with G. S. Hodgson, visiting investigator from Chile.

plantation experiments that these cells are present also in dogs, monkeys, and man. We have shown that cells circulating normally in mice have the capacity to differentiate into red cells and granulocytes as well as lymphocytes.

White cells have been concentrated from pooled heart blood from 100 to 200 F_1 hybrid mice, (C57BL/6 Cum ♀ × DBA/2 Cum ♂) or (C57BL/Cum ♀ × C3H/Anf Cum ♂), and injected into heavily irradiated inbred recipients (B10D2, D2, C3) which were either parent of or closely related to the donors.

At intervals after the transplantation, recipient mice were tested for peripheral red and white cell chimerism by standard serological methods. Some were killed for histological study and for typing of nucleated cells of bone marrow, lymph nodes, and spleen. Some chimeras were injected with Fe^{59} within 2 weeks after transplantation, and 24 hr later Fe^{59} uptakes by spleen and erythrocytes were measured.

Results and Discussion. – Transplantation and differentiation of leukocytes into erythropoietic, granulopoietic, and lymphopoietic elements have been shown in three mouse strains. The lowest cell dose that showed transplantation was 20 million, although a clear increase in iron uptake was seen, in an isologous transplantation, at a dose of 5 million. Four nonisologous chimeras are still living and retain donor hemopoiesis 6 months after transplantation. Nine of 10 mice irradiated and given 33 million isologous leukocytes are also "long-term" survivors.

Iron-59 was taken up by spleen and red cells of all chimeras tested but not by tissues or irradiated control mice. When the newly formed, Fe^{59}-containing erythrocytes were reacted with donor-specific isoantiserum in the presence of rabbit complement, the Fe^{59} was released into the supernatant. In some cases 100% recovery of the Fe^{59} activity was seen in specific serum, whereas very little was released from cells in nonimmune mouse serum.

THE SEARCH FOR TRANSPLANTATION ANTIGENS

G. S. Hodgson[11]

Throughout the world during the past 5 to 10 years a number of research groups have tried to determine the chemical nature of the substances in tissues that cause transplantation immunity and foreign-graft rejection. The problem has been extremely difficult from a conceptual and technical point of view. From what work has been done, the suggestion has been made that these antigens are mucoproteins.

Since this suggestion is not firmly established, the search for transplantation antigens is now in the state that genetics was when investigators were trying to find out what chemical substances contained the genetic information. Once it was known that the genetic information was in DNA, then the more detailed determination of the chemical basis for genetic specificity within the DNA molecule could be sought.

[11] Visiting investigator from Chile. Work done in collaboration with C. C. Congdon and D. G. Doherty.

Our present approach is that of the earlier period in genetics. We have used a histologic spleen assay to make a general search for substances that we think have chemical configuration similar to that of transplantation antigens.[12] Phenol extracts of *Salmonella typhosa* endotoxin contain such a substance. We hope to learn the general chemical properties from this system so that the more difficult chemical fractionation of mammalian tissues can be undertaken.

At the same time, we have found that rat urine contains substances that behave like transplantation antigens in a functional rat-mouse bone marrow chimera transplant test.[13] Powdered fractions obtained from ethanol and phenol extracts contain the antigenic material. An attempt to show the presence of these antigens in mouse urine failed, and we expect to shift to mouse or rat tissues as a source of the antigen as the chemical technology progresses. Our findings do not contradict the idea that mucoproteins are the transplantation antigens. Proof, however, is still lacking because of the chemical problems involved.

X-RAY-INDUCED CHROMATID ABERRATION RATE IN CULTURES OF HUMAN LEUKOCYTES

M. A Bender P. Carolyn Gooch

Introduction. – Earlier studies of chromosomal aberrations induced in human leukocytes by treating freshly drawn human blood with hard x rays showed that only chromosome-type aberrations were induced with an appreciable frequency.[14] From this we concluded that none of the leukocytes in peripheral blood have effectively doubled chromosomes. Autoradiographic studies of H^3-thymidine-labeled leukocyte cultures showed that virtually all the cells coming into metaphase in culture first went through an *in vitro* DNA synthesis (S) period.[15] The fact that a measurement of chromosome-type breakage uncomplicated the presence of cells capable of chromatid breakage can thus be made with the human leukocyte system allows direct comparison between pre- and postsplitting chromosomal aberration rates. To obtain postsplitting data for comparison with our presplitting measurements, we irradiated 66-hr leukocyte cultures with hard x rays (HVL = 2 mm Cu) and collected metaphases for the next 6 hr with colchicine. The metaphase cells examined were thus mainly in the post-DNA synthesis (G_2) period at the time of irradiation.

Results and Discussion. – Table 18 shows the pooled data for cultures of the leukocytes from three donors. No exchanges of the chromatid type were seen. There were a few chromosome-type exchanges, however, indicating that there was a small fraction of cells in the sample that still had at least some unsplit chromosomes at the time of irradiation. The fact that the chromosome-type exchanges did not increase exponentially with dose suggests that the higher doses of x rays used induced some degree of mitotic delay.

[12] C. C. Congdon *et al.*, *Radiation Res.* 14, 458 (1961).

[13] G. S. Hodgson, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 73.

[14] M. A Bender and P. C. Gooch, *Proceedings of the National Academy of Sciences of the United States* (in press).

[15] M. A. Bender and D. M. Prescott, *Experimental Cell Research* (in press).

Table 18. Chromatid Aberrations in Human Leukocyte Culture Irradiated 6 hr Before Fixation[a]

Dose (r)	Number of Cells Scored	Cells With 2n = 46	Chromatid Deletions	Isochromatid Deletions	Chromosome Exchanges	Chromatid Breaks (%)
Control	220	8	2	5	0	3.2
25	310	17	22	18	2	12.9
50	300	12	23	20	4	14.3
100	295	18	31	36	1	22.7

[a]Pooled data from cultures from three donors.

The slope of the linear regression line for the chromatid breakage data is 0.0023 breaks per cell per roentgen. This value may be compared with our previously obtained value of 0.0024 chromosome-type breaks per cell per roentgen. While the present estimate for chromatid-type breakage may be somewhat high, because the presence of some chromosome-type exchanges in the samples indicated that some fraction of the breaks scored as isochromatid deletions must in fact have been chromosome-type deletions, it is apparent that in our human leukocyte system the prechromosome doubling breakage rate is about the same as the postdoubling breakage rate.

Radiation Immunology

Takashi Makinodan[1]
J. F. Albright
E. H. Perkins
E. E. Capalbo[2]
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Paul Urso[3]
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RELATION OF CONCENTRATION AND INTERVAL BETWEEN INJECTIONS OF ANTIGEN TO SECONDARY ANTIBODY RESPONSE

Takashi Makinodan W. J. Peterson J. F. Albright

Introduction. – Previous results[4] showed that increased antigen concentrations stimulate greater production of antibody in intact animals. The quantitative effectiveness of increasing antigen when provided in a single or in divided doses has not been carefully assessed. This is, in part, due to factors[5] which obscure interpretation of results obtained from intact animals. Such information may be expected to provide additional understanding of the functional status of cells which can be stimulated by antigen.

Results. – Mice were given initial injections of 1 ml of 0.01%, 1%, or 10% suspensions of sheep erythrocytes (SRBC). At intervals thereafter, 38×10^6 spleen cells were transferred into isologous, irradiated culture animals. One group of recipients was immediately given additional antigen (1%); the second group received no antigen after spleen-cell transfer. Antibody titers were determined 5 days after spleen-cell transfer.

The quantitative differences in the concentrations of initial antigen are not revealed until 10 days after stimulation (Fig. 3). At this time peak titer is obtained from cells initially stimulated with 0.01% antigen. Much higher titer is obtained from cells initially stimulated with 1% antigen, while 10% antigen has little additional effect. The curves represent maximum antibody titers (secondary response)

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[1]Leave of absence, NSF Senior Postdoctoral Fellowship, National Institute of Radiological Sciences, Chiba-City, Japan.

[2]Visiting investigator from abroad.

[3]Consultant.

[4]T. Makinodan *et al.*, *J. Immunol.* **77**, 250 (1956).

[5]T. Makinodan and W. J. Peterson, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 76–78.

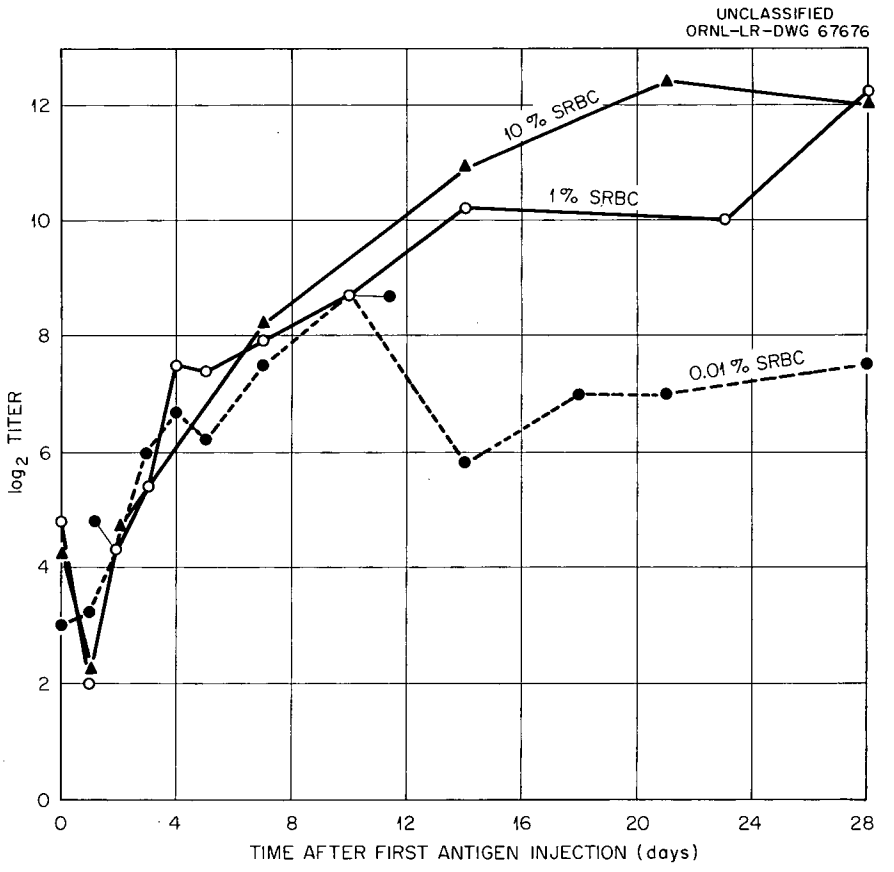

Fig. 3. Antibody Production by Spleen Cells Secondarily Stimulated at Intervals After Initial Stimulus with Varying Concentrations of Antigen.

which can be obtained at the various time intervals consistent with concentration of stimulating antigen. The variation in antibody production during the interval, 1 hr to three days, can be explained as a loss from the spleen of active antibody-producing cells and their subsequent replacement. There is a sharp change in the rate of increase of antibody after 4 days. This may be due to a qualitative change in the cells producing antibody – perhaps as a result of a regulatory mechanism or of an increasing proportion of nonfunctional cells. The titers of antibody produced by cells initially stimulated with antigen but not provided with antigen after transfer are, as expected, considerably lower. The quantitative relations between antigen dose and antibody produced are maintained, however.

Discussion. – Our data lead to the following interpretation: the number of cells stimulated toward antibody production depends upon the concentration of antigen initially provided. Beyond a certain amount (~1%), additional antigen has little effect. Of the cells initially stimulated, only a certain fraction engage in immediate antibody production. Many of the latter may be induced to migrate out of the spleen. The maximum antibody titer obtained depends, to a great extent, upon the number of cells present which can be activated to functional status by additional antigen.

MOBILIZATION OF ANTIBODY-PRODUCING CELLS BY ANTIGEN STIMULATION

J. F. Albright Rachel R. Carter

Introduction. – The exchange of cells of antibody-producing potential between organ sites has not been carefully investigated. It is clear that the introduction of competent cells into the irradiated animal or into the embryo results in selective incorporation of the cells in lymphatic tissues. This report is concerned with the movement of cells away from lymphatic tissue, specifically the spleen, as a result of specific antigenic stimulus.

Results. – Mice were immunized at 8 weeks of age with sheep erythrocytes (SRBC). Four weeks later they received a second injection of either a 1.0% or a 0.1% suspension of SRBC. At intervals of 1 hr to 10 days animals were killed, and 24×10^6 spleen cells were transferred intravenously to isologous, irradiated (800 r) recipients. Six days later the culture animals were bled, and serum agglutinin titers were determined. Separate experiments were performed, with one modification: 1 hr prior to transfer of cells at each of the time intervals after secondary immunization, 1 ml of a 0.1% suspension of SRBC was injected into the cell donors. In this way, the concentration of antigen was kept fairly constant at each cell transfer. Similar experiments were conducted in the same manner except that immediately following transfer of 24×10^6 cells the culture animals received an additional antigen injection.

Figure 4 shows that there is a sharp decrease in antibody produced by cultured cells during the 48 hr after secondary antigen stimulus. Since there is good evidence that the antibody-producing cells are functionally equivalent, it can be calculated that ~99% of the functional cells are lost from the spleen during this interval. During the next 48-hr interval there is a partial restitution of the antibody-producing cells in the spleen. Whether the subsequent loss of antibody production is due to further emigration of competent cells or the result of a quantitative decrease in antibody synthesis – owing, for example, to regulation – is not yet clear.

The amount of antibody produced by the cells which received antigen at the time of transfer is, presumably, due to the combined activity of cells activated by secondary antigen stimulation (and which are migratory) and latent cells which require additional antigen for activation. The curve of activity at intervals after secondary stimulation reflects the migratory loss of activated cells and the restitution phase.

Discussion. – The migration of antibody-producing cells from the spleen after secondary antigen stimulation also occurs after primary antigen stimulation. The data from this functional analysis appear to support the observations of Congdon and Makinodan[6] concerning the initial histological events occuring in the spleen after antigen stimulation. Our results suggest that, of the cells stimulated by antigen, only a fraction may be actively engaged in antibody liberation at a particular time.

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

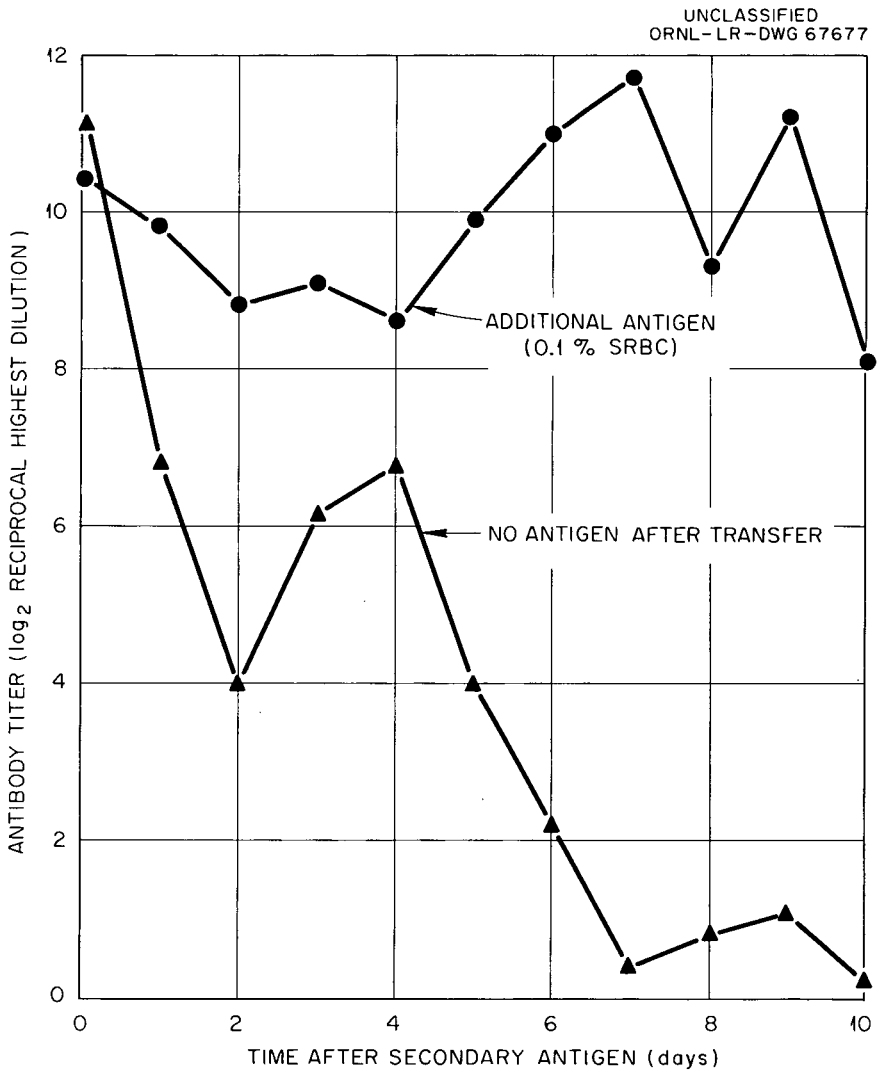

Fig. 4. Antibody Production by 24 × 10^6 Spleen Cells Cultured in vivo at Various Intervals After Secondary Antigen Stimulation. Six-day titers.

A QUANTITATIVE STUDY OF ANTIBODY PRODUCTION BY CELLS SECONDARILY STIMULATED WITH ANTIGEN

J. F. Albright Rachel R. Carter

Introduction. – It was shown previously[7] that there is a strict relation between circulating antibody titers and the number of competent antibody-producing cells. It was also shown[8,9] that active mitosis is

[7] T. Makinodan *et al.*, pp 182–89 in *Mechanisms of Antibody Formation* (ed. by M. Holub and L. Jaroskova), Czechoslovakian Academy of Science, 1960.

[8] P. Urso, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 75–76.

[9] E. E. Capalbo, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 79–80.

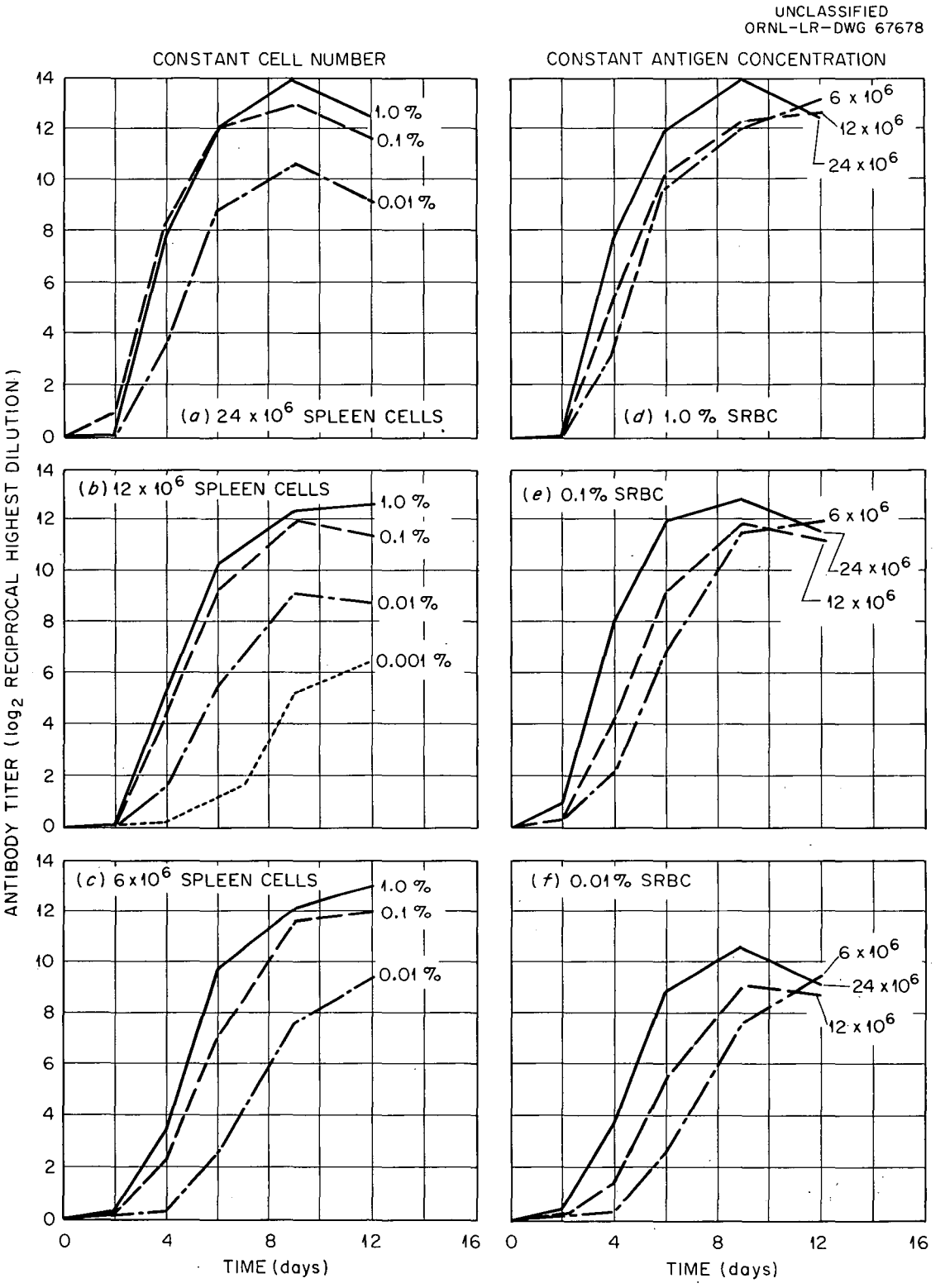

Fig. 5. Antibody Production by Spleen Cells Cultured in vivo in Response to Secondary Stimulation with Sheep Erythrocytes.

a characteristic of antibody-forming cells. It is, therefore, reasonable to assume that antibody titers at intervals after antigen stimulation indicate the extent of multiplication of functional cells. This report is an analysis of presumed multiplication of antibody-producing cells as a function of (1) quantity of stimulating antigen and (2) initial number of active cells.

Results. – Spleen cells (6, 12, or 24 $\times$ 10^6) were removed from immunized mice and cultured in irradiated (800 r) isologous recipients. The latter were injected with antigen [1.0%, 0.1%, 0.01%, or 0.001% suspension of sheep erythrocytes (SRBC)] immediately after spleen-cell transfer. Serum antibody titers were determined at intervals thereafter. The results are presented in two ways: antibody production by the same initial number of transferred spleen cells stimulated by different doses of antigen (Fig. 5*a*–*c*), and antibody production at a constant antigen concentration by different initial numbers of transferred spleen cells (Fig. 5*d*–*f*). The curves are typical of cellular multiplication. The duration of the induction phase is decreased by either (1) increasing the stimulating antigen dose or (2) increasing the number of spleen cells initially placed in culture ("inoculum size"). It is apparent that the maximum antibody titer is controlled by the quantity of stimulating antigen. Regardless of the initial number of spleen cells, the 12-day titers are equivalent for a given antigen dose. With higher numbers of cultured cells there is a pronounced regression phase prior to 12 days. Regression occurs sooner when the length of the induction phase is shortened.

Discussion. – The cellular events which occur during induction are, as yet, obscure. It seems unlikely that, in this system, the induction time is required for building up threshold concentrations of metabolic intermediates or materials required for mitosis. It is possible that this time is required for histological organization of the transferred spleen cells or for the association of some critical number of active cells into an aggregate sufficient to support specialized function. It is equally possible that part of the induction period is required for organization of intracellular reactions leading to antibody synthesis. Several explanations can be offered for the retardation and regression phases of the curves. Among these is the possibility of regulatory control operating through soluble antibody. Thus, above some critical ratio of soluble antibody to active cells, the synthesis or release of new antibody may be inhibited. Evidence for such a mechanism has been obtained. In any event, it seems unlikely that regression in this system is due to accumulation of toxic metabolites or exhaustion of nutrients. Judging from, as yet, limited data, curtailment of antibody production is not merely a matter of antigen exhaustion.

THE EFFECT OF AET ON RADIATION-PROTECTIVE AND ANTIBODY-SYNTHESIZING CAPACITIES OF HEMATOPOIETIC CELLS

E. H. Perkins Martha R. Leonard

Introduction. – Early work by Makinodan *et al.*[10] demonstrated that, in the intact animal, AET protected predominantly non-antibody-producing hematopoietic cells. We have extended this work by transfer-

[10] T. Makinodan, I. C. Shekarchi, and C. C. Congdon, *J. Immunol.* **79**, 281–87 (1957).

ring cells into lethally irradiated (800 r), genetically compatible (isologous) recipients and determining the antibody-forming and protective capacities of the transferred cells.

Results. – Injection of rat RBC-preimmunized (C57BL/Cum ♀ × C3H Anf Cum ♂) donor mice with AET (8.8 mg per 25-g mouse) prior to exposure to either 300 or 600 r failed to protect the immune capacity of transferred spleen cells (20 or 40 × 10^6) as judged by their ability to produce antibody when stimulated with rat RBC (secondary response), while bone marrow (2 × 10^6) from the same animals protected lethally irradiated recipients (Fig. 6). It was further demonstrated that while a spleen-cell suspension from AET-treated and irradiated donor mice failed to produce antibody titers significantly different from their controls, the same number of transferred spleen cells enabled lethally irradiated recipients to survive. AET failed to enhance the recovery of the immune response. When antigen was injected either 1 or 2 weeks after cell transfer, antibody titers were no greater among mice that had received transferred spleen cells from donors pretreated with AET prior to irradiation (300 or 600 r) than in animals that had received spleen cells from donors which had received no AET prior to exposure to these same levels of irradiation.

Discussion. – Using antibody production as an index of immune capacity, we found that AET failed to protect the immune response of transferred irradiated spleen cells. On the other hand, AET protected

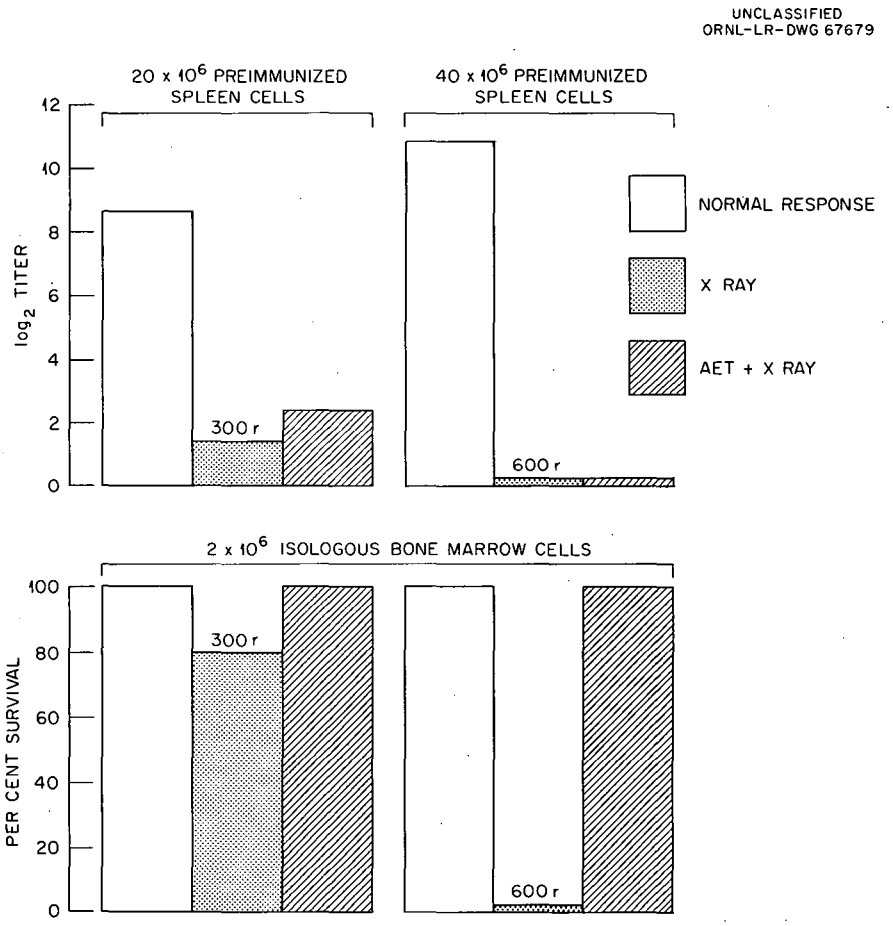

Fig. 6. Effectiveness of AET in Protecting Hematopoietic Cells Against Irradiation.

spleen or bone marrow hematopoietic cells necessary for survival. While it appears that hematopoietic cells participating in the immune response are more radiosensitive than hematopoietic cells necessary for recovery, the data do not permit quantitative evaluation of this difference. Studies are in progress which will yield this information.

HOMOGRAFT REACTION STUDIES IN DIFFUSION CHAMBERS

E. E. Capalbo

Introduction. – In previous studies where antibody formation was the criterion for rejection, it was reported that during the homograft reaction and in a certain strain combination, almost complete rejection of the target (donor) cells occurred when they were cultured in the presence of preimmunized cells in an immunologically inactive (x irradiated) recipient.[11] To study the homograft reaction on a cytomorphological basis, the fate of preimmunized cells and target cells, labeled in vivo with H^3-thymidine (H^3T), was investigated using a modified diffusion-chamber technique.[12]

Results. – Mice of C3H strain were used as donors of preimmunized (active) cells and C57BL mice as donors of immunizing (target) cells. Two control and two experimental groups were used. In one experimental group 12×10^6 C3H spleen cells preimmunized against C57BL cells 2 days before were cultured with 12×10^6 target cells labeled in vivo with H^3T. The control group consisted of chambers with 12×10^6 H^3T target cells plus 12×10^6 unlabeled target cells. In the second experimental group 12×10^6 in vivo H^3T-labeled C3H spleen cells were cultured with the same number of unlabeled target cells. For the control group, H^3T-labeled active cells were cultured with unlabeled active cells. By means of autoradiographs done at different time intervals after incubation it was possible to see inhibition of proliferation of target cells and their virtual disappearance at 48 hr. However, these cells proliferate with the expected doubling time of $\sim$24 hr when cultured in the presence of isologous unlabeled cells. In the second experiment, in which the immunized cells instead of the target cells were labeled with H^3T, it was possible to see that the active cells proliferate actively and that they have a mean generation time of $\sim$12 hr when cultured in the presence of target cells (Fig. 7).

Discussion. – These results show that in the homograft reaction with the mouse-strain combination used, the C57BL spleen cells disappear promptly after being cultured with preimmunized C3H spleen cells. Considering that the labeled target cells proliferate at a normal rate when cultured with isologous, unlabeled cells, the rapid disappearance of these cells when cultured with preimmunized cells suggests that the target cells are actually being killed by the active cells. The latter, on the other hand, proliferate at the same rate as preimmunized cells when in the presence of the test antigen.[13]

[11] Franco Celada and R. R. Carter, *Journal of Immunology* (in press).

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

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

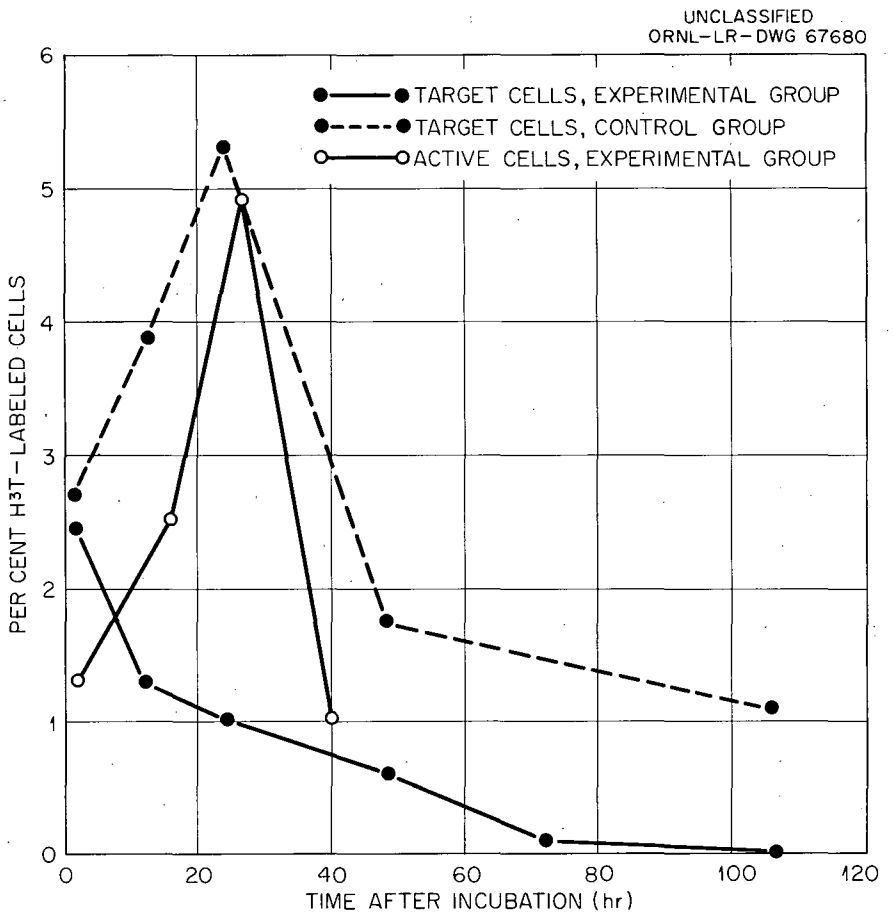

Fig. 7. Homograft Reaction and Proliferation of Target Cells in the Presence and in the Absence of Preimmunized Spleen Cells.

Pathology and Physiology

Section Chief – A. C. Upton

Pathological Effects of Neutrons and High-Energy Radiation in Mammals

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Physiological and Chemical Effects of Radiation

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THE EFFECT OF VARIATION IN RECIPIENT X-RAY DOSE ON THE INDUCTION OF THE FOREIGN SPLEEN REACTION

G. E. Cosgrove M. L. Davis

Introduction. – Parental-strain spleen cells injected into sublethally irradiated F_1 hybrid mice cause illness and death in varying numbers of the recipients, depending on the strain combination and x-ray dose. The associated pathological changes involve principally the lymphoid tissues, which show early granulomatous hyperplasia and subsequent scarring and atrophy. This condition was studied in several strains of mice, using wide variations in the x-ray dose.

Methods and Results. – Young adult (101/Cum × C3H/Anf Cum)F_1 and (C57BL/Cum × 101/Cum)F_1 mice, hereafter designated 1C3F_1 and B1F_1, respectively, were exposed to whole-body 250-kvp x rays

[1]Consultant.
[2]Visiting investigator from abroad.
[3]Research associate.

and injected intraperitoneally immediately afterward with 15 to 150 × 10^6 parental spleen cells. Increasing mortality was observed with increasing radiation dose (Figs. 8, 9). Similar results were observed with other strain combinations but will not be presented here.

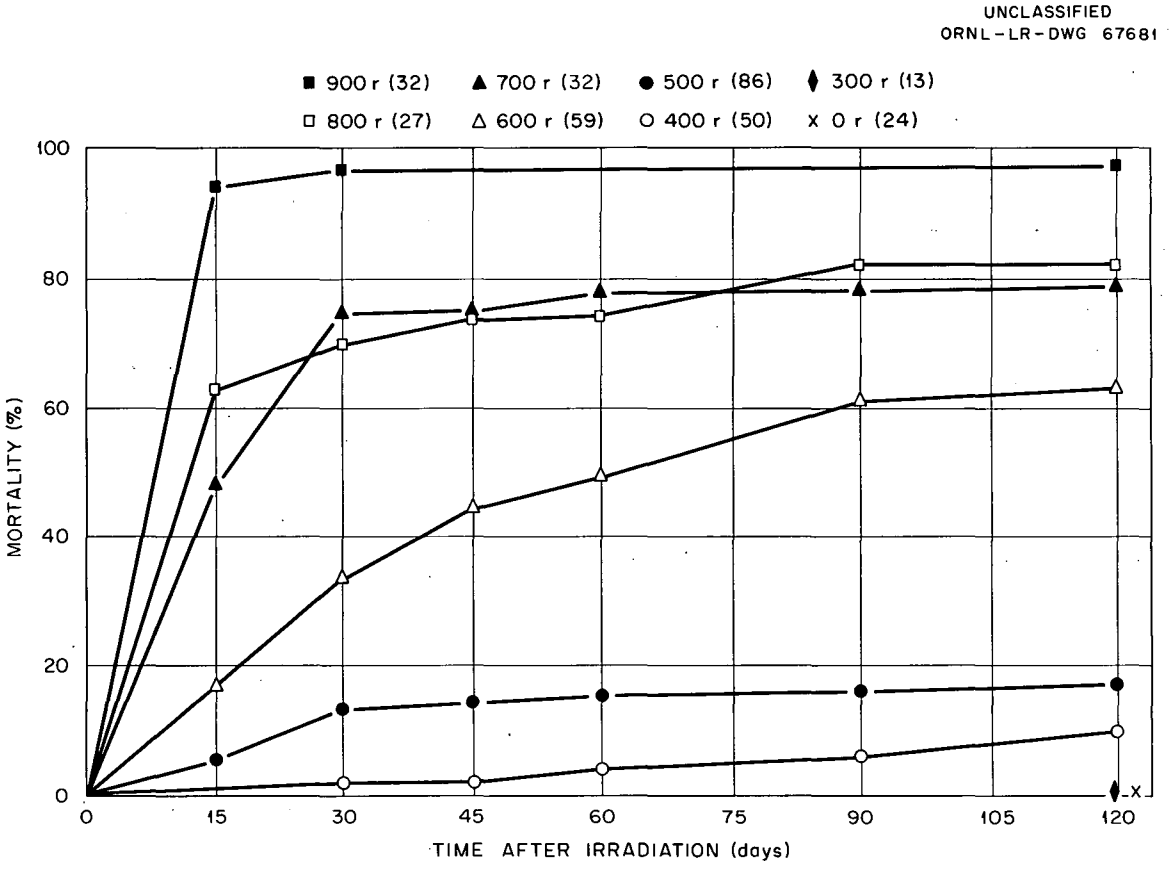

Fig. 8. Influence of X-Ray Dose on Mortality of 1C3F$_1$ Recipients of 101 Spleen Cells. Numbers in parentheses denote number of mice.

Discussion. – In the "strongly reactive" (C57BL → B1F$_1$) combination, 24% mortality was encountered even in unirradiated recipients, and 80 to 90% mortality was observed at a dose of only 300 r. In the "weakly reactive" (101 → 1C3F$_1$) combination, no mortality was encountered below 400 r and a high mortality observed only with x-ray doses in the LD_{50}/30-day range and above. These results are interpreted to mean that the host must be depressed to varying degrees by irradiation in order to allow a lethal graft-vs-recipient immunologic reaction to occur, depending on the extent of histoincompatibility between donor and host strains.

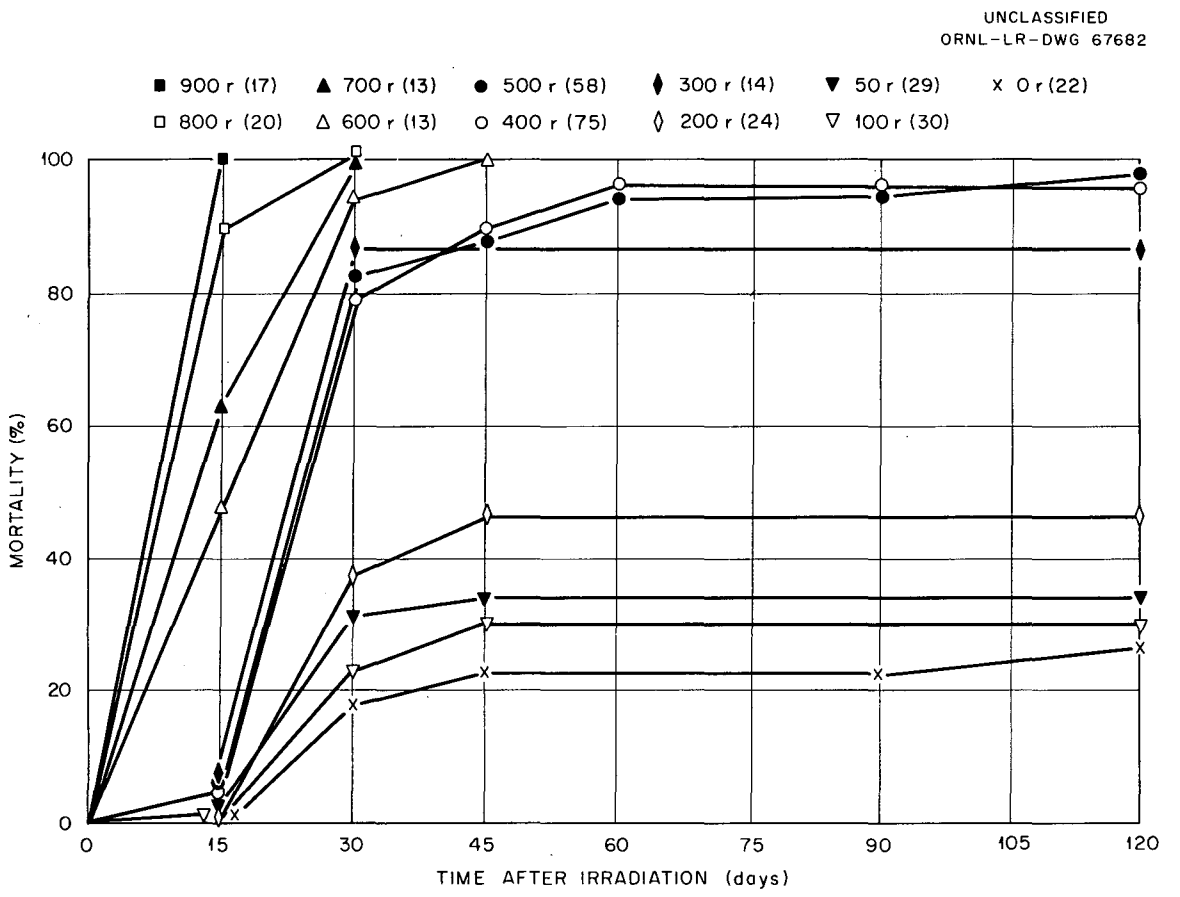

Fig. 9. Influence of X-Ray Dose on Mortality of $B1F_1$ Recipients of C57BL Spleen Cells. Numbers in parentheses denote number of mice.

INHIBITION OF THE FOREIGN BONE MARROW REACTION BY IRRADIATION OF THE DONOR CELLS

Gustavo Cudkowicz Audrey Eisenstadt S. Rebecca Graves

Introduction. – Bone marrow cells of $(C57BL/6 \times DBA/2)F_1$ mice were exposed in vitro to an x-ray dose ranging from 300 to 500 r, with and without the radioprotective drug AET[4] dissolved in the suspending medium. It was expected, on the basis of previous experiments,[5] that after transplantation of such irradiated marrow into lethally irradiated $(C3H/Anf \times 101)F_1$ recipients the surviving donor elements would still be competent for repopulation of the recipients' hemopoietic sites but not for immunological reactivity against the hosts' tissue antigens.

[4] S,β-aminoethylisothiuronium·Br·HBr, kindly supplied by D. G. Doherty.

[5] G. Cudkowicz, *Proc. Soc. Exptl. Biol. Med.* **107**, 821 (1961).

Methods and Results. – After total-body exposure to 900 r of x rays, adult recipients were injected intravenously with 10 to 20 × 10^7 nucleated marrow cells. Before injection, the donor cells were suspended in newborn agamma calf serum and exposed to x rays in plastic petri dishes. The AET was dissolved in the calf serum in the amount of 1 mg/ml. Data on the mortality of recipients 40 and 90 days after treatment are presented in Table 19. Deaths occurring within the first 40 days are attributed mainly to failure of the marrow graft, since transplantation of relatively few unirradiated donor cells (2 to 20 × 10^6 cells per mouse) is followed by a high mortality rate in this period, whereas transplantation of 40 × 10^6 or more unirradiated donor cells per mouse leads to lower mortality in the first 40 days, although secondary mortality is high 40 to 90 days after transplantation. In both instances, marrow failure and secondary disease, the clinical syndrome is characterized by weight loss, hunched position, diarrhea, and ruffling of hair. Bone marrow failure, however, can be recognized in mice surviving more than 40 days after treatment by the presence of host-type erythrocytes in the peripheral blood. Therefore, to ascertain whether the donor cells had transplanted, peripheral blood hemoglobin was analyzed,[6] the hemoglobins of the host and donor strains differing in solubility.

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

Table 19. Survival of Lethally Irradiated (C3H/Anf × 101)F_1 Mice Given (C57BL/6 × DBA/2)F_1 Marrow Cells Pre-Exposed in vitro to X Rays

Donor Cells		Recipients Surviving (%):[a]		
Pretreatment	Number per Recipient	40 Days	90 Days	Secondary Disease Syndrome
	(× 10^7)			
None	0.5–2	15	0	Present (?)[d]
None	4–8	60	24[b]	Present
300 r	10	50	10[b]	Present (?)[d]
AET + 300 r	10	90	80[b]	Transient, mild
400 r	15	50	25[b]	Present (?)[d]
AET + 400 r	15	70	70[b]	Absent
500 r	20	30	30[c]	Absent
AET + 500 r	20	70	70[c]	Absent

[a]Forty mice per group.
[b]Donor-type hemoglobin in peripheral blood of survivors.
[c]Host-type hemoglobin in peripheral blood of survivors.
[d]"Insufficient grafted marrow" and "secondary disease" symptoms not distinguishable (see text).

The results can be summarized as follows:

1. The exposure of donor marrow cells to x rays markedly reduced their ability to protect irradiated recipients against early lethality, so that cell doses of the order of 10^8 per mouse were necessary; surviving recipients of such marrow showed a reduced incidence of foreign marrow reaction.

2. AET protected hemopoietic cells against radiation injury, as judged by effectiveness of the marrow in preventing early mortality among irradiated recipients. However, the incidence and severity of the foreign marrow reaction in such recipients were as low as when donor cells were irradiated in the absence of AET, suggesting that the compound did not protect immunologically competent cells in the marrow.

3. All surviving recipients of marrow cells exposed to 300 to 400 r of x rays had donor-type hemoglobin 90 days after treatment. On the other hand, recipients of 500-r irradiated marrow had host-type hemoglobin, indicating that in the latter case the grafted cells persisted only temporarily.

Discussion. – The reduced incidence of foreign marrow reaction in mice receiving homologous marrow cells irradiated in vitro is attributed to the elimination of immunologically competent cells from the donor marrow to an extent adequate to inhibit the development of clinically significant graft-vs-host immune reactions. Irradiation of the marrow cells in the presence of AET, a radioprotective drug for hemopoietic elements but not for immune cells,[7] enabled the selective destruction of the latter cells.

COMPARISON OF DELAYED SOMATIC EFFECTS OF RADIOMIMETIC AGENTS AND X RAYS

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

Introduction. – Preliminary data were reported earlier indicating that midlethal doses of nitrogen mustard (HN2) and triethylene melamine (TEM) exerted life-shortening, cataractogenic, and oncogenic effects on surviving RF female mice, qualitatively similar to those of x rays.[8] The age distribution of mortality rates in the various experimental groups was, therefore, analyzed to ascertain whether the radiomimetic chemicals also advanced the time of death in the same manner as ionizing radiation.

Results. – Gompartz-type curves for overall mortality (Fig. 10) revealed qualitatively comparable displacement of the life table by each of the agents tested. Similar effects were also produced in the case of pulmonary (Fig. 11) and ovarian tumors. The situation was less clear-cut in the case of myeloid leukemia, which tended to develop earlier in life (Fig. 12), and in the case of thymic lymphomas, which occurred even earlier.

Discussion. – These results illustrate the complexity of the late somatic effects of the agents studied. Although effects on overall mortality and mortality with diseases of old age were qualitatively similar and consistent with the hypothesis of premature aging, effects on death rate with earlier-occurring diseases, such as leukemia, were complex and require further analysis.

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[7] G. Cudkowicz, *Transpl. Bull.* 29, 19 (1962).

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

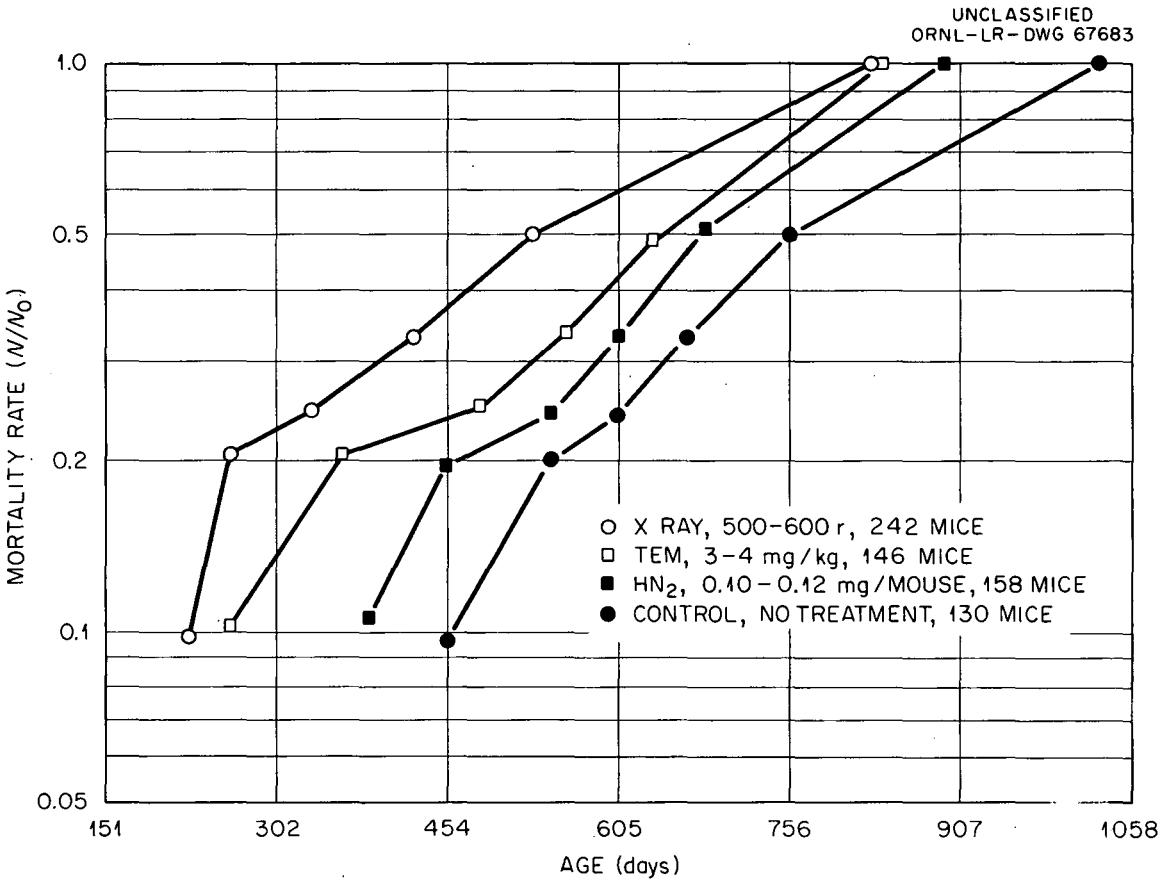

Fig. 10. Mortality Rate in Relation to Age.

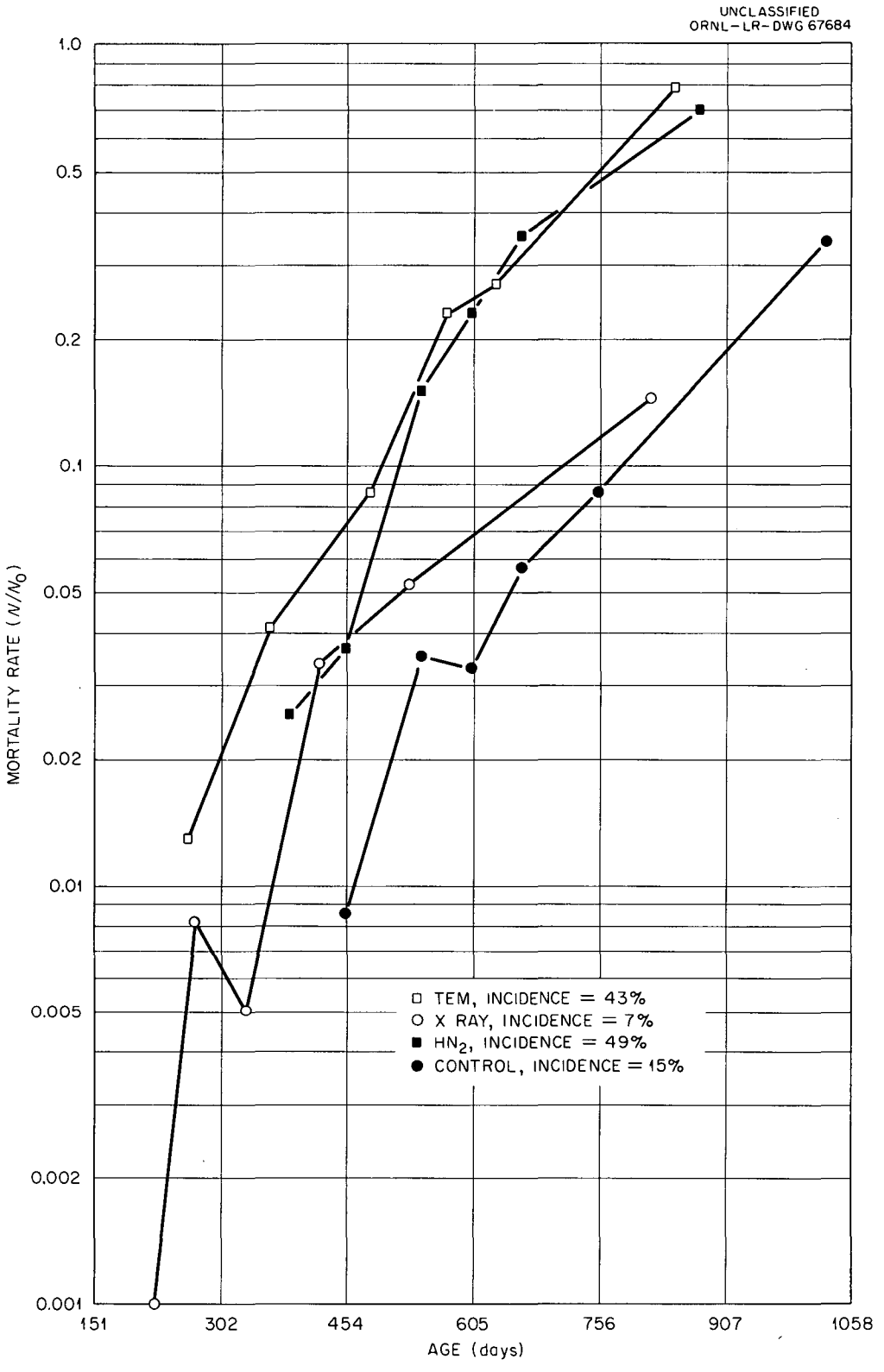

Fig. 11. Mortality Rate in Mice with Pulmonary Tumor.

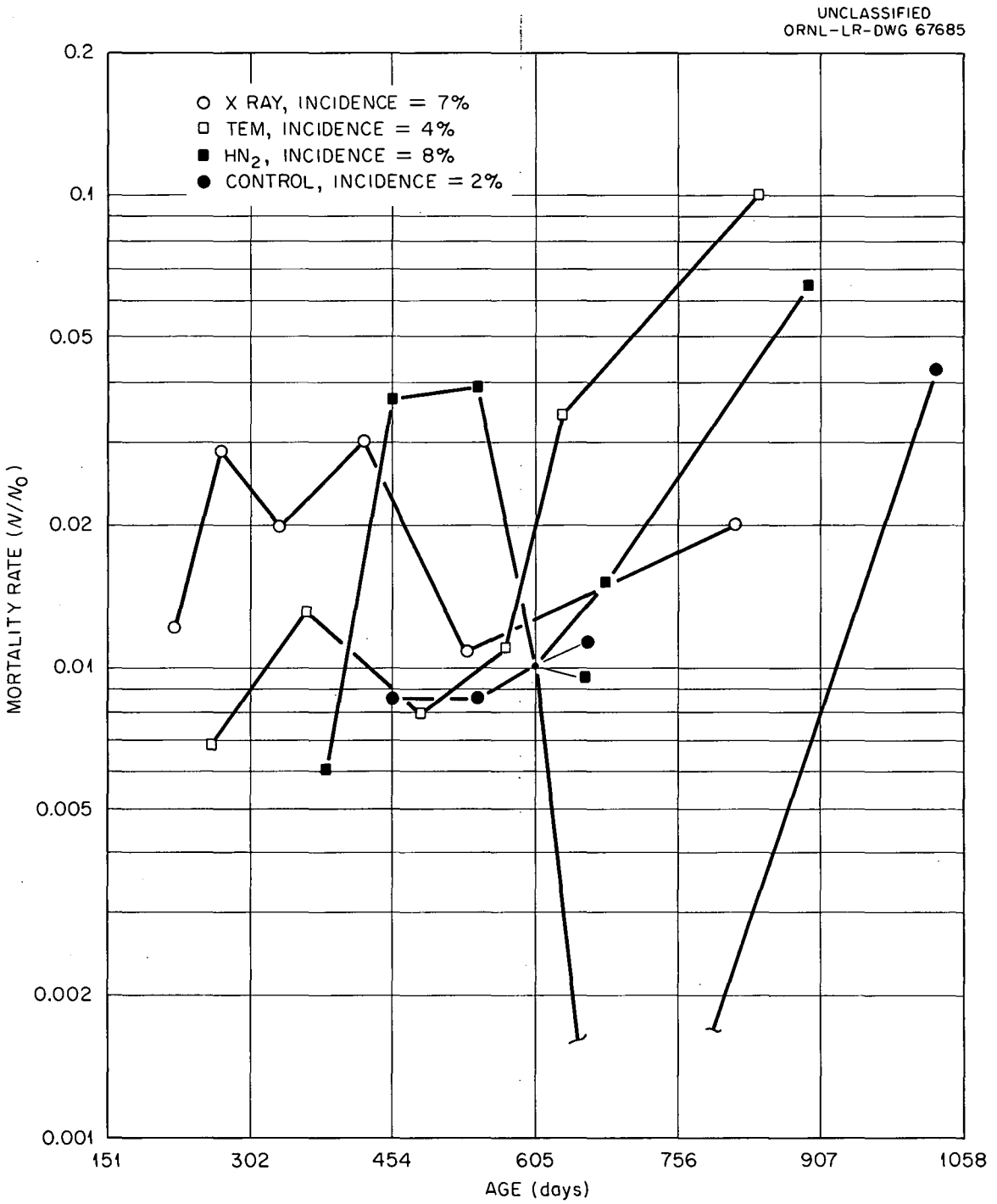

Fig. 12. Mortality Rate in Mice with Myeloid Leukemia.

STUDIES OF PLATELET REGULATION

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

Introduction. – Previous studies showed that a solution of egg albumin or a suspension of ground glass injected subcutaneously into rats produced a 40 to 50% increase in the number of circulating platelets, with the peak number occurring 5 days after the initial injection. Because this finding raised the question whether the response was mediated by a humoral agent, serum from rats injected with these two materials was assayed in normal recipients for platelet-stimulating activity.

Methods. – Donor rats were injected on the morning and afternoon of the first day and on the morning of the second day with 6 ml of either a 7% suspension of ground glass or approximately a 7% solution of egg albumin. In the afternoon of the second day the serum was collected. It was then injected subcutaneously into assay rats in the morning and afternoon for 2 days, 6 ml per injection, and peripheral platelet counts were taken at daily intervals on days 3 through 7.

Results and Discussion. – Serum from both kinds of donors produced about a 40% increase in peripheral platelet counts on the fifth day, and the count returned to normal 2 days later. This response was comparable to that produced by serum of platelet-depleted donors. It suggests that the glass and albumin stimulate the production and release into the blood stream of a humoral agent that is capable of stimulating platelet production in other hosts. Our failure to elicit such a platelet response by injection of cortisone acetate into rats suggests that the release of an adrenal cortical agent in response to a condition of stress is probably not the common factor promoting this platelet response.

ROLE OF *Hb* AND *Sol* IN THE INHERITANCE OF MOUSE HEMOGLOBINS

R. A. Popp Diana M. Popp Carlene C. Denny

Introduction. – Genetic tests have demonstrated that the molecular characteristics of mouse hemoglobin are determined by two loci (*Hb* and *Sol*) which segregate independently.[9] Comparative analyses of the tryptic peptides of mouse hemoglobin have indicated that electrophoretically diffuse hemoglobin differs in primary structure from the electrophoretically single type.[10] This report concerns the relation between strain differences in tryptic peptides and genetic substitutions at *Hb* and *Sol*.

Materials and Results. – Parental strains (C57BL and BALB/c) were used to produce two new lines of mice (HBS and HBD). The hemoglobin types of these four lines of mice differ from one another; namely, C57BL is $Hb^1/Hb^1, Sol^1/Sol^1$; BALB/c is $Hb^2/Hb^2, Sol^2/Sol^2$; HBS is $Hb^1/Hb^1, Sol^2/Sol^2$; and HBD is $Hb^2/Hb^2, Sol^1/Sol^1$. The alpha and the beta chains of the four types of hemoglobin were separated by

[9] R. A. Popp and Diana M. Popp, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 91–92.

[10] R. A. Popp, "Mammalian Hemoglobins," in *Symposium on Methodology in Mammalian Genetics, Bar Harbor, September 1960.*

column chromatography.[11] The tryptic peptides of the alpha and beta chains were then analyzed by "fingerprinting"[12] and were compared with those of native globin. Fingerprints of the tryptic peptides of the alpha chains of the four globins were indistinguishable. However, the beta chains of C57BL globin and of HBS globin differed from those of HBD globin and BALB/c globin in the position of one tryptic peptide (Fig. 13).

Discussion. – The demonstration that a peptide in the beta chain of globin from mice of Hb^1/Hb^1 genotype differs from that in mice of Hb^2/Hb^2 genotype suggests that the molecular structure of the beta chain of mouse globin is controlled by alleles at *Hb*. The possibility cannot at present be excluded, however, that another locus, as yet undiscovered or linked with *Hb*, may be involved.

[11] S. Wilson and D. B. Smith, *Can. J. Biochem. Physiol.* **37**, 405 (1959).

[12] V. M. Ingram, *Biochim. et Biophys. Acta* **28**, 539 (1958).

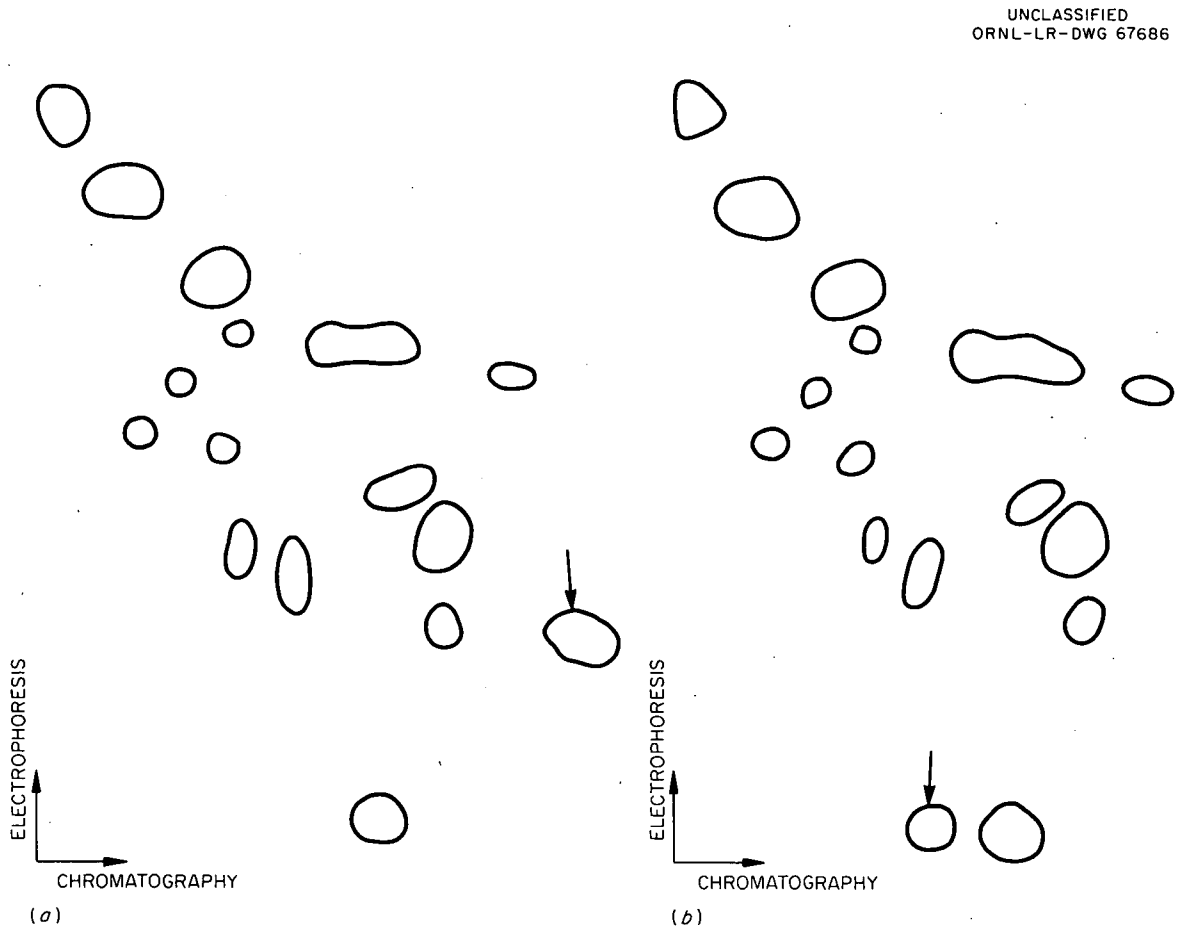

Fig. 13. Beta-Chain Tryptic Peptides: *A*, **of C57BL or HBS Globin;** *B*, **of BALB/c or HBD Globin.** Chromatography in 4:1:5 butanol-acetic acid-water; electrophoresis in 1:10:289 pyridine-acetic acid-water.

THE COMBINED APPLICATION OF PEROXIDASE STAINING AND AUTORADIOGRAPHY

R. A. Popp W. D. Gude Diana M. Popp

Introduction. – In general, histochemical stains have not been successfully applied to tissues after autoradiography, owing to the tendency for organic acids and reducing agents in photographic developers and fixatives to denature enzymes and alter their reaction products. An effort was made to find procedures permitting the combined use of peroxidase and autoradiography, so that isotopes, such as S^{35}-sulfate, that preferentially labels eosinophilic granulocytes[13] might be used as an aid for identifying the small percentage of intensely peroxidase-positive granulocytes in rat peripheral blood. A more direct identification of these cells was difficult since the peroxidase stain interferred with subsequent identification of eosinophilic granules using Giemsa stain. Procedures permitting the combined application of peroxidase staining and autoradiography are reported here.

Materials and Results. – Peripheral blood was taken from the tail vein of normal rats and rats in which eosinophilia had been induced by *Trinchinella spiralis* infection. One half of the blood films were stained for peroxidase as described below:

Solution A	100 ml 95% ethanol
	0.25 g benzidine
	0.50 g sodium nitroprusside dissolved in 5.0 ml H_2O
	5.0 ml 0.2 *M* acetate buffer (pH 4.5)
Solution B	Above ingredients plus 0.2 ml H_2O_2

Blood films were flooded with 1.5 ml of solution A for 1 min. Then 2 drops of solution B were added and thoroughly mixed with solution A by means of a medicine dropper. After 4 min, the film was rinsed with distilled water to remove the reaction mixture. Blood films were coated with NTB_2 liquid emulsion and exposed in the darkroom for periods of 1 to 7 weeks. Although the peroxidase stain was decolorized during routine developing and clearing of the autoradiographs, the differential peroxidase staining of rat granulocytes developed when the films were treated once again with ingredients used for the initial peroxidase reaction; that is, 1.5 ml of solution A and 2 drops of solution B. The ratio of more-to-less peroxidase-positive cells in peroxidase-stained autoradiographs was compared (Table 20) with the eosinophil-neutrophil ratios of Giemsa-stained autoradiographs.[14] The positive correlation of eosinophil-neutrophil ratios obtained from Giemsa-stained preparations and ratios of more-to-less peroxidase-positive granulocytes suggest that the eosinophils were the intensely peroxidase-positive cells.

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

[14] W. D. Gude, A. C. Upton, and T. T. Odell, Jr., *Stain Technol.* **30**, 161 (1955).

Table 20. Comparison of Eosinophil-Neutrophil Ratios in Autoradiographs of Peripheral Blood Stained with Giemsa or Peroxidase, of Normal and *T. Spiralis*-Infected Rats

Series	Days After Infection with *T. spiralis*	Giemsa		Standard Deviation[a]	Number of Granulocytes Counted	Peroxidase		Standard Deviation[a]	Number of Granulocytes Counted
		Eosinophils (%)	Neutrophils (%)			Eosinophils[b] (%)	Neutrophils[b] (%)		
A	10	14.33	85.67	±2.08	300	15	85	±2.00	300
	21	32.75	67.25	±4.79	400	36	64	±0.00	200
B	Control	3.16	96.84	±1.34	600				
	8	30.75	69.24	±6.85	400	28.50	71.50	±2.12	200
	12	29.33	70.67	±5.35	300	31.50	68.50	±2.12	200
	14	35.00	65.00	±5.65	200	35.00	65.00	±5.65	200

[a]Several differential counts of 100 cells each were made. Averages and standard deviations were calculated from these differential counts.

[b]The more- and the less-intensely stained cells were called eosinophils and neutrophils, respectively. Samples of peripheral blood that were compared were obtained from the same animal.

Discussion. – Although the quinhydrone color of the peroxidase stain was lost during fixation of the autoradiographs, probably because of the reducing action of the thiosulfate in the acid fixative, the redevelopment of the color on posttreatment with appropriate reagents permitted the combined use of the peroxidase stain and autoradiography. Many other applications of this combined staining approach are envisaged.

FURTHER STUDIES ON VIRAL FACTORS IN RADIOGENIC LEUKEMIA

D. F. Parsons V. K. Jenkins W. D. Gude M. A Bender[15]

Introduction. – Previous reports presented preliminary data suggesting that viruses are involved in the pathogenesis of radiogenic myeloid leukemia in the RF mouse. The following experiments were carried out with primary and serially passaged radiation-induced myeloid leukemias to explore further the viral aspects of leukemogenesis.

Methods and Results. – Virus-like particles were detected with the electron microscope (Figs. 14 and 15) in the tissues of a significant number of mice with primary leukemia but were much more numerous in serially passaged leukemia cells, especially when they were incubated in vitro (Table 21). In association with the increased particle content of such serially passaged cells, there was an increase in the leukemogenic potency of their cell-free filtrates (Table 21).

[15]Member of Mammalian Recovery Group.

Table 21. Evidence of Viral Activity in Radiogenic Myeloid Leukemias of RF Mice

Diagnosis	Frequency of Appearance of Virus-Like Particles Shown by Electron Microscopy[a] (number of positives/number examined)		Leukemogenic Activity of Cell-Free Filtrates in Nonirradiated Isologous Recipients[b] (number of positives/number injected)			
			Newborn Mice		Adult Mice	
	in vivo	in vitro	Myeloid Luekemia	Thymic Lymphoma	Myeloid Leukemia	Thymic Lymphoma
Nonleukemic control	0/6	1/15	0/63	0/63	0/193	0/193
Primary leukemia	5/18	1/15	0/58	0/58	0/93	0/93
Passaged leukemia	7/18	13/28	18/274	10/274	9/279	0/279

[a]Sections of spleen or cultured spleen cells.

[b]Cell-free filtrates of brain or spleen homogenates were injected intravenously into recipients less than 24 hr old (newborn) or 10 weeks old (adult); ratios denote proportion of recipients developing leukemia during the first 6 months of life.

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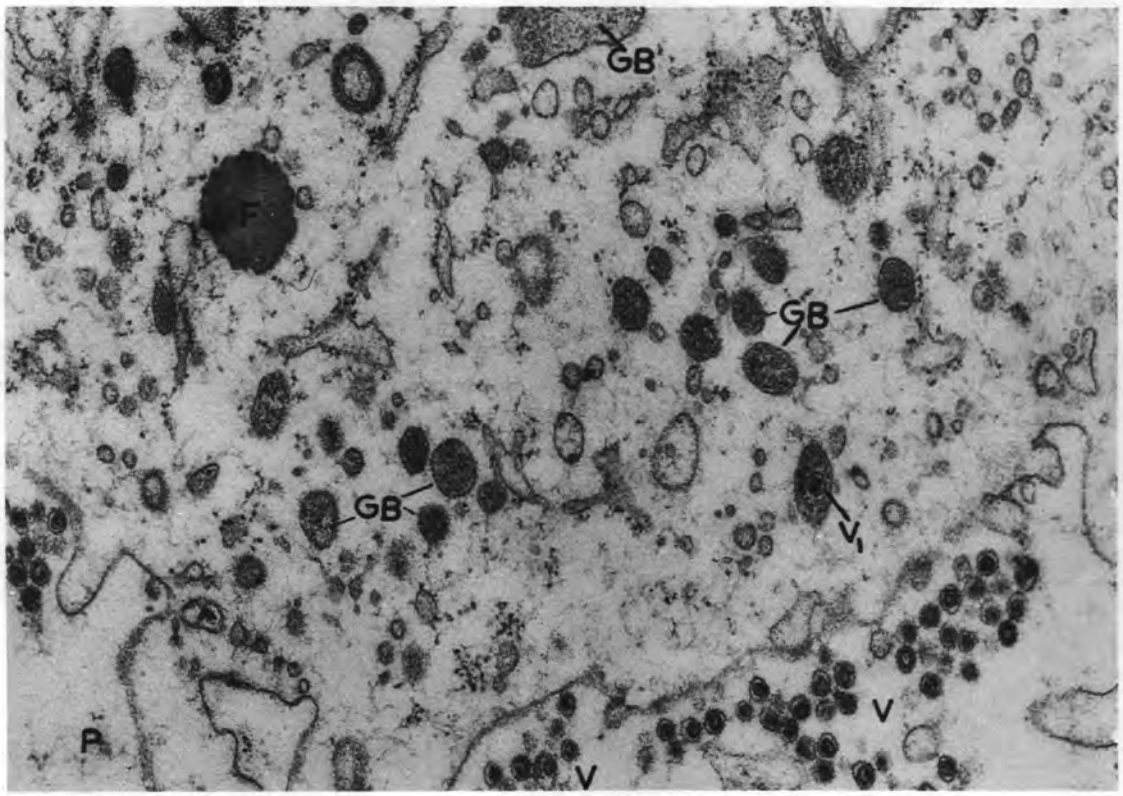

Fig. 14. Portion of a Large Mononuclear Cell in a 14-day Myeloid Leukemia Spleen Culture in the Second-Passage Generation. Large numbers of virus-like particles (*V*) are present at the outside of the cell, and an increased number of granular bodies (*GB*) are present in the cytoplasm, one of which contains a similar particle (V_1). (*P* = plasma membrane; *F* = fat bodies; *ER* = endoplasmic reticulum). 38,000X.

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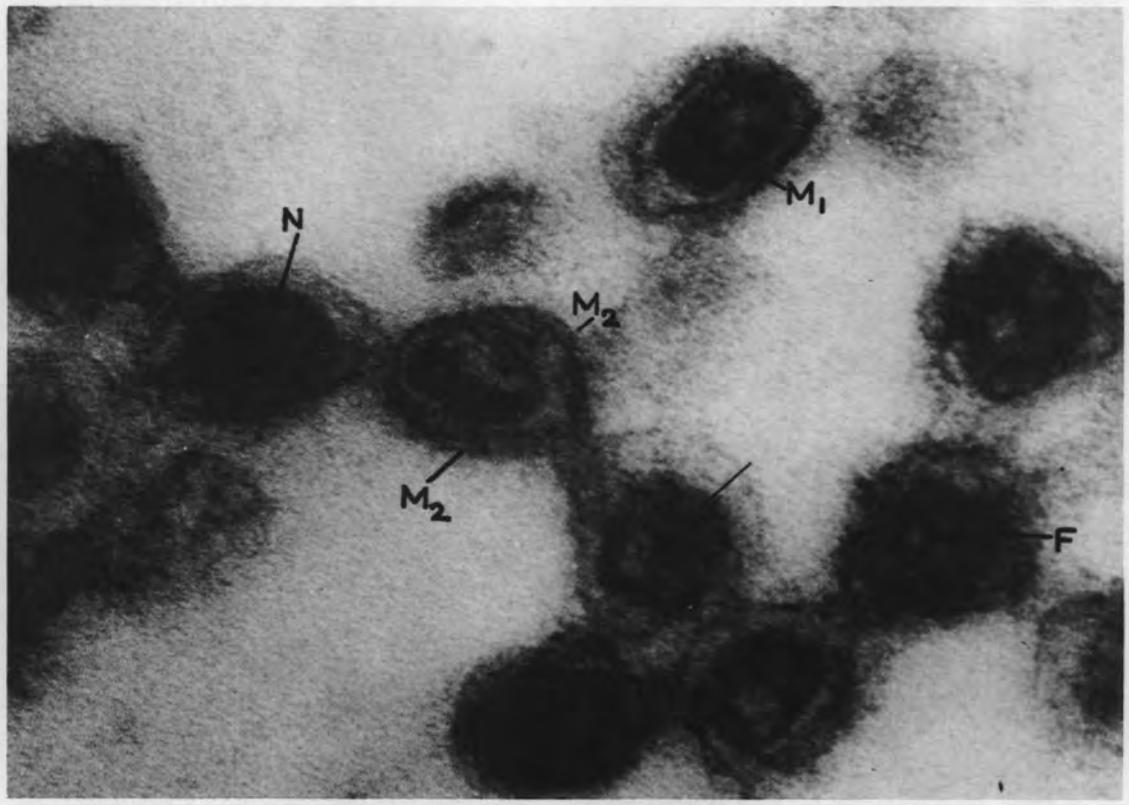

Fig. 15. Part of Another Large Group of Particles Found in the Same Tissue Culture as Shown in Fig. 1 The outer membrane is 35 A thick and appears single when sectioned normally (M_1). In other particles it appea double (M_2), but this may be due to oblique sectioning and to the slight thickness of the section. The dense cente (N) appears to have no definite membrane and to contain filaments (F) of 40-A diameter and fibrils of 20-A diameter (indicated by arrows). 300,000X.

Discussion. – These results support earlier observations that implicated viral factors in radiation leukemogenesis. The induction of lymphomas as well as myeloid leukemias by cell-free filtrates suggests, among other possibilities, that the same virus may induce either type of neoplasm, depending on factors that condition host susceptibility. This interpretation is supported by data from studies on other leukemia viruses.[16] The precise roles of radiation, virus, and host factors remain, however, to be elucidated.

[16] L. Gross, *Oncogenic Viruses*, Pergamon, New York, 1961.

Cell Physiology

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J. G. Green[1]
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Fumio Sawada[3]
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E. Jane Holloway
R. Elizabeth Lybarger
C. T. Rankin, Jr.
Mary L. Anderson[2]
L. M. Krausz[5]
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THE ZONAL ULTRACENTRIFUGE: ZONE-STABILITY STUDIES

N. G. Anderson C. T. Rankin, Jr.

The zonal ultracentrifuge[6] was developed to be the true preparative counterpart of the analytical ultracentrifuge. In practice, however, it is also an analytical instrument capable of resolving mixtures of particles ranging in size up to whole cells. The centrifuge may be used for rate-zonal density-gradient centrifugation or for isopycnic-gradient centrifugation. The latter method has wide applications and may be used to isolate cells having different densities or, at much higher speeds, to fractionate DNA by banding in dense salt gradients. Rate-zonal density-gradient centrifugation will be most useful in cell fractionation generally, especially in separating different classes of ribosomes, and in protein purification. A combination of the two types of centrifugation is used in scanning tissue homogenates, as previously reported.[6] In all applications it is essential that gradient systems be constructed so as to ensure stability of the particle zones. Experimental studies have therefore been undertaken to determine the amount of broadening of the sample zone as it moves in and out of the spinning rotor, and during acceleration to, and deceleration from, operating speed.

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[1]Research associate.

[2]Consultant.

[3]Visiting investigator from abroad.

[4]USPHS Fellow.

[5]ORINS Fellow.

[6]N. G. Anderson, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 100.

Experimental. – The volume of the rotor as a function of the radius (correcting for core and septal volumes) has been determined. The position of a zone in the rotor can therefore be calculated from the position of the zone in the effluent stream as recovered from the rotor at unloading speed (up to 5000 rpm). If the stream is passed through a continuous analytical device and the concentration of material in the zone as a function of volume determined, then the width of the zone in the spinning rotor may be calculated. A sample gradient made from 10 ml of 8% bovine serum albumin in distilled water and 10 ml of 17% sucrose was layered over a 1200-ml gradient that varied linearly with rotor radius from 17 to 55% sucrose (w/w). After the sample was introduced, the rotor was accelerated to 25,000 rpm for 5 min, decelerated to 5000 rpm, and unloaded. The ultraviolet absorbance of the effluent stream (Fig. 16) was recorded, and the results were recalculated in terms of the rotor radius. The sample zone at a radius of 25.3 mm would be

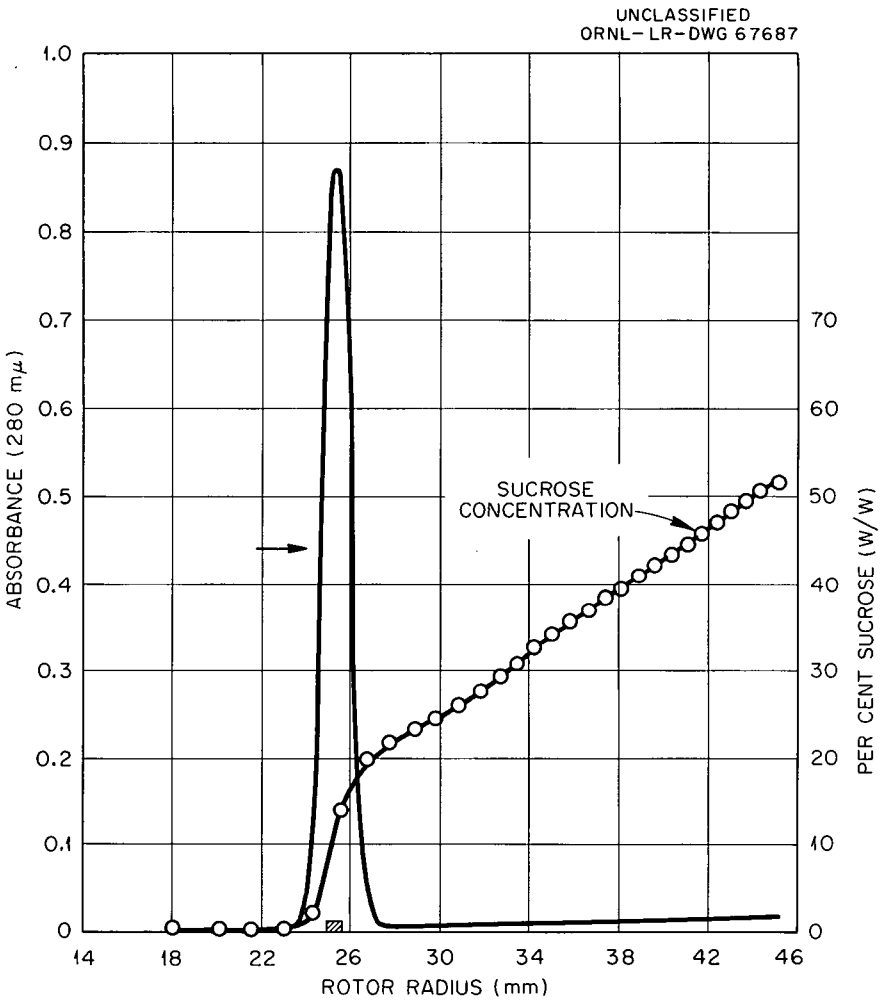

Fig. 16. Widening of Sample Zone in Zonal Ultracentrifuge. Sample introduced as gradient containing 10 ml of 8% bovine serum albumin and 10 ml of 17% sucrose (w/w). Bar indicates width zone would have in the absence of any diffusion or mixing. Sample introduced at 5000 rpm, accelerated to 25,000 rpm for 5 min, and unloaded at 5000 rpm (5°C).

0.65 mm thick if no mixing or diffusion occurred. The peak recovered had a width of 1.6 mm at half height, indicating that only a small amount of band-spreading had occurred.

Discussion. – The small amount of spreading observed may be due, in part, to the rotor core's being designed without regard to the fact that no zone is absolutely vertical in the rotor but is rather part of a long parabola, according to the equation

$$L = \frac{\omega^2 x^2}{2 \times 980}, \tag{1}$$

where L is the length of parabola in cm, x is the core radius in cm, and ω is the angular velocity in radians per second. With the core of rotor II, the maximal diameter is 3.785 cm. A rotor having a core of this upper diameter, according to Eq. (1), cannot be longer than 500 cm if it is to be loaded and unloaded at 5000 rpm. The difference in radial position between the top and the bottom of the rotor at the maximal core diameter is approximately 1 mm. This could account for a large fraction of the spreading seen. Making the core according to the principle shown in Eq. (1), therefore, should markedly increase the resolution.

These results demonstrate that very stable, thin starting zones can be formed in the zonal ultracentrifuge.

DETECTION OF INTRACELLULAR ICE IN "FROZEN" YEAST CELLS BY DIFFERENTIAL THERMAL ANALYSIS AND CONDUCTOMETRY

Peter Mazur

Introduction. – The belief that death of yeast cells at subzero temperatures is associated with the presence of intracellular ice has heretofore rested on indirect evidence. However, data was presented[7] showing that frozen yeast suspensions behave like frozen solutions during warming in that they absorb considerably more heat at temperatures below 0°C than a thermally equivalent sample of pure ice. In a frozen solution, this heat absorption is the result of the progressive melting of ice between the eutectic point and the melting point. If in the frozen suspensions it is also a result of progressive melting, the suspensions must in fact be frozen solutions. (A frozen solution consists of solutes in contact with ice.) If the solutes are inside the yeast cell, then the ice would have to be inside the cells, and the existence of intracellular ice would be demonstrated.

Methods. – The procedures and apparatus for determining heat absorption by the technique of differential thermal analysis were described previously.[7]

Results. – The results presented are (*a*) the relation between heat absorption by frozen suspensions and the melting of ice, (*b*) the proportion of cell solutes in contact with ice, and (*c*) the location of solutes in contact with ice.

[7] P. Mazur, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 105–8.

Evidence that Heat Absorption by Frozen Suspensions Is Due to Melting of Ice

1. With true solutions, the maximum difference in temperature developing between the sample of frozen solution and the reference ice sample is related to the concentration of solutes as

$$\log (\Delta T_{max})^2 = \log c_o + \log \frac{b\Delta H_f}{C},$$

where c_o is the concentration, b the molal freezing point lowering, ΔH_f the total latent heat of fusion of the sample, and C the heat capacity of the sample. Similarly, if a suspension of cells in water behaves like a frozen solution, the relation between ΔT_{max} and the concentration of cells a would be

$$\log (\Delta T_{max})^2 = \log a + \log \frac{b\Delta H_f c_o}{C}.$$

Since the last term in each equation is nearly constant, these equations predict a linear relation of unit slope between $\log (\Delta T_{max})^2$ and the logarithm of the solute concentration in a solution or the logarithm of the concentration of cells in the suspensions. These predictions were borne out experimentally (Fig. 17).

2. In theory, the difference in temperature between frozen solutions and a reference ice sample will increase hyperbolically during warming; that is, $\Delta T \cdot T_s$ = constant, when T_s is the temperature of the solution in °C. This equation also was approximated by a frozen cell suspension.

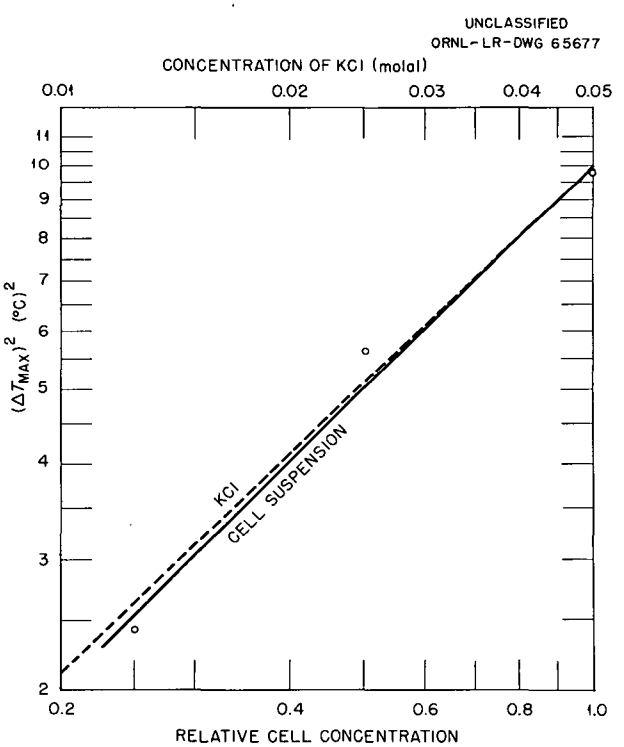

Fig. 17. Relation Between the Concentration of KCl or of Yeast Cells in a Suspension, and the Maximum Temperature Difference Developed in Differential Thermal Analysis Against Pure Ice.

Proportion of Cell Solutes in Contact with Ice

1. From Fig. 17 it can be seen that the heat absorbed by a full-strength cell suspension was equal to that absorbed by 0.05 *M* KCl. The cells in this suspension occupied about one-third of the volume. If they had occupied all the volume, they would have absorbed heat equal to 3×0.05, or 0.15 *M* KCl. The total intracellular solute concentration in yeast is equivalent to 0.3 *M* KCl, according to Conway and McArmstrong;[8] thus, these calculations suggest that some 50% of the yeast solutes were in contact with ice.

2. Yeast suspensions were subjected to multiple freezing and thawing, plus sonication, a procedure producing gross destruction of 80% of the cells and hence free diffusion of all cell solutes throughout the suspension. Table 22 compares the melting point and $(\Delta T_{max})^2$ for this suspension with the values obtained after the initial freezing. The magnitude of both parameters is proportional to the concentration of solutes in contact with ice. Since their magnitude after the initial freezing is some 70% of that after multiple freezing, then some 70% of the solutes in the yeast must have been in contact with ice.

Location of Solutes in Contact with Ice

1. Before freezing, more than 90% of the solutes in the suspension were inside the cell. This is shown by the fact that when the water surrounding the cells was removed by centrifugation, its electrical conductivity, melting point, and $(\Delta T_{max})^2$ were less than 10% of the values obtained after the cells were broken open by freezing, thawing, and sonication (Table 22).

2. The solutes inside the cells do not contribute to the conduction of electric current because of the high resistance of the cell membrane. Thus, before freezing, the conductivity of the whole suspension was of the same low value as that of the suspending fluid. It was equivalent to about 0.0015 *M* KCl. If the solutes remain inside the cells after freezing, and during warming, the frozen suspension should continue to act electrically like 0.0015 *M* KCl.

On the other hand, if after freezing, the cell solutes are free to diffuse throughout the frozen suspension, the frozen suspension would behave electrically like a much more concentrated solution, namely, 0.025 *M*.

Figure 18 shows the changes in electrical resistivity of frozen 0.0015 *M* and 0.025 *M* KCl solutions during warming. The bottom curve in Fig. 19 shows the changes in electrical resistivity of a frozen-thawed cell suspension that has been frozen a second time. As a result of the initial freezing and thawing, the intracellular solutes escaped into the extracellular water, and, after the second freezing, the resistivity of the suspensions, as expected, dropped during warming in a manner similar to that of 0.025 *M* KCl. In contrast, after the initial freezing (top curve of Fig. 19) the changes in electrical resistance during warming were like those observed for 0.0015 *M* KCl. That is to say, there was no resistance drop until the temperature rose to nearly 0°C. This behavior indicates that the solutes were still inside the cell.

Since the cellular solutes were inside the yeast cell at the time that heat absorption was observed, and since the heat absorption was such that at least 70% of the solutes had to be in contact with ice, then

[8] E. J. Conway and W. McD. Armstrong, *Biochem. J.* 81, 631–39, 1961.

Table 22. Effect of Repetitive Freezing and Thawing on Thermal and Electrical Characteristics of Suspensions of Yeast Cells

Treatment	Conductivity (mho/cm)	Melting Point (°C)	$(\Delta T_{max})^2$ (°C)^2
	$\times 10^{-4}$		
Before freezing			
Cell suspension	2.0		
Supernatant	2.0	−0.04	0.44
After first freezing			
Cell suspension	35	−0.56	10.0
Supernatant	40		9.8
After four freezings and sonication			
Cell suspension	46	−0.77	12.7
Comparison solutions			
0.025 *M* KCl	35		
0.0015 *M* KCl	2.1		

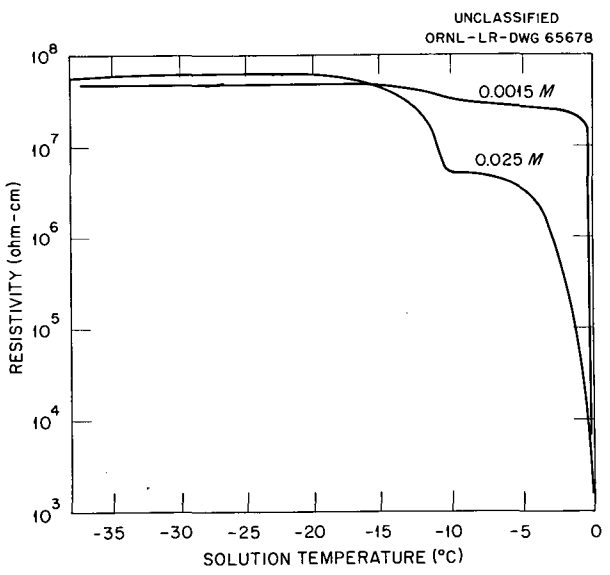

Fig. 18. Changes in Electrical Resistivity of Frozen KCl Solutions During Warming and Thawing. Measurements made at 1000 cps.

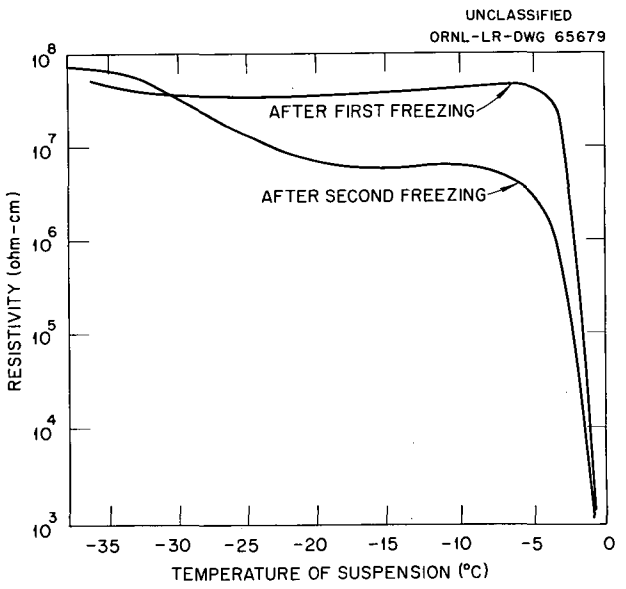

Fig. 19. Changes in Electrical Resistivity of Frozen and Twice Frozen Yeast Suspension During Warming. Measurements made at 1000 cps.

the ice must have been inside the cell. In other words, it is concluded that the cells did, in fact, undergo intracellular freezing. This conclusion is valid so long as more than 10% of the cellular water had remained within the cells after cooling. The actual percentage remaining was 40%, as calculated from the measured volumes of freeze-substituted cells.[9]

CHARACTERIZATION OF THE SUPERNATANT FROM FROZEN-THAWED YEAST CELLS

J. G. Green

Introduction. – It has been shown[10] that yeast cells in suspension lose a portion of their contents during freezing and thawing. As a result of these initial studies, it was believed that the characterization of the materials appearing in the supernatant from centrifuged frozen-thawed cell suspensions might yield information on the nature and severity of intracellular damage.

Methods and Results. – To obtain a simple and reliable cell-count assay procedure, hemacytometer counting was correlated with nitrogen analyses, dry weights, and results of turbidimetric measurements. For routine application, turbidimetric assay at 600 mμ was selected, since the procedure is convenient and reliable.

In the preliminary studies, only a single freezing and warming treatment is being employed, a severe treatment causing death of virtually all of the cells. The thoroughly washed, resuspended cells are frozen rapidly in a dry ice-ethanol bath and warmed slowly to 4°C. As far as practicable, all subsequent operations are conducted at that temperature level. After treatment, the cells are sedimented by centrifugation and washed once, and the supernatant and washing are combined.

After treatment, 60% of the yeast phosphorus appears in the supernatant. This fraction consists almost entirely of inorganic and acid-labile phosphate. Cold acid extraction of the cell residue removes an additional 18% of the cellular phosphate compounds having a higher proportion of organic, acid-stable phosphate and very little acid-labile material.

Anthrone kinetics and Prussian-blue colorimetric studies indicate that a a reducing sugar is the major carbohydrate appearing in the supernatant. Additional reducing activity appears after acid hydrolysis but not after incubation with invertase or gluc-amylase preparations. Glycogen and similar long-chain polysaccharides appear to be absent from the supernatant but may be obtained by hydrolysis of the cell residue.

Numerous free amino acids are found in the supernatant. In addition, biuret, amino acid, and ultracentrifuge studies indicate that the supernatant may contain proteinaceous materials.

A full complement of known bases and nucleosides occurs in the supernatant. Although the nucelotide diphosphates, especially ADP, appear in quantity, the nucleotide triphosphates are not present. Cold acid extraction of the cell residue yields only trace quantities of ATP. Soluble RNA precipitable at pH 3.6 appears in the supernatant. In addition, extended cold acid extraction reveals the retention of an RNA fraction in the cell residue.

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[9] P. Mazur, *J. Bacteriol.* 82, 662–72 (1961).

[10] P. Mazur, *J. Bacteriol.* 82, 673–84 (1961).

This study has just begun, and other analytical procedures are being developed. Although conclusive results await experimental variation of the freezing and warming techniques, the developmental studies indicate that the materials lost from the cells are soluble compounds having relatively low molecular weights.

ENZYMATIC PROPERTIES OF POLY-L-VALYL RIBONUCLEASE

Fumio Sawada R. R. Becker

Introduction. – It has been reported elsewhere[11] that poly-L-valyl ribonuclease retained enzymatic activity when yeast RNA was used as substrate. We have now extended this investigation to include the determination of the pH optimum in RNA hydrolysis and a more detailed study using cytidine-2′,3′-cyclic phosphate and uridine-2′,3′-cyclic phosphate as substrates.

Results and Discussion. – The rate of hydrolysis of yeast RNA as determined titrimetrically is slightly less when polyvalyl RNase is used in place of the native enzyme, although hydrolysis is optimal at pH 7.7 in both cases. Table 23 summarizes the data for native and polyvalyl RNase using cytidine-2′,3′-cyclic phosphate and uridine-2′,3′-cyclic phosphate as substrates. Once again the optimal pH is the same for the modified and the native enzymes, but a rather surprising change in relative rates of hydrolysis of the two substrates has occurred. Thus, comparing modified with native ribonuclease, one observes an increased activity for the polyvalyl RNase-cytidine cyclic phosphate system, but a decreased activity for the modified enzyme when the uridine derivative is used as substrate. These changes are reflected in the K_m values, with no significant differences in the maximal velocities.

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

Table 23. Comparison of Enzymatic Activity of RNase and Poly-L-Valyl RNase

Substrate	Enzyme	Optimal pH[a]	Enzymatic Activity[a,b]	K_m (10^{-3} M)[c]	V_m[b,c]
Cytidine-2′,3′-cyclic phosphate	Native	6.8	11	2.1	29
Cytidine-2′,3′-cyclic phosphate	Polyvalyl	6.8	16	0.86	23
Uridine-2′,3′-cyclic phosphate	Native	6.6	3.9	1.9	6.7
Uridine-2′,3′-cyclic phosphate	Polyvalyl	6.6	1.3	7.7	7.7

[a] Substrate, 8×10^{-4} M; enzyme, 2×10^{-6} M.
[b] 10^{-8} mole hydrolyzed per minute per milliliter.
[c] Enzyme, 1×10^{-6} M.

The change in relative activity of the modified enzyme toward the cytidine and uridine cyclic derivatives may permit selective cleavage of various RNA samples during the early periods of hydrolysis. This possibility is being investigated in addition to continuing the experiments regarding the binding of nucleotide inhibitors to the native and polyvalyl enzymes.

ACIDIC PHOSPHATASES IN RAT LIVER BREI

Herbert Schuel N. G. Anderson

Introduction. – Previous work suggests that several nonspecific acidic phosphatases with activity optima over a wide pH range may be present in rat liver.[12] Considerable effort has been expended to determine the distribution of "acid phosphatase" in rat liver by histochemical or tissue fractionation procedures. Out of these experiments has developed the concept that "acid phosphatase" is primarily confined within a special class of cytoplasmic granules known as lysozomes.[13] However, the enzymatic assays were conducted at a single hydrogen ion concentration. As a prelude, therefore, to a study of the distribution of acidic phosphatase activities in liver components separated in the zonal ultracentrifuge, we reinvestigated the effects of pH and the nonionic detergent Turgitol NPX on acid phosphatase activity in liver brei.

Experimental. – Acid phosphatase activities in 0.5% rat liver brei in 0.25 *M* sucrose were determined automatically in the Technicon Autoanalyzer, using phenyl phosphate as substrate. The assay measured the amount of free phenol (ϕ) colorimetrically by the reaction with 4-amino antipyrene and potassium ferricyanide. Sodium malonate (0.2 *M*) was employed to buffer over a range from pH 2.6 to 6.8. The Turgitol NPX, to a final concentration of 0.2%, was added to the substrate solution to release the enzymatic activities confined within the cytoplasmic granules. The results of a typical experiment can be seen in Fig. 20. In the untreated brei, pH maxima were observed at 3.2 and 5.5. The addition of the detergent caused the release of considerable additional activity between these optima. A plot of the percentage of activity change induced by the detergent shows that most of the additional activity was between pH 3.4 and 4.8. The effect of brei concentration at pH 3.2, 4.0, 5.0, and 5.5 on enzymatic activity was also determined. The activities were a direct linear function of brei concentration over a range from 0.2 to 1.6%. This corresponded exactly to the linear range of the phenol assay, 10 to 120 μg/ml.

Discussion. – These observations confirm the presence of several acidic phosphatases in rat liver brei. The heterogeneity of this family of enzymes must be considered in attempting to determine the distribution of acidic phosphatases in cells and tissues.

[12] G. A. J. Goodlad and J. T. Mills, *Biochem. J.* 66, 346–54 (1957).

[13] C. de Duve, pp 128–59 in *Subcellular Particles*, ed. by T. Hyashi, Ronald Press, New York, 1959.

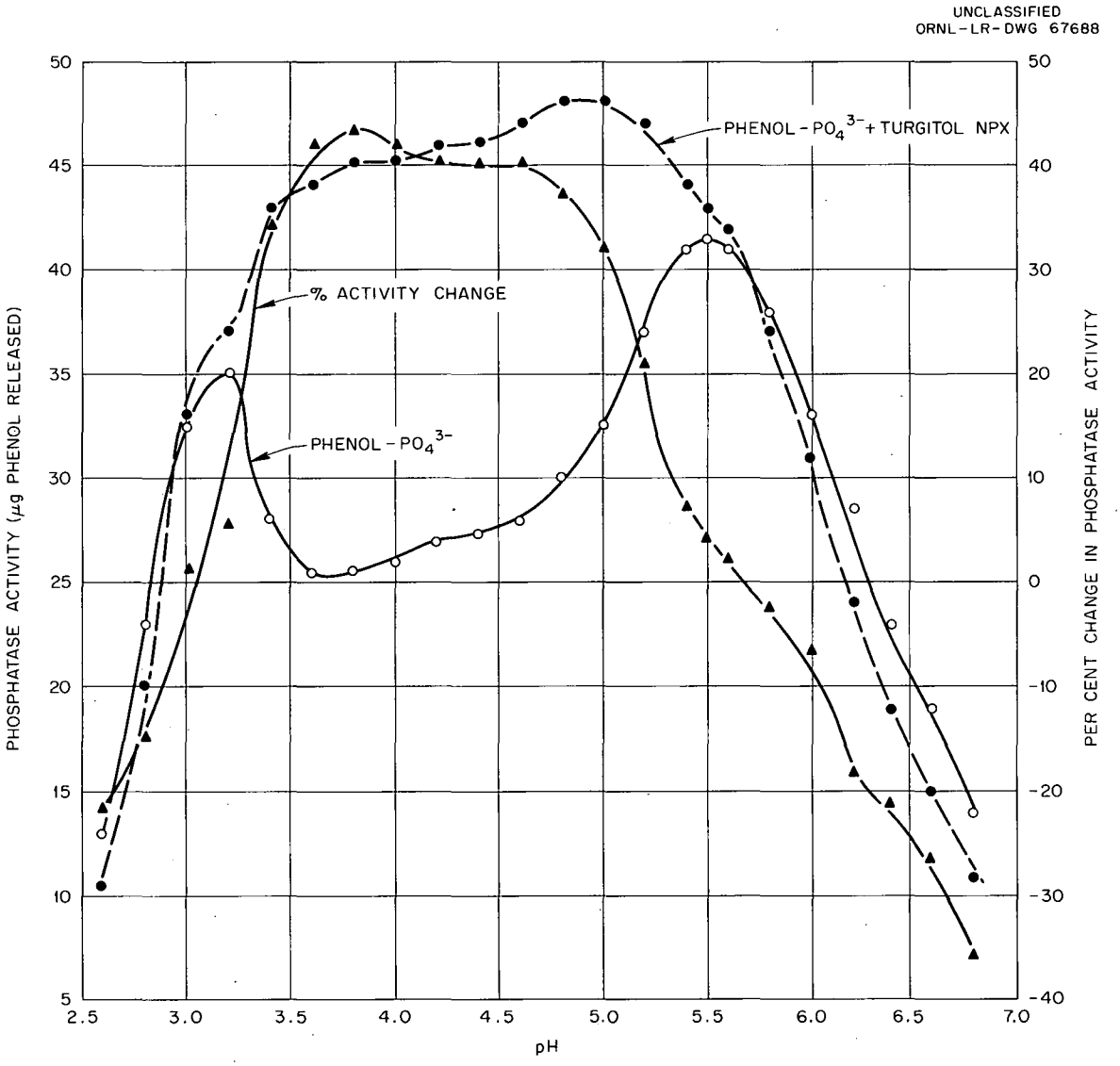

Fig. 20. Effect of Turgitol NPX on pH Activity Curve of Rat Liver Brei Acid Phosphatases.

DISTRIBUTION OF ACIDIC PHOSPHATASES IN RAT LIVER BREI FRACTIONS SEPARATED IN THE ZONAL ULTRACENTRIFUGE

Herbert Schuel N. G. Anderson

Introduction. – One of the major objectives of contemporary research in cellular physiology is to determine the distribution of enzymes within cells. The development of the zonal ultracentrifuge has made it possible to isolate during one centrifugation and without extensive handling the components of rat liver

brei; that is, soluble phase, microsomes, mitochondria, "cell-wall fragments," and nuclei.[14] Since it is now clear that several acidic phosphatases are present in rat liver,[15] an attempt has been made to determine the distribution of these enzymes in liver components separated in the zonal ultracentrifuge.

Experimental. – Separations of liver brei components were accomplished in rotor II, using a 1200-ml sucrose gradient that was linear with radius. The gradient went from a 17% to a 55% sucrose solution (w/w), with a 66% sucrose "cushion" at the rotor edge. Speeds ranged to 30,000 rpm. Acid phosphatase activities were measured continuously and automatically in the gradient as it was pumped out of the centrifuge with the Technicon Autoanalyzer. Phenyl phosphate, containing 0.2% of the nonionic detergent Turgitol NPX, was the substrate, and 0.2 *M* sodium malonate buffers at pH 3.2, 4.0, 5.0, and 5.5 were employed. The results of a typical experiment (22,000 × *g* for 2 hr) can be seen in Fig. 21. The pH 3.2 activity was associated with the cytoplasmic particles, while the pH 4.0, 5.0, and 5.5 activities were present in the soluble phase as well as in the particulate fractions. A relatively small amount of activity was present in the microsomes, but none was seen in the nuclei. Ribosomes were separated from the soluble phase by using a gradient that was developed for virus isolation, which extended linearly with radius from 10 to 33% (w/w) sucrose. The ribosomes did not contain any phosphatase activity at the hydrogen ion concentrations employed. The high sucrose concentrations used in the gradients did not appear to affect the phosphatases adversely. Comparison of the pH activity curves (pH 2.6 to 6.8) in the presence of 8 and 55% sucrose reveals an approximately 5 to 7% reduction in the amount of phenol released, in spite of the fact that the viscosity of the 55% sucrose is about 23 times that of 8% sucrose at room temperature.

Discussion. – The data suggest that the various acid phosphatases are not equally distributed in the components of rat liver brei. The pH 3.2 activity appears to be associated with the cytoplasmic particles, while the pH 4.0, 5.0, and 5.5 activities are associated with both the soluble phase and particulate fractions. Additional work is necessary to determine the effect of these observations on the hypothesis proposed by de Duve that "acid phosphatase" is confined within a special class of cytoplasmic granules known as lysozomes.[16] In this connection, an automated procedure to assay cytochrome oxidase in the brei fractions is being developed.

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[14] N. G. Anderson and C. L. Burger, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, pp 102–4.

[15] See the preceding section, "Acidic Phosphatases in Rat Liver Brei," this report.

[16] C. de Duve, pp 128–59 in *Subcellular Particles*, ed. by T. Hyashi, Ronald Press, New York, 1959.

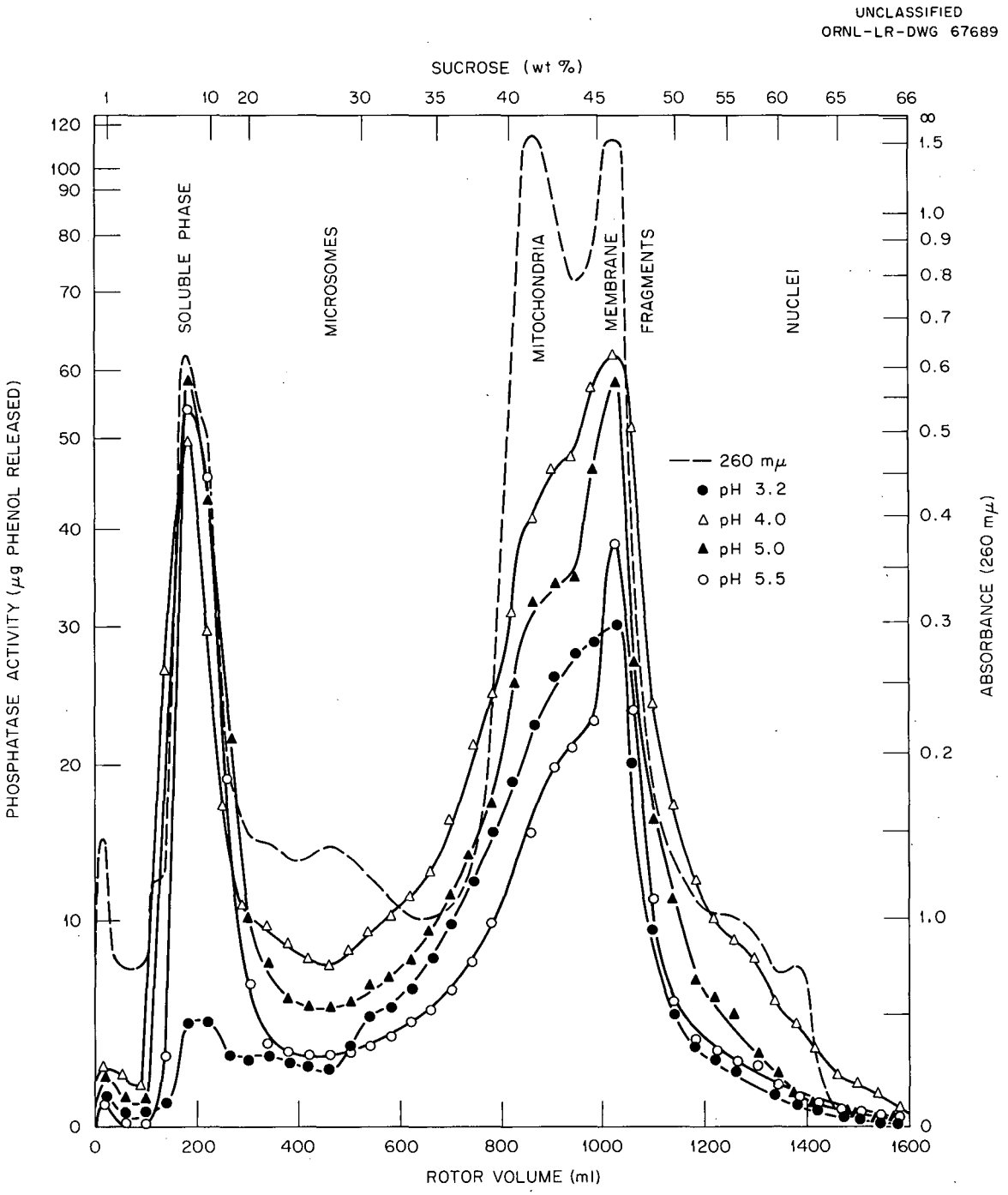

Fig. 21. Distribution of Acid Phosphatase Activities in Rat Liver Brei Components Isolated in the Zonal Ultracentrifuge.

THE ISOLATION AND ANALYSIS OF AMPHIBIAN YOLK PLATELETS

R. A. Wallace

In the early oocyte of *Rana pipiens*, a hexagonal, crystalline structure has been found to gradually fill out the mitochondrial membrane system.[17] This structure is subsequently found as the main body of the amphibian yolk platelet. Isolation of the crystalline body by homogenization and centrifugal methods revealed that it is composed of two components with corrected sedimentation values of 12.1 *S* and 3.18 *S*. The faster sedimenting component appears to be a lipoprotein with an isoelectric point of approximately 8.9, whereas the slower component is a phosphoprotein with an isoelectric point below 3.5. Further preliminary measurements indicate that the molecular weights of these two proteins are approximately 400,000 and 35,000 respectively. Since the phosphoprotein comprises 15% of the protein recovered, it is further assumed that 2 moles of phosphoprotein complex with 1 mole of lipoprotein to form the crystalline structure.

Methods for the complete separation of these entities are presently being perfected so that further physical-chemical properties and interactions can be studied. It is hoped that such studies will provide an understanding of both the formation of this structure within mitochrondrial membranes[17] and also the observed appearance of many membrane structures around the crystalline complex conjunctive with its dissolution.[18,19]

In the late oocyte and early embryo, the crystalline structure is further surrounded by a less dense, noncrystalline, "granular layer," which in turn is enclosed within a unit membrane[18] – this complex is defined as the yolk platelet. During early phases of differentiation, the "granular layer" is frequently seen to disappear.[19] Early work[20] indicated that the composite platelets contain one-third of the RNA in the early embryo, a possibility that has led several people (see Flickinger[21]) to suggest a special role for the platelet during inductive processes. Since this postulate has received some attention, it was considered important to isolate yolk platelets in yield, morphologically intact and free from contamination – conditions which hitherto have not been strictly met.

A method of sucrose layering in 5% polyvinylpyrrolidinone has been perfected (together with S. Karasaki) to give a 50% yield of highly purified platelets that still maintain a crystalline structure and the outer "granular layer." Component analysis of these platelets is in progress. It has thus far been determined that the RNA content of isolated material is 0.05%. This would mean that, at most, the yolk platelets contain 2.5% of the RNA in the amphibian egg. The reality of this figure is also being further considered in the light of nonspecific material that would give a positive test under the conditions employed.

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[17] R. Ward, Ph.D. thesis, Columbia University (1961).

[18] See the section, "The Ultrastructure of Yolk Platelets in the Amphibian Egg," p 42, this report.

[19] T. Yamada, *Adv. in Morphogen.* 1, 1 (1961).

[20] P. Grant, *J. Exptl. Zool.* 124, 513 (1953).

[21] R. Flickinger, *Am. Naturalist* 41, 373 (1957).

PHYSICAL PROPERTIES OF POLYPEPTIDYL PROTEINS

L. M. Krausz F. C. Scaduto Fumio Sawada R. R. Becker

Introduction. – It has been reported[22] that poly-*L*-valyl chymotrypsin behaves as an aggregating system when examined by the Archibald technique in the ultracentrifuge. The object of the work of the past 6 months was to study this derivative further and determine its partial specific volume ($\bar{V}$), diffusion coefficient (D), sedimentation coefficient (S_{20}), and molecular weight (M).

Results and Discussion. – The values determined for native chymotrypsin and for poly-L-valyl chymotrypsin are included in Table 24.

Table 24. Physical Constants of α-Chymotrypsin and Poly-L-Valyl Chymotrypsin

Substance	$\bar{V}^{(a)}$ (cc/g at 20°)	$D_{20,w}{}^{(a)}$ (cm²/sec)	$S_{20}{}^{(b)}$
		$\times 10^7$	
Chymotrypsin	0.713	8.99	2.42[c]
Poly-L-valyl chymotrypsin	0.733	7.76	2.96

[a]The data was obtained from 1.5% solutions in phosphate buffer at pH 6.20 and ionic strength 0.2.
[b]The values of S_{20} are those extrapolated to infinite dilution.
[c]Taken from the data of G. W. Schwert, *J. Biol. Chem.* 179, 655 (1949).

Sedimentation velocity experiments revealed that the poly-L-valyl chymotrypsin preparation contained about 10% of a heavy component (S_{20} = 27.0). This material was apparently responsible for the high molecular weight obtained in sedimentation-equilibrium experiments employing the Archibald technique.[22] Once separated, however, the lighter component, S_{20} = 2.96, behaved essentially as monomer.

The main difference observed in recent experiments between α-chymotrypsin and the major component of the polyvalyl derivative is the lack of aggregation of the latter even under conditions of pH, concentration, and ionic strength where the native enzyme exists as a dimer. Indeed, the low-ionic-strength polymerization of chymotrypsin described by Massey *et al.*[23] is not observed in the polyvalyl derivative. In 1% solution in 0.01 *M* phosphate buffer, pH 7.9, the native enzyme exists largely (70%) as a hexamer; under identical conditions, polyvalyl chymotrypsin remains a monomer. It thus appears that the addition of valyl peptides has interfered with the electrostatic interaction which leads to polymer formation. Recent experiments have shown that the temperature-reversible aggregation reaction which has been observed in polyvalyl protein derivatives[22,24] involves the polymerization of the S_{20} 27.0 component for hydrophobic interaction to a very-high-molecular-weight species.

[22]L. M. Krausz, Florence C. Scaduto, and R. R. Becker, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, pp 110–11.

[23]V. Massey, W. F. Harrington, and B. S. Hartley, *Discussions Faraday Soc.* 20, 24 (1955).

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

Nucleic Acid Chemistry

W. E. Cohn
F. J. Bollum
J. X. Khym[1]
Keiichi Kusama[2]
B. C. Pal[3]
A. Jeannine Bandy
Patricia A. Williams

THE CONFIGURATION OF PSEUDOURIDINE[4] AND THE PREPARATION OF ITS O-4:C-5′ANHYDRIDE

W. E. Cohn A. M. Michelson[5]

Pseudouridine (I in Fig. 22) was established as 5-ribofuranosyluracil by chemical and physical methods[4] and as the *D* isomer by synthesis.[6] Although the properties of its four isomeric forms (A_f, A_s, *B*, and *C*) were taken to indicate α and β-anomers of the furanosyl and pyranosyl isomers, no direct proof of the configuration of any one of these has been available. We have now synthesized the O-4:C-5′ cyclo (anhydro) derivative (IV) of pseudouridine *C*, the naturally occurring isomer (I) obtained from human urine, by treating the 5′-*p*-toluenesulphonyl-2′,3′-isopropylidene derivative (III) with Na *t*-butoxide. Models indicate that only the β configuration can form such a cyclo derivative; therefore, pseudouridine *C* is a β-*D*-ribofuranosyl, as are all nucleosides derived from nucleic acids. From the older evidence,[4,6] the β form is the α-ribofuranosyl, and the *A* forms are the α- and β-pyranosyluracils.

The new anhydride has a single pK at 8.4, considerably below the 9.3 of the isopropylidene derivative (II) or the 9.5-9.6 of the other forms (but like that reported for 2-ethoxyuracil). The ultraviolet absorption maxima of both forms are at 288 mμ, in contrast to 262 (pH 2 to 7) and 286 (pH 12) for acyclic forms.[4] It is hydrolyzed by dilute acid at 23°C ($t_{1/2}$ = 60, 17, and 5 min in 0.01, 0.05, and 0.2 *N* HCl) to pseudouridine *C* (I).

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[1]Reporting in Nucleic Acid Enzymology.

[2]Visiting investigator from abroad.

[3]Research participant.

[4]See previous reports, also W. E. Cohn, *J. Biol. Chem.* 235, 1488 (1960).

[5]Chemist's Laboratory, Arthur Guinness Son and Co., Dublin, Ireland.

[6]Robert Shapiro and R. W. Chambers, *J. Am. Chem. Soc.* 83, 3920 (1961).

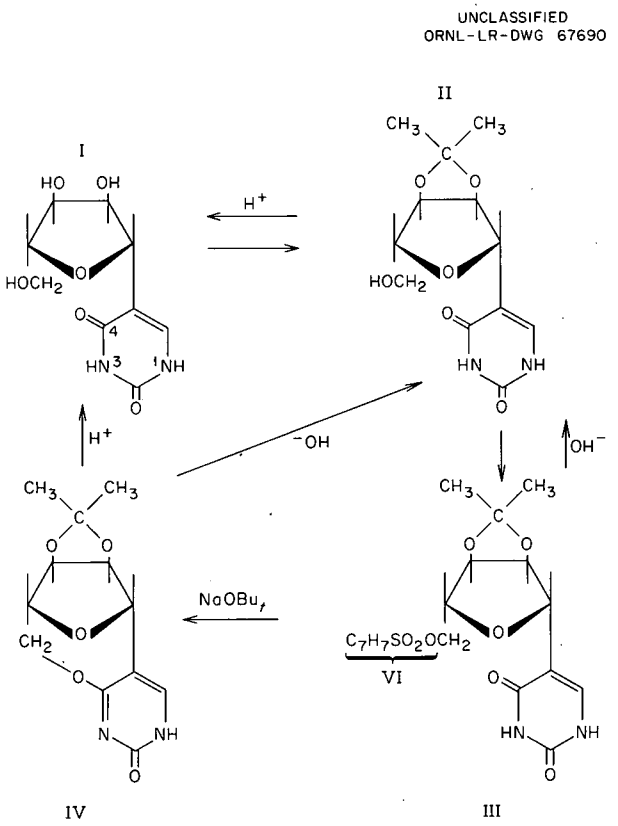

Fig. 22. Synthesis of Cyclopseudouridine (Isopropylidene Derivative).

PURIFICATION OF CALF THYMUS POLYMERASE

F. J. Bollum Bruce Alberts[7] P. A. Williams

The purification of calf thymus DNA-polymerase on a large scale was greatly facilitated by procurement of large-scale mixers, pumps, and a pilot-plant-scale centrifuge whose bowl opens automatically. Suitable cold-room space is also available. Our initial run with the new facilities permitted a 20-kg fractionation to proceed to completion in 1 week, compared with 2 weeks for a 10-kg preparation in the old facility.[8] It is anticipated that experience will permit us to achieve fractionation of 30 kg of calf thymus gland in about 3 days.

The large-scale enzyme-isolation project provides starting material for studies on further purification, with an immediate objective of obtaining the DNA-polymerase in a nuclease-free form. The most promising new development in this direction has been chromatography on hydroxyl-apatite columns, which resulted in high resolution *and* quantitative recovery of enzyme activity, the latter result in marked contrast to the losses observed earlier with DEAE-cellulose.[8] Figure 23 illustrates an analytical-scale run demonstrating

[7]Harvard University, loanee.

[8]F. J. Bollum and Patricia A. Williams, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 112.

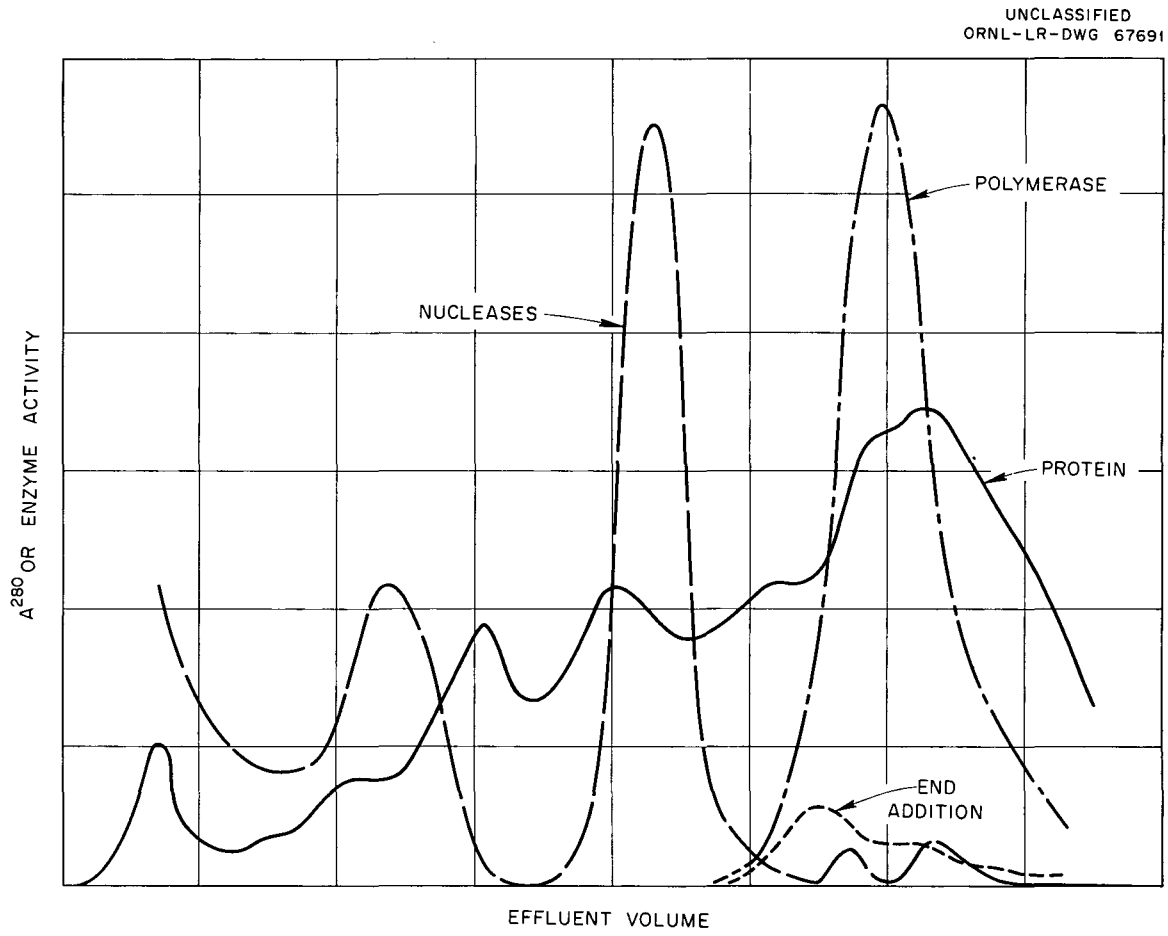

Fig. 23. Chromatography of Calf Thymus Polymerase on Hydroxyl-Apatite.

a reasonably good separation of three DNA-degrading proteins from the DNA-polymerase peak. The activity resulting in end-addition of single deoxyribonucleoside triphosphates to polydeoxyribonucleotide chains[9] is not resolved from the polymerase. The material obtained from hydroxyl-apatite columns is very unstable at 0° to 4°C or frozen at −20°C. It will be necessary to find conditions for stabilizing the enzyme protein before proceeding further with the fractionation.

RADIATION EFFECTS ON "PRIMER" DNA

F. J. Bollum R. B. Setlow

Denatured DNA acts as a primer for calf thymus DNA-polymerase.[10] Irradiation of denatured DNA or native DNA (the latter subsequently denatured in order to act as a primer) by 2800-A light results in a

[9] *J. Biol. Chem.* **235**, PC 18 (1960).

[10] F. J. Bollum, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1959*, ORNL-2813, p 154.

marked loss of this priming ability. Subsequent irradiation by 2400-A radiation gives a partial restoration of priming activity. The magnitude of the inactivation as a function of dose and its wavelength dependence indicate that it probably arises from the formation of dimers between adjacent thymine residues in the DNA molecules, a polymerization known to be effected by 2800-A radiation.[11]

The results obtained from the UV irradiation of primer DNA are in marked contrast to those obtained from x irradiation of DNA solutions. X-ray doses up to 200 kr given to denatured (priming) DNA (2 mg/ml) do not decrease the priming activity of the DNA when tested with the most highly purified calf thymus polymerase. This finding disagrees with that of Stacey,[12] who used a crude enzyme fraction to test for priming activity.

In an attempt to verify our interpretation of the results on irradiation of primer DNA's, we have examined P^{32}-labeled *Escherichia coli* DNA for a radiation-induced "lesion." After irradiation, the DNA was degraded with diesterase and the enzyme-resistant products were isolated by chromatography on DEAE paper. Compounds with the structure pXpTpT (pTpT = thymidylic acid dimer) were isolated from the UV-irradiated DNA. Their structures were demonstrated by photolysis with 2400-A radiation followed by further degradation with diesterase and/or alkaline phosphatase to mononucleotides or nucleosides.

In collaboration with R. F. Kimball, we also isolated enzyme-resistant material from x-irradiated DNA. These compounds probably arise from chain breakage rather than from the base alteration found in UV-DNA. The outstanding observation in this x-ray irradiation study is the marked inverse dependence of chain breakage on DNA concentration during irradiation. At high concentrations, such as used in the studies on priming activity, chain breakage is small. Since chains exceeding 20 monomer units serve reasonably well as primers, the chain breaks produced at high concentration will not affect priming activity.

PSEUDOURIDINE METABOLISM

Keiichi Kusama
(with the aid of David Prescott)

Introduction. – The study of the metabolism (formation, degradation, and exchange of parts with other pyrimidine components) of pseudouridine[4] in *Tetrahymena pyroformis*, a species that requires a preformed pyrimidine for growth, has continued with the aid of various labeled precursors.

Results. – Four precursor materials were employed, with the following results.

1. Uracil-2-C^{14} and uridine-2-C^{14} were incorporated into RNA-UMP and -ψMP, somewhat less efficiently into RNA-CMP, in a 10-day growth period (cell multiplication factor over 1000). The uracil was essentially undiluted from source to acid-soluble material to RNA or DNA pyrimidine nucleotides. This showed the preservation of the pyrimidine ring intact and the absence of appreciable synthesis of pyrimidine rings.

[11] R. B. Setlow and A. J. Coughlin, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 150.

[12] K. Stacey, *Biochem. Biophys. Research Communs.* 5, 486 (1961).

2. Uracil-5,6-H^3, in a 2-week growth period, appeared in the RNA-UMP, -CMP, and -ψMP, with specific activities diluted by factors of 18, 70, and 4000, respectively. In the acid-soluble material, these drops in specific activity were 30 (for UMP, uridine, and uracil, equally), 70 for CMP, and 7000 for pseudouridine (even greater for thymine). Considerable difficulty was experienced in proving that the starting material was indeed 5,6-labeled, but with that point settled affirmatively, two questions arise from the results. (*a*) Why the large loss in label from the (equally labeled) 6 position when the 5-carbon is substituted (by ribose, to make pseudouridine, or by methyl, to make thymine)? We have established considerable (unexpected) chemical lability in the 5 and 6 hydrogens of uracil. Apparently this instability, leading to exchange, is biological as well. (*b*) Why does the 6 hydrogen become even more exchangeable during or after the replacement of the 5 hydrogen by a carbon?

3. Uridine-5,6-H^3 appears after one day (cell multiplication factor of about 10 times) in RNA-UMP, -CMP and -ψMP in specific activities reduced by 350, 850, and 130,000, respectively, relative to the starting material. This again shows a large loss in the 5 and 6 hydrogens on formation of the UMP and CMP, and a still larger loss of the 6 label when the ribose replaces hydrogen at the 5 position.

Discussion. – These experiments all indicate that the C and N ring of the pyrimidine portion of uracil and uridine can serve as a precursor of pseudouridine (and other pyrimidines) in RNA (and DNA) in *Tetrahymena,* with exchange of the 5 and 6 hydrogens far beyond what can be accounted for by carbon substitution on the 5 position. The mechanism and significance of this exchange remain to be determined.

The previous qualitative observation[13] that pseudouridine can donate its ribose moiety to purines, was made under conditions that did not permit determination of the extent of direct pseudouridine-uridine isomerization. Another sample of tritiated pseudouridine has been obtained in the hope of settling this point. Ribose-labeled uridine and pseudouridine, and carbon-labeled ribose precursors, will be used to investigate the possibility of isomerization.

THE STRUCTURES OF 3-METHYLADENINE AND 3-METHYLADENOSINE AND THEIR POSSIBLE RELATION TO MUTAGENESIS BY ALKYLATING AGENTS

B. C. Pal

Introduction. – Although attempts to detect any alkylation of the adenine moiety in DNA by alkylating agents have been unsuccessful so far,[14,15] the possibility of such a reaction as a probable cause of mutagenesis cannot be ignored altogether. Adenosine is alkylated at N-1 and at N-3 by alkylating agents.[16] The compounds 3-alkyladenine and 3-alkyladenosine may exist either in the amino or imino form. These possibilities have been investigated.

[13] K. Kusama, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 114.

[14] P. Brookes and P. D. Lawley, *Biochem. J.* **80**, 496 (1961).

[15] R. J. Rutman *et al.*, *Federation Proc.* **20**(1), 354 (1961).

[16] P. Brookes and P. D. Lawley, *J. Chem. Soc.* 539 (1960).

Results and Discussion. – Contrary to earlier belief,[17] present investigations support the amino form of 3-methyladenine. The compound 3-methyladenine (1) does not show an anionic pK, as is evident from its UV spectra and electrophoretic mobility at pH 12.0, and (2) fails to react with the mercury reagent, which failure is characteristic of those purines having no unsubstituted imidazole -NH-. Like adenine, 6-dimethylaminopurine is methylated at N-1, N-3 (highest yield), and N-9 by dimethyl sulfate. The products can be separated by paper chromatography in isopropanol-NH_3 mixture, followed by rechromatography in isopropanol-HCl. A method of separation of the products by ion exchange chromatography has been developed in which unreacted 6-dimethylaminopurine is separated by adsorption on Dowex-1 at pH 10.0. The eluate containing the three methylated products is fractionated on Dowex-50, using a gradient of ammonium formate. The 3-methyl-6-dimethylaminopurine thus obtained has spectra very similar to those of 3-methyladenine, which conclusively proves the amino form of the latter. The infrared spectrum also indicates the presence of the amino group and the absence of the imidazole -NH- in 3-methyladenine.

The compound 1-methyl-6-dimethylaminopurine loses its UV spectrum in 0.1 *N* NaOH, and the spectrum is not regenerated on acidification. This behavior is not unexpected; 1-methyladenine rearranges into 6-methylaminopurine in alkali possibly by a ring opening and closing mechanism.[16] Such a rearrangement cannot take place in 1-methyl-6-dimethylaminopurine.

Since adenine is alkylated at N-1, N-3, and N-9 at ordinary temperature and at pH 7.0 (ref 18), corresponding tautomeric forms are proposed for adenine under these conditions. This is interesting in view of the amino form of 3-methyladenine inasmuch as it involves a proton shift from the imidazole ring to the pyrimidine ring and vice-versa.

The compound 3-methyladenosine, on the other hand, appears to exist in the imino form (II in Fig. 24). The amino form (I) is possible only if the molecule can exist in the form of a cation; 3-methyladenosine does not migrate as a cation on electrophoresis at pH 10.0. This finding indicates that when the adenine

[17] G. B. Elion, p 43 in *Ciba Foundation Symposium on the Chemistry and Biology of Purines*, 1957.

[18] B. C. Pal, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 115.

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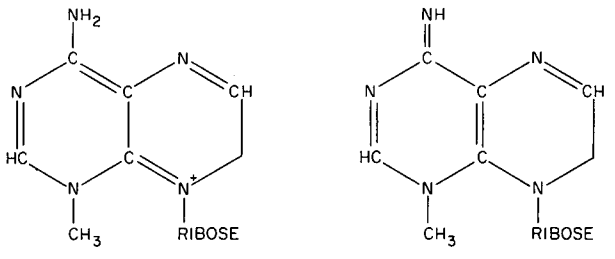

3-METHYLADENOSINE (6-AMINO FORM) I

3-METHYLADENOSINE (6-IMINO FORM) II

Fig. 24. Possible Forms of 3-Methyladenosine.

moiety of the DNA is alkylated, the 6-NH_2 group is converted into an =NH group. This compound would act as an acceptor instead of a donor in the Watson-Crick hydrogen-bonding scheme of the DNA and would not bond with thymine, as it usually does.

Summary. – The compound 3-methyladenosine does not behave as a cation at pH 10 and thus appears to exist as a 6-imino compound that would be a hydrogen acceptor rather than donor in hydrogen bonding. On the other hand, and contrary to earlier conclusions, 3-methyladenine is regarded as an amino compound by virtue of proton migration from the imidazole ring to the 6-amino position, thereby giving spectral properties similar to 3-methyl-6-dimethylaminopurine as well as other reactions characteristic of the absence of protons in the imidazole ring. The three methylated derivatives of 6-dimethylaminopurine (N-1, N-3, N-9) have been prepared and separated by chromatography. The N-1 derivative is destroyed by alkali, as it cannot undergo the rearrangement of 1-methyladenine.

Nucleic Acid Enzymology

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Katherine H. Stephenson

NUCLEIC ACID METABOLISM OF GERMINATING CORN SEEDLINGS

Elliot Volkin Drew Schwartz[2] M. Helen Jones

The work of Drew Schwartz with gene-directed esterase synthesis and that of R. J. Mans on cell-free amino acid incorporation reveal a very strong dependence of these activities on time after germination of maize. Insofar as these functions may be a reflection of the synthesis of transfer RNA or messenger RNA, or both, metabolic studies on the nucleic acids of germinating corn seedlings have been initiated.

Although the corn seed is primarily self-sufficient with respect to its nutritional requirements, it readily assimilates $P^{32}O_4$ ions from water and incorporates the isotope into nucleic acids as well as other phosphorylated organic constituents. Both root and plumule materials become labeled.

Preliminary experiments designed to follow the accumulative uptake of P^{32} into RNA at 3, 6, and 10 days after the start of germination revealed a very definite tendency for the formation of messenger-like RNA in the roots. That is, based on constituent mononucleotide radioactivities, the newly formed RNA had an apparent composition similar to maize DNA. The data from the plumules were inconclusive in this respect.

Experiments under way are designed to follow the labeling properties of RNA after short-term periods of $P^{32}O_4$ assimilation (pulsed experiments). Roots of seedlings were immersed in solution containing $P^{32}O_4$ for only 4-hour periods at 1, 2, 3, 4, 6, and 8 days after the start of germination. Although the data are presently incomplete, the following observations have been noted.

1. Between the second and third days a great surge of RNA synthesis by the plumule takes place. In spite of this large net synthesis of RNA, the radioactivity incorporated into RNA during the P^{32} pulse at the 3-day time was by far the lowest of all the pulsed experiments. This low extent of labeling took place in spite of a large increase in radioactivity into the acid-soluble pool at this time. It is our contention that an endogenous source of RNA (or probably RNA breakdown products) is being utilized from another tissue, probably the scutellum. Experiments to test this hypothesis have been designed.

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2. A marked change in base content of the total plumule RNA takes place between the first and second day. It appears that RNA of high guanine and cytosine content is being synthesized, possibly a reflection of transfer RNA production. At the third day and thereafter, the total RNA composition reverts to a normal pattern.

3. Although the RNA content of the plumule increases rapidly at the third day of germination and continues to increase at least through 8 days, the RNA content of the primary root remains relatively constant. At 1 and 2 days the specific activities of plumule and root RNA's are about equivalent, but at the 3-day time and thereafter the specific activity of the primary root RNA becomes some 50-fold higher than the plumule RNA. Since there is little, if any, net synthesis of RNA evident in the primary root, these data may indicate an extensive RNA turnover in this tissue.

THE PRESENCE OF AN RNA ASSOCIATED WITH THE DNA OF *DROSOPHILA MELANOGASTER*

C. G. Mead

As a consequence of the isolation of *Drosophila* DNA free of cytoplasmic RNA, a small fraction of RNA is consistently found associated with the DNA. The isolation procedure results in a complete separation of microsomal and soluble RNA from the DNA. The nature of this DNA-associated RNA has been further investigated.

The molar nucleotide ratio (Table 25) of this RNA is quite different from that of the microsomal RNA. Notably the purine-pyrimidine ratio and the 6-amino-6-keto ratio are both found equal to 1.0, a value characteristic of DNA. The molar nucleotide ratio that this RNA approaches, however, is not identical with that of the DNA.

Table 25. Nucleotide Molar Ratios of *Drosophila melanogaster* Nucleic Acids

Nucleotide	Molar Ratio		
	DNA	RNA Associated with DNA	Microsomal RNA
Cytidylic	19.6	22.3	16.4
Adenylic	28.6	27.6	21.2
Uridylic or thymidylic	31.4	26.6	29.8
Guanylic	20.4	23.4	33.6
Purines / Pyrimidines	0.96	1.00	1.21
6-Amino / 6-Keto	0.93	0.99	0.86
A + U(+) / G + C	1.50	1.18	1.04

A study of the rate of incorporation of P^{32} into the nucleic acids of third instar larvae (Table 26) demonstrates that the RNA associated with the DNA incorporates P^{32} at a higher rate than the microsomal RNA.

It is possible that this DNA-associated RNA fraction is actually a "DNA-like RNA." The difference in the molar nucleotide ratios of the DNA and this RNA is, however, somewhat atypical.

Table 26. Incorporation of P^{32} into the RNA of Third Instar Larvae of *Drosophila melanogaster*

Time After P^{32} Addition (min)	Specific Activities (counts/min/mg nucleic acid)	
	RNA Associated with DNA[a]	Microsomal RNA
5	148	13
10	308	18
30	368	53
60	390	106

[a]Removed from DNA as 2′,3′ nucleotides.

THE USE OF AMINES IN LIQUID-LIQUID EXTRACTIONS OF NUCLEIC ACIDS AND RELATED PRODUCTS FROM AQUEOUS SALT SOLUTIONS

J. X. Khym[3] Katherine H. Stephenson Elliot Volkin

Introduction. – Liquid-liquid ion exchange, a development in ion exchange technology, was first u by Smith and Page[4] who employed long-chain, high-molecular-weight amines as acid-binding extractants. In this process, amines dissolved in water-immiscible solvents are converted to a desired salt form by shaking with aqueous solutions of a given acid or buffer. Thus converted, the anion of the amine salt can be replaced by another anion by subsequent equilibration with a fresh aqueous phase. Liquid-liquid anion exchange studies have been initiated here to test the feasibility of this technique for (1) the extraction of RNA, DNA, nucleoside mono-, di-, and triphosphates, and intermediate-molecular-weight oligonucleotides from aqueous salt solutions, and (2) the separation of the compounds listed from each other as well as further separations within a group.

Results and Discussion. – The initial experiments were carried out with calf liver RNA dissolved in aqueous salt solutions which were then equilibrated with an amine salt of known concentration in a water-immiscible solvent. After equilibration, the layers were separated and each examined spectrophotometrically for RNA content. Extreme differences in the extractability of RNA from the aqueous phase can be

[3]From Nucleic Acid Chemistry Group.

[4]E. L. Smith and J. E. Page, *J. Soc. Chem. Ind.* **67**, 48–51 (1948).

related to (1) the type of immiscible solvent, (2) molecular weight, base strength, and structure of the amine, (3) the salt form of the amine, and (4) the concentration and pH of the salt in the aqueous phase.

It was found that chloride, bromide, and sulfate salts of several amines gave poor performance in several of the practical solvents which are available and that organic salts such as formate, acetate, and citrate gave a reasonable performance in that emulsions, third-phase formations, colloids, etc., were minimized. With respect to the solvents, a series of tests using the formate salt of several amines showed that butyl ether; esters such as amyl acetate, isoamyl acetate, and butyl acetate; and hydrocarbons such as decane and dodecane could be used best as solvents for the extraction of RNA. Thus far, about 20 amines, differing markedly in molecular weight and structure, have been assayed for possible use as reagents. Only two show promise as good extractants for RNA. They are Primine JMT and 81R (Rohm and Haas Company trade names) which are *t*-alkyl primary aliphatic amines with highly branched alkyl chains and molecular weights of 315 and 191, respectively.

It was found that RNA once extracted into the organic phase (from dilute formate, extraction coefficients greater than 30 can be obtained) could be removed quantitatively by shaking the organic phase with borax solution. The RNA can be recovered by dialysis followed by precipitation with ethanol. A series of decreasing extraction coefficients was obtained by increasing the formate concentration in the aqueous phase in a given extraction sequence. Removal of the RNA from the organic phase by the latter method has thus far always resulted in some degree of third-phase formation that depends on the solvent systems and amines used and may contain 5 to 20% of the 260-mμ absorbancy compounds.

Chemical Protection and Enzyme Catalysis

D. G. Doherty
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CHEMICAL PROTECTION

D. G. Doherty J. D. Bales, Jr. Cynthia M. Park

Introduction. – One approach toward obtaining improved radiation protection is through the use of mixtures of protective compounds. These mixtures may be selected with the view that the compounds supplement one another by their different mechanisms of action (e.g., using sulfhydryl compounds plus serotonin or sulfhydryl compounds plus *p*-aminopropiophenone). Another way is to attempt to increase the concentration of an active compound by either reducing its toxicity or by maintaining or transporting more of it in an active form to radiation-sensitive sites. We explored this latter approach. Glutathione, the nontoxic sulfhydryl peptide that has been implicated in a variety of biological reactions, including thiol-disulfide exchange in both simple and protein disulfides,[2] was selected as the most likely compound to use in conjunction with *S*,2-aminoethylisothiourea (AET) as the prime protective agent.

Results and Discussion. – Glutathione (GSH) has been extensively investigated and its protective activity, when given intravenously or subcutaneously, established by Patt *et al.*[3] and Chapman and Cronkite.[4] We obtained a considerably greater elevation of the radiation LD_{50} than the latter authors by administering GSH intraperitoneally (i.p.). In the $BC3F_1$ strain of mice (LD_{50} 700 r), 1.6 g of GSH per kilogram, i.p., 15 min prior to x irradiation raised the LD_{50} to 950 r, while 3.2 g/kg gave an LD_{50} of 1300 r. Toxicity studies of combinations of GSH and AET (given as 2-mercaptoethylguanidine MEG) revealed that the maximum i.p. dose of AET could be increased one-third, from 360 mg/kg to 480 mg/kg, when 1 to 2 g of GSH per kilogram was administered at the same time. This proved to be the optimum combination dose, as higher or lower GSH doses resulted in toxic death of the animals. The radiation

[1]Former research associate.

[2]W. E. Knox, chap. 8 in *The Enzymes*, vol 2, ed. by P. D. Boyer, H. Lardy, and K. Myrbäck, Academic Press, New York, 1960.

[3]H. M. Patt *et al.*, *Proc. Soc. Exptl. Biol. Med.* **73**, 18–21 (1950).

[4]W. H. Chapman and E. P. Cronkite, *Proc. Soc. Exptl. Biol. Med.* **75**, 318–22 (1950).

LD_{50} for the maximum dose of AET alone (360 mg/kg) was 1500 r. Combined treatment with 2 g of GHS per kilogram and 480 mg of AET per kilogram increased this value to 1700 r. A similar effect on toxicity was observed for GSH mixtures with 2-mercaptoethylamine (MEA), the maximum tolerated dose of MEA being increased one-third from 150 mg/kg to 200 mg/kg. Preliminary indications are that the combined treatment LD_{50} is higher than that for MEA alone. Oxidized glutathione (GSSG) had a similar effect on the toxicity of AET, although the radiation LD_{50} for the combination was lower than that for AET alone (1300 r). GSH also reduced the toxicity of the disulfide of MEG [diguanidoethyl disulfide (GED)], although there was no increase in radiation protection obtained from the increased quantity of GED that could be administered. Thus, it is quite clear that GSH can reduce the toxicity of sulfhydryl compounds and disulfides, and, in the former case, this leads to increased radiation protection at the higher radiation dose levels. The evidence at hand at present is not sufficient to indicate by what mechanism these effects are obtained.

ENZYME CATALYSIS

D. G. Doherty Cynthia M. Park

Introduction. – Previous studies, a recent one being that of Inagami and Sturtevant,[5] established that chymotrypsin and trypsin mutually hydrolyze some substrates, the rate being 10% of that with the preferred enzyme. To find additional substrates that would be useful in defining the radiation damage to the active centers of these enzyme systems, a search was made for a better combined substrate.

Results and Discussion. – Methyl *m*-aminophenylpropionate, containing structural requisites and spacing of groups common for both enzymes, was readily hydrolyzed, the rate with trypsin being one-fourth that with chymotrypsin. Conversion to the corresponding cyclohexyl derivative gave an 8-fold increase in rate with trypsin and, as might be expected from specificity requirements, a decrease with chymotrypsin. Methyl *p*-aminophenylpropionate was split at a rate similar to the rate for the *meta* derivate by chrymotrypsin but, with the amino group further removed from the sensitive bond, at a greatly reduced rate by trypsin. The methyl *m*- and *p*-hydroxphenylpropionates were very slowly split by trypsin, the *meta* compound very slowly by chymotrypsin, and the *para* compound rapidly. Substitution of the amino group for the hydroxy group had little effect on the K_S for chymotrypsin but reduced the k_3 by a factor of 3. An investigation of the susceptibility of the corresponding amides and hydroxamides is in progress. These active substrates, bound presumably at two points to the enzyme rather than three, should prove to be useful in exploring the nature of radiation damage to these enzymes.

[5] T. Inagami and J. M. Sturtevant, *J. Biol. Chem.* 235, 1019–23 (1960).

STRUCTURE OF GLYCOPROTEINS

A. Bezkorovainy D. G. Doherty

Introduction. – During the investigation of bovine plasma as a source of glycoproteins for structural studies, it became apparent that the procedure developed for the isolation of the bovine M-2 glycoprotein could be equally well applied to the preparation of bovine orosomucoid in quantity.

Results and Discussion. – Ammonium sulfate fractionation followed by column chromatography on a carboxymethyl cellulose ion exchanger readily yielded a pure bovine orosomucoid. The preparation appeared homogeneous on filter paper and on cellulose acetate paper electrophoresis at pH 4.5, 7.0, and 8.6, as well as in the ultracentrifuge at concentrations from 3.2 mg/ml to 10.0 mg/ml, giving an $s_{w,20}$ of 3.2 at infinite dilution. The intrinsic viscosity was 0.076, partial specific volume 0.714, and the molecular weight calculated from this data is 49,000. Its chemical constitution was compared with that of human orosomucoid, and only minor differences in amino acid, hexose, and hexosamine were found. Its sialic acid content, however, was somewhat higher than that of the human preparation. This procedure is a rapid simple method for preparing relatively large quantities of homogeneous bovine orosomucoid.

Enzymology

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STUDIES ON PLASMA LACTIC DEHYDROGENASE IN MICE WITH MYELOID LEUKEMIA

K. B. Jacobson Kazuo Nishio[8] V. K. Jenkins[8]

Introduction. – A correlation between elevated lactic dehydrogenase (LDH) activity in blood plasma and the growth of a tumor in a mammal has been reported, and a similar correlation exists for a radiation-induced myeloid leukemia as well.[9] We demonstrated a correlation between the extent of invasion of the

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[8]Pathology and Physiology Group.

[9]K. Nishio, K. B. Jacobson, and V. K. Jenkins, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 94.

liver by the tumor cells and the LDH level. The LDH level was abnormally high when the liver was extensively infiltrated.

Results and Discussion. – From the hypothesis that the elevated LDH activity resulted from a release of this enzyme as well as other components from the displaced liver cells, the possible identity of the elevated plasma LDH was sought. Although initial experiments did not differentiate liver LDH from that appearing in the plasma,[9] succeeding experiments were able to show such a difference.

The characteristics of the LDH in the plasma of a tumor-bearing animal clearly were not similar to those from the normal liver or spleen but most closely resembled those from leukemic spleen and leukemic liver. The latter are composed largely of tumor cells, the spleen to the extent of approximately 85%, and the livers, 30 to 80%. Thus it would seem that the LDH appearing in the plasma is produced by the tumor cells. The foremost question at present concerns the factor(s) that influence tumor cells to release large amounts of this enzyme, although very little information is available concerning such control mechanisms.

HORMONAL CONTROL OF ENZYME SYNTHESIS

F. T. Kenney

Introduction. – Our previous studies of the induction of tyrosine-α-ketoglutarate transaminase of rat liver have established (1) that induction is mediated by adrenocortical hormone,[10] and (2) that increased enzyme activity reflects enzyme synthesized *de novo* during induction.[11] The present experiments were undertaken to ascertain whether this represents a "true" induction, in the sense that inducer actually stimulates the synthesis of this particular protein.

Results. – All experiments utilized animals adrenalectomized 2 to 5 days prior to the experiment, to ensure that noninduced controls remained deficient in adrenal steroids. Induction was accomplished by intraperitoneal administration of hydrocortisone, 10 mg per 100 g body weight. Induced and noninduced animals were given C^{14}-leucine in three injections, 1½ hr apart. After 4½ hr, labeling of the total soluble proteins and of the transaminase (specifically precipitated by anti-transaminase) was compared in the two experimental conditions.

In induced animals, transaminase activity had increased 25-fold during the labeling period, and specific radioactivity of the enzyme was 5 to 10 times greater than that of the total soluble proteins. This then reflects the radioactivity of the enzyme when all of it has been synthesized during the experimental period. It was surprising to find that in noninduced controls, wherein there had been no net increase in enzyme, radioactivity of the enzyme after 4½ hr was equal to that in the induced condition. This result reflects an exceptionally rapid turnover of this enzyme in the noninduced, steady-state condition, a turnover sufficiently rapid that all the enzyme is renewed in 4½ hr.

[10] F. T. Kenney and R. W. Flora, *J. Biol. Chem.* **236**, 2699 (1961).

[11] F. T. Kenney, *Journal of Biological Chemistry* (in press).

It is possible, then, that induction simply blocks the degradative reaction, permitting the newly synthesized enzyme to accumulate. However, induction results in a 25-fold increase in $4\frac{1}{2}$ hr. If this reflects the true rate of enzyme synthesis, unobscured by concurrent degradation, maintenance of the steady-state level in the noninduced condition would require that all the enzyme be renewed approximately each 11 min. This possibility was investigated by following the time course of enzyme labeling after a single injection of C^{14}-leucine in noninduced animals. Measurements of the labeling of total soluble proteins showed that labeling continued at a steadily decreasing rate for 1 hr and then stopped, indicating that the pool of precursor leucine was unlabeled after 1 hr. Isotope incorporation into the enzyme continued to increase throughout this period, which is evidence that the time for renewal of the enzyme is greater than 1 hr. The enzyme then lost radioactivity as the labeled portion was replaced by that newly synthesized from unlabeled precursors.

While measurements of the rate of loss of radioactivity are as yet incomplete, the available data permit the conclusion that the half-life of this enzyme in the steady-state condition is greater than 1 hr, and probably lies between 2 and 3. It is thus apparent that induction cannot be explained as an inhibition of the enzyme degradation and therefore must represent an increase in the rate of transaminase synthesis.

Discussion. – Since the synthesis of a specific protein is stimulated, the hormonal action must be at a stage in the assembly of the protein wherein specificity is directed upon the synthetic mechanism. This suggests that the mechanism of hormonal action in this instance, at least, may be by gene activation. Experiments designed to test this possibility are in progress.

STUDIES ON PROTEIN SYNTHESIS BY CELL-FREE PREPARATIONS FROM MAIZE

Rusty J. Mans

Introduction. – The cell-free amino acid incorporating system isolated from maize seedlings[12] is being used to investigate several aspects of protein biosynthesis in higher plants. The system is well defined as to requirements: It is very active in incorporating amino acids, moderately stable upon storage, and consistently reproducible. The system should be amenable to investigation of the following questions concerning the mechanism and control of protein biosynthesis in higher plants:

1. What is the nature and what are the requirements for the formation of soluble protein from particle-bound protein?
2. What is the nature of the stimulation of incorporation by DNA?
3. What is responsible for the rapid decline in incorporating activity with maturation of a developing seedling?

Results and Discussion. – The lack of stability of the supernatant fraction has been correlated with a relatively high level of ribonuclease activity in preparations from the entire stem of the germinated

[12] R. J. Mans and G. D. Novelli, *Biochim. et. Biophys. Acta* 50, 287 (1961).

seed. By a more judicious dissection of the seedling, that is, removal of the plumule to the exclusion of the scutellar node, the stability of the supernatant fraction is considerably enhanced, the activity is increased twofold, and the ribonuclease level is decreased about 75%.

The stabilization of the supernatant permitted the use of a Sephadex G-25 column to remove endogenous amino acids as well as other low-molecular-weight components from this fraction. When combined with washed particles in the incorporating system, a complex mixture of amino acids is required for the incorporation of a single labeled amino acid into protein.

The aerosol procedure of E. Volkin was used to isolate DNA from developing maize kernels and maize seedlings. This material, when included in the incubation mixture, results in a 30% increase in incorporation. The effect depends on the inclusion of UTP and CTP as well as ATP and GTP. Furthermore, the effect is reversed by addition of deoxyribonuclease. However, the stimulation of incorporation is not specific for maize DNA since DNA prepared from other sources is at least as active as the maize material. The nature of the DNA effect is being investigated, as is the role of the four ribonucleotides in potentiating the effect.

Studies are being initiated to discern what is responsible for the rapid decline in incorporating activity with maturation of the developing seedling. The approach is similar to that used with the developing endosperm.[13] However, more rigorous examination of the various components now known to be involved in the sequence of events leading to incorporation of an amino acid into protein will be made. It is likely that these studies will yield some insight into the mechanisms controlling protein synthesis in the maturing plant.

With the involvement of DNA in the maize incorporating system, it seems propitious to bring together this work and the findings of Schwartz[14] of a genetically controlled esterase in maize. Preliminary experiments, performed jointly, demonstrate that (1) the specific esterase is present in the biochemical preparations used in the incorporating system and (2) Schwartz's electrophoretic assay is of sufficient resolution and sensitivity as to make feasible a cell-free study of the biosynthesis of this specific enzyme.

A major limitation in pursuing the studies outlined above is the difficulty in obtaining large amounts of plant material required for biochemical preparations. Efforts are now being directed toward scaling up the laboratory methods to pilot-plant size analogous to that of J. Bonner's group in making similar preparations from pea seedlings.[15]

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[13] R. Rabson, R. J. Mans, and G. D. Novelli, *Arch. Biochem. Biophys.* **93**, 555 (1961).

[14] D. Schwartz, *Genetics* **45**, 1419 (1960).

[15] J. Bonner, R. C. Huang, and N. Maheshwari, *Proc. Natl. Acad. Sci. U.S.* **47**, 1548 (1961).

PROPERTIES OF ADENOSINE 5′-PHOSPHOSULFATE REDUCTASE

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

Introduction. – Adenosine 5′-phosphosulfate (APS) reductase, an enzyme found in the *Thiobacilli* and "sulfate-reducing bacteria," catalyzes the reversible oxidation of sulfite (in the presence of AMP) to APS as follows:

$$AMP + SO_3^{2-} \rightleftharpoons APS + 2e.$$

The enzyme catalyzing this reaction appeared to be a flavoprotein, and a portion of the flavin in the enzyme preparation was reduced by sulfite in the absence of added AMP. This latter observation suggested that an "enzyme-sulfate" or "cofactor-sulfate" may be an intermediate in the reaction. The oxidation represents a new mechanism for the conversion of chemical energy into biologically utilizable energy, and the mechanism of action of APS-reductase is being studied in detail.

Results. – The enzyme, enriched 75-fold, exhibits a yellow color that can be bleached upon the addition of sulfite, even after extensive treatments with charcoal and resins to remove AMP. The difference spectrum of the oxidized and reduced enzyme has typical flavin maxima at 385 and 440 mμ. Upon boiling or precipitation with acid, the amount of flavin dissociated from the enzyme is equal to the amount of flavin reduced by sulfite, and the released flavin has been identified as FAD by chromatography and by activity in the *D*-amino acid oxidase assay. The involvement of flavin in the enzyme is also indicated by the observation that the ratio of enzyme activity, when measured in the presence of β-mercaptoethanol (2×10^{-4} *M*), to flavin reduced by sulfite has been constant in the enzyme preparations tested. All attempts to resolve the enzyme for flavin have been unsuccessful.

Attempts have been made to demonstrate the formation of an S^{35}-labeled enzyme or cofactor, and, although protein in the enzyme preparation is labeled by S^{35} sulfite, the extent of labeling is not related to activity but rather to the amount of protein in the preparation. However, other experiments with S^{35} sulfite indicate that there may be an intermediate labeled with S^{35} in this oxidation.

Discussion. – APS reductase seems to be a flavoprotein, but final proof of this must await resolution of the enzyme. The "active site" of the enzyme does not appear to involve a free sulfhydryl group, and it has not been possible to detect S^{35}-labeled flavin. Studies with inhibitors have suggested that an additional cofactor may be present in the enzyme, and this aspect is now being investigated.

RNA METABOLISM DURING ANTIBODY SYNTHESIS

C. J. Wust L. Astrachan[16]

Introduction. – One school of thought regarding antibody synthesis suggests that information pertaining to specificity in the combining portion of an antibody is introduced into a cell by the antigen. The

[16]Nucleic Acid Enzymology Group.

antibody (serum globulin) is synthesized by the immunologically responsive cell influenced by the antigen, which connotes specificity, and by the protein-synthesizing mechanism, which is determined genetically. Studies on the cell-free synthesis of protein have implicated a specific RNA, called "messenger RNA,"[17] which is formed under the direction of DNA. The specificity conveyed by antigen would seem to alter the message directly or alter the combination of messenger with ribonucleoprotein particles, the site of protein synthesis. Examination of nucleotide composition was undertaken to determine if the antigen did effect a change in the RNA during the immune response either quantitatively or qualitatively.

Results and Discussion. – A number of $C3BF_1$/Cum mice were immunized with an enzyme, triose phosphate dehydrogenase, crystallized from rabbit muscle. One and two days after the last injection each of the animals was given 100 μc of P^{32}-orthophosphate. After 2 hr the animals were killed, their livers and spleens were removed, weighed, and minced in perchloric acid. The acid-insoluble material was extracted with hot salt and subjected to alkaline hydrolysis by the method of Volkin and Astrachan.[18] Analysis of the hydrolysate on Dowex-1 revealed a DNA with an (A +T)/(G +C) ratio of 1.55 and an RNA with an (A + U)/(G + C) ratio of 0.59, with no apparent differences between immunized and control animals. The total RNA was slightly increased in immunized animals, but qualitatively it was the same as in controls. In immunized animals, RNA was present at a level of 16.5 μmoles per gram of spleen, while the level was 15.0 μmoles per gram in controls. The DNA level was 35.4 μmoles per gram of spleen in immunized mice and 47.2 μmoles per gram in controls. The radiospecific activity (counts/min per μmole) was increasingly higher in the first and second day after immunization. In the nucleotide examination, controls had a specific activity of 133 to 150; first day after last injection of antigen, 194 to 220; and, second day after last injection, 305 to 374. It was apparent that the RNA was quantitatively increased during the immune response but qualitatively the same as in controls.

An examination of the RNA in subcellular fractions was made. A number of $C3BF_1$/Cum mice injected intravenously with sheep erythrocytes were given P^{32}-orthophosphate intraperitoneally on the second and sixth days after injection. Control animals were given P^{32} only. (This antigen elicits serum antibody on the second day in the primary response.[19]) The animals were killed after 1.5 hr, and the spleens were excised, homogenized, and fractionated in a Tris, pH 8.1, sucrose, Mg^{2+}, K^+ medium. RNA was isolated from 12 subcellular fractions that included chromatin, nuclear ribosomes, microsomes, mitochondria, and soluble phases. RNA extracted by phenol treatment from ribosomes, microsomes, and desoxycholate-solubilized material from microsomes were subjected to centrifugation in a 3 to 20% sucrose gradient. Such treatment yielded 4-8S, 16S, and 23S subfractions in which the 4-8S had a specific activity 1.5 times that of the other fractions. The activity of these subfractions of 2-day immunized spleens was 1.5 to 2 times higher than that of control and 6-day immunized spleens. Desoxycholate released high-specific-activity 4-8S RNA. Nucleic acids were purified from the chromatin fraction

[17] J. M. Eisenstadt and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 130.

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

[19] C. C. Congdon and T. Makinodan, *Am. J. Pathol.* 39, 697–709 (1961).

and examined in CsCl equilibrium centrifugation. Each bimodal peak of DNA observed had specific activity but no apparent indication of a hybrid RNA.[20] Mononucleotide composition of all these subfractions was examined after alkaline hydrolysis. Nuclear soluble, cytoplasmic, nucleolar, and microsome 4-8S RNA had specific activities 2 to 3 times higher than other RNA from other subfractions. The 2-day immunized RNA was found to have higher specific activities throughout, with the exception of cytoplasmic soluble RNA, which was very high in controls as well. Again, there were quantitative increases but no apparent qualitative change. In general, the nucleotide composition in terms of (A + U)/(G +C) specific activity ratios was 0.95 to 1.17. However, nuclear soluble RNA had a ratio of 1.34 to 1.45, and cytoplasmic soluble RNA had a ratio of 0.56 to 0.8. This qualitative difference was noted in controls and immunized mice.

The effect of these fractions in an amino acid-incorporating system comprised of a spleen ribonucleoprotein particle and a liver pH 5.0 fraction is being studied with particular attention to soluble, nucleolar, chromatin, and desoxycholate-soluble RNA. These fractions inhibit the incorporating system in initial experiments. They have been deproteinized and are being examined in the role they play in enhancing amino acid incorporation and synthesis of antibody.

THE ROLE OF RNA IN THE SYNTHESIS OF β-GALACTOSIDASE

J. M. Eisenstadt G. D. Novelli

Introduction. – Initial experiments with cell-free preparations from cells of *Escherichia coli* showed that synthesis of β-galactosidase was sensitive to DNase treatment. UV and x-irradiation inhibition studies had indicated that the DNA was the sensitive component of the synthesizing system. Only DNA prepared from cells containing the genetic information necessary for the synthesis of β-galactosidase is effective in reversing the irradiation damage.[21]

The observations, together with the absolute requirement for nucleoside di- and triphosphates, suggested that synthesis of RNA may be necessary for enzyme formation and are consistent with the idea that a separate RNA species may be directly involved in the synthesis of protein.[22]

Recently an enzyme called RNA polymerase has been described[23] that carries out a DNA-dependent synthesis of RNA. The RNA species has a base composition reflecting the base composition of the primary DNA. These studies suggest that the synthesis of an RNA containing the information for the amino acid sequence of enzyme protein may be necessary for enzyme formation.

Results and Discussion. – RNA polymerase was prepared from cells of *E. coli* by the method of Hurwitz[24] and tested in the β-galactosidase synthesizing system.

[20] M. Hayashi and S. Spiegelman, *Proc. Natl. Acad. Sci. U.S.* **47**, 1564 (1961).

[21] J. M. Eisenstadt and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 130.

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

[23] J. Hurwitz, A. Bressler, and R. Diringer, *Biochem. Biophys. Research Communs.* 3, 15 (1960).

[24] J. Hurwitz, personal communication, 1961.

The experiments to be described involve the use of a three-part reaction. In the first step are combined purified RNA polymerase, DNA extracted from cells induced to form β-galactosidase, the inducer, and the nucleoside triphosphates. After a 20-min incubation there is a 10-min DNase treatment to destroy the DNA added initially. After the preceding incubations, the reaction mixtures were added to the β-galactosidase-synthesizing system composed of the 100,000 × *g* supernatant and particles, amino acids, and a source of energy. It is during this final incubation that the experimental data are obtained.

The results indicate that the synthesis of RNA is involved in the cell-free formation of β-galactosidase. If RNase is included during the RNA polymerase incubation, little or no enzyme synthesis is observed upon subsequent incubation with the protein synthesizing system. Similar results are obtained if either DNA, RNA polymerase, or the nucleoside triphosphates are eliminated from the initial incubation, followed by DNase treatment. However, if the RNA polymerase is allowed to act for 20 min and then DNase is used to destroy the DNA, the product of the DNA polymerase is capable of evoking the synthesis of β-galactosidase. If the DNase treatment is omitted after RNA polymerase incubation, enzyme synthesis is even better. Kinetic measurements indicate that, in the presence of RNA polymerase, enzyme synthesis proceeds linearly for at least 45 min. These experiments suggest that the RNA synthesized by RNA polymerase in the presence of preinduced DNA does indeed contain information and further that the information can be transmitted to the protein-synthesizing system.

These studies are being continued in order to delineate the role of the inducer in the synthesis of β-galactosidase. It is hoped the work will lead to an understanding of the control mechanisms of protein synthesis.

FRACTIONATION PROCEDURES APPLICABLE TO CELL-FREE β-GALACTOSIDASE SYSTEMS

W. R. Finnerty G. D. Novelli

Introduction. – A study was initiated to evaluate fractionation procedures and the corresponding effect on cell-free β-galactosidase synthesis. The fractionation techniques used were streptomycin precipitation, pH, DEAE column chromatography, and ammonium sulfate precipitation. The organism used in all studies was *Escherichia coli* ML 308.

Results. – *Streptomycin Precipitation.* – Supernatants prepared by high speed centrifugations were treated with 0.1 *M* streptomycin sulfate.[25] The precipitate was separated into salt-soluble and water-soluble fractions. Using either DNA purified from whole cells or the salt-soluble fraction, significant increases (100–300%) in cell-free β-galactosidase could be observed. RNA prepared from whole cells plus purified DNA also resulted in the stimulation of β-galactosidase activity.

pH Fractionation. – Supernatants adjusted to pH 4.8 yielded a precipitate consisting of 50% of the protein and 90% of the DNA contained in a normal supernatant. The precipitate was separated into salt-soluble and water-soluble fractions and all fractions adjusted to pH 7.2. Increases in cell-free β-galactosidase activities were observed, ranging from 100 to 400%. The salt-soluble fraction was an absolute

[25] S. Cohn and J. Lichtenstein, *J. Biol. Chem.* 235, PC 55 (1960).

requirement, while the water-soluble fraction in high concentrations exhibited inhibition of β-galactosidase activity. This phenomenon was also characteristic of the water-soluble fraction obtained by streptomycin precipitation.

DEAE Chromatography. – A supernatant was fractionated on a DEAE column with a linear salt gradient. Three fractions were obtained, the second containing all the β-galactosidase activity. By combining fractions I and III, ribosomes, and purified DNA, increases in cell-free β-galactosidase activities were noted.

Ammonium Sulfate Precipitation. – Supernatants were precipitated to 0.8% of saturation, using saturated ammonium sulfate. The precipitate dissolved in buffer and then exhaustively dialyzed, does not show appreciable increases in cell-free β-galactosidase activities.

SUBMERGED TISSUE CULTURE OF MAIZE ENDOSPERM

J. E. Graebe G. D. Novelli

Introduction. – The growth of endosperm from *Zea maize* in submerged tissue culture is under investigation. If a sufficiently high growth rate could be obtained, this tissue would make a convenient source of genetically defined, fresh plant material for studies of protein biosynthesis. At present, genetic stocks must be maintained in the field, hand-pollinated, harvested, and stored.

Results and Discussion. – The growth of maize endosperm, derived from a tissue culture which had been maintained on agar for many years, was studied in liquid culture. Various media were tried. The cultures were aerated by shaking or by forced aeration with air-CO_2 or oxygen-CO_2.

The growth obtained in these cultures was not of the desired magnitude.

Attempts are being made to increase the growth rate by frequent transfers of the tissue. Other parts of the maize plant than the endosperm are being considered as possible starting material.

PREPARATION OF TRANSFER RNA

C. L. Robinson G. D. Novelli

Introduction. – One of the main efforts of this laboratory is to study the enzymic mechanisms involved in the synthesis of protein. As a part of this program it became necessary to have a source of purified transfer RNA to study some of the terminal steps in protein synthesis.

Results. – Using the method of Ofengand *et al.*,[26] we have prepared transfer RNA from 200 g of *Escherichia coli* cells. The yield was 10.4 millimoles of RNA nucleotide. The activity of the transfer RNA was tested by determining the extent to which it could be charged with C^{14}-leucine. For this purpose we prepared a partially purified amino acid activating fraction from a supernatant that had been largely freed of nucleic acid by treatment with streptomycin sulfate. In the presence of this enzyme

[26] E. J. Ofengand, M. Diekmann, and P. Berg, *J. Biol. Chem.* **236**, 1741 (1961).

fraction the transfer RNA can be charged with C^{14}-leucine to the extent of 1 mole of leucine per 25 moles of RNA.

Attempts are in progress to further purify the transfer RNA to obtain a better molar yield of activated leucine. We are also trying to prepare an enzyme fraction that will transfer the leucine from the RNA to the ribosomes.

CELL-FREE SYNTHESIS OF TRYPTOPHAN SYNTHETASE

O. H. Smith G. D. Novelli

Introduction. – The attempts to establish an enzymatic system, from cell-free extracts of *Escherichia coli*, capable of the synthesis of the tryptophan synthetase "A" protein have been continued. As reported previously,[27] preliminary experiments had indicated what appeared to be cell-free synthesis of tryptophan synthetase.

Results and Discussion. – Increases in the enzymatic activity of the "A" protein of tryptophan synthetase have been observed in a number of experiments. However, these apparent increases in activity have been of such an unpredictable and variable nature that it was felt some effort should be made to ascertain the optimal conditions for a number of the reactions believed to participate in protein synthesis before any attempt would be made to measure and isolate newly synthesized "A" protein.

Thus, a number of experiments measuring the incorporation of leucine-C^{14} into subcellular fractions of *E. coli* have been performed. These experiments have been noteworthy in several respects. It was found that the specific activity for amino acid incorporation of particle preparations could be increased some two to three times by several washing procedures. In addition, these washed particles were virtually free of any "A" protein, as measured by enzymatic and immunological assay. A preparation of soluble RNA from *E. coli* has been found to stimulate the incorporation of radioactive amino acids into protein up to 6-fold over control values. This soluble-RNA fraction will be tested for its effect on in vitro enzyme synthesis.

It is now hoped that the problem of measuring small increases in "A" protein enzyme activity may be resolved by the use of washed particles and a supernatant fraction from an *E. coli* mutant in which the genetic region controlling tryptophan synthesis is deleted. Much of the evidence indicates that it is the various nucleic acid components of the cell that control protein specificity; thus, through supplementation of the above particles and supernatant with the proper nucleic acid fractions, enzyme synthesis may be possible. A sample of tritiated indole has been prepared, and this will allow the measurement of small amounts of "A" protein which have been formed.

[27] O. H. Smith and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 135.

THE ROLE OF RIBOSOMES IN PROTEIN SYNTHESIS

W. H. Spell, Jr. G. D. Novelli

Studies on the effect of Mg^{2+} concentration and spermine on the incorporation of leucine-C^{14} were continued.[28] *Escherichia coli* ML-308 was grown in media containing leucine-C^{14} and spermine or no spermine. The ribosomes obtained from the two cultures were analyzed by means of a sucrose density gradient after lowering the Mg^{2+} concentration to 10^{-4} *M*. In low magnesium, 70S particles break down to 50S and 30S, except for a so-called "active fraction," which is stable toward low magnesium concentrations and incorporates amino acids rapidly.[29] The results from this experiment indicated that there was an effect of spermine on the specific activity of the 70S particles. The specific activity of the 70S particles from cells grown in spermine was at least twice that of 70S particles from cells without spermine. However, attempts to repeat these observations were unsuccessful.

RNA Polymerase. – RNA polymerase was prepared according to the method of Hurwitz.[30] A study of the enzyme assay is being made. Ribonucleotides labeled with P^{32} were prepared for use as substrates. Tissières[31] has shown that a 16S RNA, which is presumably messenger RNA, is rapidly formed in the cell-free system. We hope to synthesize the 16S RNA with all nucleotides labeled and subsequently study the disposition of messenger RNA during the cell-free synthesis of protein.

AMINO ACID INCORPORATION BY CELL-FREE EXTRACTS OF *SACCHAROMYCES FRAGILIS*

A. K. Williams G. D. Novelli

Introduction. – Attempts by various investigators to obtain cell-free systems from yeast that are capable of incorporating radioactive amino acids into protein have for the most part been unsuccessful. The desirability of such a system lies in the availability of large quantities of biological material and in the versatility resulting from the numerous mutant strains of the organisms which are obtainable.

We have been successful in isolating from *Saccharmoyces fragilis* a cell-free system that is capable of incorporating radioactive leucine into acid-insoluble protein.

Results and Discussion. – Log phase cells of *S. fragilis* are broken by shaking 10 min with two volumes of glass beads in a "Buhler-Vibrogen-Zellmühle." The cell juice so obtained is centrifuged at 10,000 × *g* for 30 min to remove residual cells and cellular debris. The ribosomes are collected by centrifugation at 105,000 × *g* for 90 min. The supernatant is dialyzed and the ribosomes are washed by another cycle of centrifugation. The ribosomes will incorporate amino acids into protein when supplemented with the dialyzed supernatant and a source of energy. The incorporation is inhibited by

[28] W. H. Spell, Jr., and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 136.

[29] A. Tissières, D. Schlessinger, and F. Gros, *Proc. Natl. Acad. Sci. U.S.* **46**, 1450 (1960).

[30] J. Hurwitz, private communication.

[31] A. Tissières and J. W. Hopkins, *Proc. Natl. Acad. Sci. U.S.* **47**, 2015 (1961).

RNase, but not by DNase or chloramphenicol. The addition of a mixture of amino acids and/or a mixture of nucleotide di- and triphosphates has essentially no effect on the incorporation.

The kinetics of the reaction are rapid, being complete in about 10 min. Eighty percent of the incorporated radioactivity remains associated with the particulate fraction, while 20% is found in the acid-insoluble fraction of the supernatant.

In many respects the incorporating system from *S. fragilis* is similar to those from mammalian liver and plant tissue.

The short time course of the reaction as well as the lability of the system on storage suggests that RNase may be a contaminant of the preparation. Efforts are being made to stabilize the system and to achieve conditions that will permit a study of DNA involvement in protein synthesis.

STUDIES ON THE FORMIC HYDROGENLYASE OF *DESULFOVIBRIO DESULFURICANS*

Joy P. Williams H. D. Peck, Jr.

Introduction. – Cell-free extracts of *Desulfovibrio desulfuricans* decompose formate to H_2 and CO_2, and the multienzyme complex catalyzing this reaction has been termed formic hydrogenlyase. A cytochrome, cytochrome c_3, has been shown to be required for formic hydrogenlyase activity in these extracts, as well as in sulfate reduction. It was, therefore, of interest to ascertain whether the formic hydrogenlyase of *D. desulfuricans* was similar to the formic hydrogenlyase system from coli-aerogenes organisms since cytochromes have not been shown to be required for formic hydrogenlyase activity in coli-aerogenes organisms.

Results and Discussion. – We have confirmed the observations of Ishimoto *et al.*[32] that indicated cytochrome c_3 was a component of this formic hydrogenlyase in which the cytochrome apparently functions in the transfer of electrons between formic dehydrogenase (assayed with methylene blue and benzyl viologen) and hydrogenase. Since cytochrome c_3 appeared to function like the X factor that has been postulated to couple formic dehydrogenase and hydrogenase in other systems, the coupling activity of cytochrome c_3 in various formic hydrogenlyase systems was investigated. Cytochrome c_3 did not stimulate hydrogenlyase activity in extracts of *Escherichia coli* that were treated with Amberlite IRC-50 to remove cytochrome, in extracts of a hemin-deficient *E. coli*, or in a system composed of formic dehydrogenase plus a hydrogenase derived from *Clostridium butylicum*. In addition, extracts from two coli-aerogenes bacteria that exhibited either formic dehydrogenase or hydrogenase activity (but not formic hydrogenlyase unless combined) did not produce H_2 and CO_2 from formate when added singly to extracts of *D. desulfuricans* from which cytochrome c_3 had been removed. Extracts of *D. desulfuricans* did not inhibit the formic hydrogenlyase activity observed when the extracts of the coli-aerogenes bacteria were combined. The observations indicate that the formic hydrogenlyase complex of coli-aerogenes bacteria and that of *D. desulfuricans* are not identical, and the possible points of difference are under investigation.

[32] M. Ishimoto *et al.*, *J. Biochem. (Tokyo)* 44, 707 (1957).

Microbiology

Enzyme and Tracer Studies on Bacterial Metabolism

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PRIMARY REACTIONS IN BACTERIAL PHOTOSYNTHESIS

R. K. Clayton

The chromatophores (subcellular particles) of photosynthetic bacteria contain several hundred bacteriochlorophyll (BC) molecules and smaller numbers of accessory substances, notably electron-transport enzymes. When light is absorbed by this system, cytochrome becomes oxidized and pyridine nucleotide is reduced. ATP can be formed when the cytochrome is again reduced by electron-donating substrates. In this way the chemical energy and reducing power needed for photosynthetic metabolism are generated.

Work in this laboratory has been aimed at elucidating the mechanism by which light energy, absorbed in a set of BC molecules, is channeled to the sites of electron-transport enzymes and there promotes a transfer of electrons from cytochrome to pyridine nucleotide. It has been established in other laboratories that the electron-donor reacting directly with the chlorophyll unit is a cytochrome. In green plants the cytochrome appears to be associated with a special form of chlorophyll (P700), forming a reactive center to which excitation energy is channeled. Evidence for a comparable system in the BC unit of photosynthetic bacteria has heretofore been lacking.

The past 6 months have seen a continuation of earlier work that was done in collaboration with W. Arnold.[3] In the earlier investigation, reversible light-induced absorbancy changes were observed in bacterial chromatophores (suspended in water or dried as films on glass plates) at temperatures from 300° to

[1] Research participant.

[2] Visiting investigator from abroad.

[3] W. Arnold and R. K. Clayton, *Proc. Natl. Acad. Sci. U.S.* **46**, 769 (1960).

1°K. The changes corresponded to an alteration of the BC. Their kinetics were indicative of photoionization, followed by recombination of electrons and holes in the BC unit. It was suggested that photosynthesis begins with ionization in a BC unit having properties of an organic semiconductor, the separate electrons and holes finding their way to appropriate electron-acceptor and donor enzymes.

The current work has established that the spectrum of light-induced absorbancy changes consists mainly of five features: (1) oxidation of a cytochrome (in sufficiently dark-adapted preparations, (2) bleaching and red-shift of carotenoid absorption bands, (3) bleaching of the long-wave band of BC (870 to 890 mμ), (4) blue shift of the BC bands at 375, 590, and 800 mμ, and (5) appearance of absorption bands at 435, 980, 1140, and 1250 mμ. Chemical oxidation causes almost the same absorbancy changes as light; the correspondence is especially striking from 700 to 1300 mμ.

The absorption spectrum of BC *in vivo* is characterized by bands at 375, 590, 800, 850, and 870–890 mμ. The relative heights of the bands at 800, 850, and 870–890 mμ show great variation in different bacterial phenotypes, and the variation can be exaggerated by such treatments as drying and exposure to detergents. The spectra of absorbancy changes, caused either by light or oxidation, remained remarkably constant throughout these variations in the BC absorption spectrum. It was concluded that the absorbancy changes associated with BC reflect the gross alteration of a special BC component in the chromatophore (SpBC) rather than a small change in the spectrum of most of the BC molecules. It follows that if these changes arise from ionization, the ionization occurs at the site of SpBC and not necessarily elsewhere.

The quantum requirement for the light-induced alteration of SpBC was determined to be about 2 quanta per molecule altered. This figure agrees with the quantum requirements for the oxidation of cytochrome[4] and the reduction of the pyridine nucleotide[5] in photosynthetic bacteria. Furthermore, the concentration of both the SpBC and the light-reacting cytochrome in photosynthetic bacteria is about 3 molecules per 100 molecules of "ordinary" BC.

Finally, it was shown that the light-induced reactions of SpBC and cytochrome are intimately related – in dark-adapted preparations, with the cytochrome reduced and thus ready to donate electrons to the BC, the charge of SpBC is attenuated and its onset is delayed while the cytochrome is becoming oxidized.

These findings provide evidence that the BC unit in photosynthetic bacteria possesses a reactive center, consisting of SpBC in association with cytochrome, where electronic excitation energy promotes an electron-transfer process. As a working hypothesis it is proposed that excitons, arriving at the SpBC site, are dissociated into electrons and holes which are captured by electron-handling enzymes (the holes are accepted by a cytochrome).

[4]B. Chance and M. Nishimura, *Proc. Natl. Acad. Sci. U.S.* **46**, 19 (1960).

[5]L. N. M. Duysens and J. Amesz, *Plant Physiol.* **34**, 210 (1959).

SOME NEW PHENOTYPES OF *RHODOPSEUDOMONAS SPHEROIDES*

R. K. Clayton R. P. Feldman Carolmarie Smith

Earlier work in this Laboratory[6] yielded several classes of mutants of *Rhodopseudomonas spheroides:* (1) forms showing hyperproduction of catalase, (2) forms lacking bacteriochlorophyll (BC), and (3) forms having abnormal carotenoid pigmentation. The third class includes types that accumulate (*a*) neurosporene, (*b*) phytoene and phytofluene, (*c*) phytoene only, and (*d*) no identifiable polyene. These types demonstrate a block at almost every step of the currently accepted pathway for carotenoid biosynthesis (precursors → phytoene →→→ spheroidenone). The missing link has been a mutant that accumulates zeta carotene; a phenotype has now been isolated that accumulates phytoene, phytofluene, and zeta carotene. The wild-type carotenoids prevent photo-oxidative injury sensitized by BC; they also cause a modification of the *in vivo* absorption spectrum of BC. Photoene, phytofluene, and zeta carotene appear not to show these signs of interaction with BC.

The traits listed above as (1), (2), and (3) have been combined in double mutants. Auxotrophic mutants incorporating these traits are now being sought to provide material advantageous for genetic recombination studies. Two types of auxotroph have been isolated, one requiring histidine and the other apparently requiring a growth factor. These auxotrophs have in turn yielded forms lacking BC or carotenoids.

POLYNUCLEOTIDE PHOSPHORYLASE OF *CLOSTRIDIUM PERFRINGENS*

M. I. Dolin

Polyadenylate synthesis, as catalyzed by purified polynucleotide phosphorylase of *Clostridium perfringens*, takes place only in the presence of a polybase such as polylysine.[7] This behavior suggested that there might exist naturally occurring basic activators of the enzyme or that the native polynucleotide phosphorylase might be bound to a basic protein carried by a particulate element of the cell. By methods similar to those used for the isolation of histones or protamines, it was shown that acid-soluble enzyme preparations can be obtained from *C. perfringens* homogenates. The latter, however, are insoluble at neutral pH and, furthermore, do not activate polyadenylate synthesis when tested with the purified phosphorylase. To date, no strongly basic activators have been found in extracts of *C. perfringens*.

With regard to the second possibility, it has been found that approximately 10% of the polynucleotide phosphorylase activity (polyadenylate synthesis) of *C. perfringens* is present in a particulate form. The particulate form of the enzyme is almost equally divided among particles that sediment at 10,000 × *g*, 33,000 × *g*, and 100,000 × *g*. This activity cannot be accounted for by occlusion of the soluble phosphorylase; however, after five washes with Tris-Mg buffer, most of the activity can be removed from the particle. The particulate form of the enzyme has one of the properties expected of an "activated" form of the enzyme, namely the K_s for ADP is lower than that found for the soluble enzyme and approaches

[6] R. K. Clayton and C. Smith, *Biochem. Biophys. Research Communs.* 3, 143 (1960).

[7] M. I. Dolin, *Biochem. Biophys. Research Communs.* 6, 11 (1961).

that found for the soluble enzyme activated by polylysine. However, the fact that only a small proportion of the activity is present in a particulate form, coupled with the finding that this activity can be removed on repeated washing, makes it difficult to assess the importance of the bound form of the enzyme.

Preliminary experiments with a synthetic mixed polypeptide as activator suggest that polybases do not act as templates for the synthesis of specific polyribonucleotides. The experiments do not rule out the possibility that polylysine may serve as a template for polyadenylate synthesis.

DPNH OXIDASE OF *CLOSTRIDIUM PERFRINGENS*

M. I. Dolin

Previous work demonstrated that *Clostridium perfringens* contains a flavoprotein that catalyzes the rapid reduction of molecular oxygen to water, with DPNH as electron donor.[8] On storage or ultraviolet irradiation of the enzyme, the oxidase is converted to a form which is virtually devoid of oxidase activity, but which retains the cytochrome reductase activity of the original enzyme.[8] Growth conditions have now been devised which result in high yields of the altered form of the enzyme. Preliminary characterization of this enzyme suggests that it is very similar to the cytochrome reductase formed *in vitro* from the oxidase. The nature of this conversion should throw some light on the characteristics of the native four-electron-transfer enzyme.

STRAIN DIFFERENCES IN METHYLMALONYL ISOMERASE OF *PROPIONIBACTERIA*

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

Introduction. – Numerous differences have been noted in the methylmalonyl isomerase obtained from various strains of *Propionibacteria*. Barker *et al.*[9] have reported dimethylbenzimidazolylcobamide (DMBC) to be the principal B_{12} coenzyme of *P. shermanii*; we have found only adenylcobamide (AC) in *P. pentosaceum*. Swick and Wood[10] purified the enzyme from *P. shermanii* about 20-fold, but we find the *P. pentosaceum* enzyme unstable under similar conditions. We have made a study of the stability and other properties of the enzymes from both strains and have attempted to relate the differences in these properties to the specific coenzymes or apoenzymes.

Results and Discussion. – Heat-stability studies were made on cell extracts of both of the above strains. The temperature at which half of the isomerase activity survives after heating for 3 min was used as an index ($T_{1/2}$). Maximum stability occurs at pH 5.8 in both extracts and at protein concentrations above 1 mg/ml (optimum enzyme activity occurs near pH 7). Table 27 shows stability at various conditions: a drop in $T_{1/2}$ with lowered protein concentration or with rise of pH; a further drop when in the form of the apoenzyme. However, if the apoenzymes are heated to a temperature at which less than

[8] M. I. Dolin, *J. Bacteriol.* **77**, 393 (1959).

[9] H. Weissbach, J. Toohey, and H. A. Barker, *Proc. Natl. Acad. Sci. U.S.* **45**, 521 (1959).

[10] R. W. Swick and H. G. Wood, *Proc. Natl. Acad. Sci. U.S.* **46**, 28 (1960).

10% of the activity remains, the presence of AC furnishes 70 to 90% protection against heating, whereas DMBC is without effect. The coenzyme concentration during heating is that which saturates in the activity assay. No other agents were found that would give significant protection. We did not expect these coenzyme effects because DMBC is the more stable coenzyme and has a 50-fold lower K_m in the bacterial isomerase. Greater heat stability appears to be primarily a property of the apoenzyme (*P. shermanii*), but both apoenzymes can be protected by the same coenzyme (AC).

The enzymes of both strains are sensitive to freezing when the protein concentration is below about 1 mg/ml. No coenzyme effect has been noted, but addition of bovine serum albumin gives considerable protection.

The pH activity curves of the isomerase also were compared in the two strains. Extracts of *P. pentosaceum* show a broad optimum from pH 6 to 7. *P. shermanii* has a sharper optimum above pH 7. However, when we added AC or DMBC to the *P. shermanii* apoenzyme, curves more like that of *P. pentosaceum* extract resulted. Thus, the optimum pH of the enzyme has been lowered in the process of reconstitution from the coenzyme and apoenzyme, but little difference is noted in the effect of the coenzymes.

There is a gradual loss of coenzyme in the purification of the *P. shermanii* enzyme, and the degree of purification is limited by the instability under the conditions of the procedure. However, the above results indicate that proper use of AC and protecting protein may allow further purification.

Table 27. Isomerase Survival Temperatures

Preparation	pH	Protein (mg/ml)	$T_{1/2}$ for Species Shown (°C)	
			P. pentosaceum	*P. shermanii*
Cell Extract	5.8	1.0	55	62
		0.02	49	55
	7	1.0	48	55
Apoenzyme	5.8	0.02	39	50

Plant Physiology and Photosynthesis

Plant Physiology

A. H. Haber
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Helen J. Luippold
H. A. Roesel[3]
Barbara L. Parks[4]

Photosynthesis

W. A. Arnold
W. F. Bertsch

NONESSENTIALITY OF CONCURRENT CELL DIVISIONS FOR DEGREE OF POLARIZATION OF LEAF GROWTH

A. H. Haber W. L. Carrier[5]

Introduction. – In general, when polarized growth is accompanied by cell division, the new cell plates formed during cytokinesis are mostly perpendicular to the axis undergoing greatest relative extension. It is not known, however, what role such "oriented" cell divisions play in morphogenesis (origin of geometric form). During germination and seedling growth, the first foliage leaf of wheat shows polarized growth, with striking changes in shape. Nearly all the cell plates formed during cytokinesis are perpendicular to the longitudinal axis. We have studied leaf length, y, and leaf width, x, in (1) tissues of normal wheat leaves growing with much cell division, and (2) leaves growing with no detectable increase in cell numbers after gamma irradiation (800 kr) of the unsown grain.

Results. – The gamma irradiation, which inhibits subsequent cell division, alters x, y, dx/dt, and dy/dt. However,

$$\frac{\frac{1}{y}(dy/dt)}{\frac{1}{x}(dx/dt)},$$

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[4]Biology student trainee.
[5]From Biophysics Group.

which appears to be constant during growth of these leaves, has a value (4.4) that is unaffected by the radiation treatment. Thus, although the gamma irradiation, which inhibits cell division, alters leaf growth rates and final size, it does not change the degree of polarization of growth, that is, the ratio of the rate of linear extension in length per millimeter of length to the rate of linear extension in width per millimeter of width.

Discussion. – We therefore conclude that the role of cell divisions in determining leaf form at maturity is merely to permit sustained growth, during which shape changes. Since the "oriented" cell divisions do not contribute to the degree of polarization, however, they play no role in the basic mechanism by which growth is polarized and which determines the manner in which leaves change shape.

USE OF GROWTH CHARACTERISTICS IN STUDIES OF MORPHOLOGIC RELATIONS

D. E. Foard A. H. Haber

Introduction. – Interpretations of morphologic relations between plant structures have been based on the mode of origin and the positional relations. Anatomic observations alone, however, have not always resolved such matters. For example, there is disagreement on the nature of the epiblast, a small, scale-like structure found in certain grass embryos. Most investigators have interpreted the epiblast as a modified leaf or leaf part.[6] A few have considered the epiblast an outgrowth of the coleorhiza.[6] We have compared various aspects of growth of the wheat epiblast, coleorhiza, and leaves during germination.

Results. – Although the epiblast and coleorhiza grow without cell division during germination, they sometimes produce hairs that resemble root hairs but are different from leaf hairs in form and mode of origin, as shown by studies using radiation-induced mitotic inhibition. Despite the similar orientation of the epiblast and leaves with respect to the main axis of the plant, during growth the epiblast changes shape such that the axis of greater linear extension per millimeter is perpendicular to the main axis of the plant. Leaves, on the other hand, change shape such that the greater rate of linear extension per millimeter is parallel to the main axis of the plant. Indoleacetic acid treatment produces excessive and disorganized growth of both the epiblast and coleorhiza but retards leaf growth. Gibberellic acid stimulates, whereas (2-chloroethyl)trimethylammonium chloride retards leaf growth. Neither substance significantly affects epiblast or coleorhiza.

Discussion. – Diverse lines of evidence are necessary in studies of morphologic relations. This study illustrates that diverse anatomic evidence may be complemented by such diverse physiologic and morphogenetic characteristics as roles of cell division in (and radiosensitivity of) differentiation of epidermal hairs, orientation of axes of polarized growth, and responses to three different types of growth regulators. The many fundamental similarities between the epiblast and coleorhiza, and the fact that they are contiguous indicate that they are parts of a single, continuous structure. Perhaps the greater significance of this study lies not in the problem of the nature of the epiblast but in suggesting that a wide

[6] W. V. Brown, *Phytomorphology* 10, 215–23 (1960).

variety of growth characteristics may be used to complement classical anatomic approaches in other studies of morphologic relations.

EFFECTS OF VERY HIGH DOSES OF GAMMA RADIATION ON GERMINATION AND GROWTH OF WHEAT

T. J. Long

Introduction. – There is a reversal in the height-dose curve for the first foliage leaf, but not for the coleoptile, after gamma irradiation of air-dry grain.[7] The lengths of the first leaf after 400 to 1000 kr of gamma irradiation, and the coleoptile after 50 to 150 kr, are essentially dose independent.[8] To complete the description of the behavior of irradiated wheat, these studies have been repeated and extended to include root growth and germination percentages.

Results. – The germination percentage of irradiated grain is not reduced by doses less than 400 kr. From 600 to 1000 kr the germination decreases from 90 to 40%. The dose-response curve for root growth does not indicate a reversal, but decreases slightly and steadily with increasing dose from 50 to 1000 kr. After 800 and 1000 kr, the percentage of seedlings without primary root emergence is large (20 to 80%). When embryos are excised from grain given 1000 kr and cultured on a medium of inorganic salts and sucrose, 82% grow, as opposed to 43% if the embryos are left on the endosperm. The percentage of seedlings without primary root emergence is the same in either case (78%). For unirradiated grain 90% grow if left on the endosperm, and 91% of the excised embryos grow, and no seedlings observed lacked root emergence.

Conclusions. – After 600 to 1000 kr, the lower germination percentages may be attributed to radiation damage of the endosperm similar to that reported by Meletti and D'Amato,[9] whereas the lack of root emergence may be due to radiation effects on the embryo itself. Absence of a reversal in the dose-response curve of roots is consistent with the explanation of the reversal advanced by Schwartz and his coworkers.[7]

STUDIES ON GIBBERELLIN SENSITIVITY

Hilde A. Roesel A. H. Haber

Introduction. – It has generally been assumed that gibberellin is most effective for promoting elongation in immature, rapidly elongating plant tissues. We have tested this generalization, using intact wheat coleoptiles in which growth is unequal in different segments.

Results. – Germination and growth of wheat took place in water either in a dark chamber or in a growth room, with a 16-hr photoperiod (~500 ft-c of white light). After 3 days, pairs of plants of identical coleoptile length were selected. Coleoptiles were marked with India ink into three segments. One of each pair was treated with gibberellic acid (GA), the other was the water control. All plants were kept in the

[7] M. Sicard and D. Schwartz, *Radiation Res.* 10, 1–5 (1959).
[8] A. Haber and H. J. Luippold, *Am. J. Botany* 47, 140–44 (1960).
[9] P. Meletti and F. D'Amato, pp 47–55 in *Effects of Ionizing Radiations on Seeds*, IAEA, Vienna, 1961.

growth room after treatment, and segments were remeasured after 24 hr. In light-pretreated coleoptiles, growth was greatest in basal and least in apical segments. In dark-pretreated coleoptiles, on the other hand, growth was greatest in apical and least in basal segments. The relative GA effect (i.e., the ratio of increase in GA to increase in water) was virtually the same for all three segments of coleoptiles that had received the same pretreatment. The overall GA effect, however, was greater after dark than after light pretreatment. This difference cannot simply be attributed to a lesser maturity of etiolated coleoptiles, because dark-pretreated coleoptiles returned to the dark chamber gave the same small relative GA effect as light-pretreated ones.

Discussion. – These results demonstrate that: (1) Gibberellin sensitivity, as measured by relative GA effect, is independent of control growth in this system, in which different parts of the same organ can be compared. The misinterpretation that gibberellin sensitivity is greater in rapidly growing tissues probably arose from consideration of absolute rather than percentage increases in length resulting from gibberellin treatment. (2) Gibberellin sensitivity is not necessarily greater in immature tissues.

DISSIMILAR EFFECTS OF STORAGE ON SEEDS INJURED BY GAMMA IRRADIATION, FREEZING, AND HEAT

Barbara L. Parks A. H. Haber

Introduction. – Inhibition of germination and seedling growth after irradiation of seeds in some instances increases after postirradiation storage.[10] Using decreased germination percentage of lettuce seeds as an index of damage, we compared the effects of storage after gamma irradiation with the effects of storage after seeds were injured by heat or by freezing treatments.

Results. – There is a postirradiation-storage effect on the germination of lettuce after seeds have suffered various extents of injury from gamma irradiation. No such pronounced storage effect was obtained after heat or freezing treatments which initially produced comparable damage, as shown by germination percentages when seeds were sown immediately after injury.

Discussion. – These results indicate that the pronounced storage after-effect of gamma-irradiated seeds is not a typical property of damaged seeds. Neither our freezing nor our heat treatments should produce significant numbers of free radicals,[11] although x-ray doses that produce comparable damage produce relatively large numbers of free radicals.[12] Our results thus provide further evidence in favor of the theory that radiation-induced free radicals play a role in the mechanisms underlying the postirradiation storage effect.

[10] A. D. Conger, *J. Cellular Comp. Physiol.* 58 (suppl 1), 27–32 (1961).
[11] D. J. E. Ingram, *Free Radicals as Studied by Electron Spin Resonance*, Butterworths, London, 1958.
[12] M. L. Randolph and A. H. Haber, pp 57–65 in *Effects of Ionizing Radiations on Seeds*, IAEA, Vienna, 1961.

PHOTOSYNTHESIS

W. A. Arnold W. F. Bertsch

The initial steps in photosynthesis involve conversion of energy from light quanta into some form which is available for utilization in the biochemical reduction of carbon dioxide to sugars. Since eight light quanta are needed to reduce one carbon dioxide molecule and since the absorption cross section for photosynthesis is known to be the size of several hundred chlorophyll molecules, it appears that some type of cooperation exists between chlorophyll molecules in the photosynthetic apparatus.

Experiments at this laboratory showed that the photosynthetic apparatus of higher plants and photosynthetic bacteria, when dried, has electrical properties which are usually associated with organic semiconducting crystals. It was also shown that upon illumination the photosynthetic apparatus (whether dried or in living cells) emits delayed light which has the characteristics of delayed light emission due to untrapping of electrons in a semiconducting crystal. The emission spectrum of this delayed light shows that it is emitted by chlorophyll undergoing a transition between the first excited singlet state and the ground state. The long lifetime of delayed light emission could be explained if absorption of a light quanta results in an excited electron which may travel freely throughout a chlorophyll semiconducting system in a high-energy conduction band. The electron could be trapped, and upon untrapping would have some probability of falling back to the ground state and emitting the long-lifetime delayed light that is observed. The present experiments, involving electron spin resonance and delayed-light studies, are concerned with the mechanism by which high-energy conduction-band electrons might be utilized in photosynthesis.

With cooperation of the Biophysics Group, electron spin resonance studies were inaugurated in the hope of directly detecting unpaired electrons (a conduction-band electron would be unpaired) in living photosynthetic systems. Light caused the production of unpaired electrons in the dried photosynthetic apparatus of higher plants and bacteria, as had been previously reported by other workers. The dark decay of unpaired electrons was similar to the dark decay of delayed light. Certain living leaves were found to possess a small electron spin resonance signal which was not altered by light. The lack of a light effect in the living system may be due to utilization of unpaired electrons in photosynthesis. Poisoning of respiration with cyanide reduced the number of unpaired electrons present in darkness, but no effect of light could be demonstrated. These experiments were temporarily discontinued until the sensitivity of the electron spin resonance equipment for water-containing samples can be improved.

Recent studies of the delayed light emission from living green algae demonstrated that the dark-decay curve of delayed light is dependent on the wavelength of the exciting light. This result implies that the various colors of exciting light are not absorbed by the same photosynthetic pigment system and is consistent with other recent evidence that at least two photoreactions are present in higher-plant photosynthesis.

We have found that simultaneous excitation by wavelengths which cause enhancement (or "Emerson effect") of oxygen evolution results in a reduction of delayed light emission. That is, the intensity of delayed light emitted after excitation with one exciting wavelength is reduced when the plants are excited

by both wavelengths. In the red region of the spectrum this negative enhancement of delayed light has the same action spectrum as the enhancement of oxygen evolution.

Chromatic transients similar to those reported for oxygen evolution have been observed in the delayed light emission of living green algae. Under conditions where both wavelengths of exciting light result in equal amounts of delayed light, a shift from one exciting wavelength to another causes changes in the delayed light emission, which then gradually returns to the steady-state value. The most effective wavelength combinations for producing chromatic transients are the same for oxygen evolution and delayed light emission, and are also the most effective wavelengths for producing the Emerson effect and the negative enhancement of delayed light emission.

We conclude that delayed light is emitted by the two pigment systems, which are responsible for the Emerson effect and chromatic transients in photosynthetic oxygen evolution. Chromatic transients presumably reflect some type of unbalance between the two pigment systems that occurs when one system is excited to a larger degree than the other. The reduction in delayed light emission caused by simultaneous excitation with both wavelengths suggests that when both pigment systems are operative there is a much smaller number of excited electrons available to produce delayed light. Since the two pigment systems may be separated by the use of relatively precise delayed light measurements, we are continuing to investigate the role of the two pigment systems in photosynthesis.

Biophysics

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MODIFICATION OF ULTRAVIOLET-INDUCED CHROMOSOMAL ABERRATION FREQUENCIES IN *TRADESCANTIA* POLLEN

J. S. Kirby-Smith Mitzi G. Miller

Introduction. – The magnitude of the synergistic action of ultraviolet and x radiation on *Tradescantia* pollen chromosomes has been found to be dependent upon the amount of visible radiation received before further radiation treatment. Owing to the complexities of dealing with three different types of radiation in any one experiment, current work aimed at an understanding of these phenomena is temporarily restricted to combinations of ultraviolet with visible or near-visible radiation.

Results and Discussion. – Cromroy[2] observed that pretreatment with visible radiation in the 5000 to 6000 A range decreased the 2650-A ultraviolet-induced aberration frequencies, while 7500 A resulted in an enhancement. These results were interpreted in terms of two independent photochemical processes, the protective action of the 5000- to 6000-A band being ascribed to a mechanism analogous to that for photoprotection in bacteria, and the action of 7500-A radiation to an independent photosensitization. These findings have been confirmed, using pollen grown under controlled conditions of light, temperature, and humidity. In addition, it has been observed that the effects of far-red (7500 A) and red (6000 A) radiation are reversible. Pretreatment with red (6000 A) or far-red (7500 A) + red (6000 A) results in fewer ultraviolet-induced aberrations; with far-red (7500 A) or red (6000 A) + far-red (7500 A) an increased number of aberrations were observed. It thus appears that the original hypothesis assuming two independent processes governing the ultraviolet sensitivity of pollen is untenable. The response observed is quite similar to that reported for seed germination and floral initiation and indicates that a photoreversible plant pigment is responsible for the sensitization of pollen to ultraviolet chromosomal damage and may be the same as that involved in other photobiological responses in plants.

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[1]Visiting investigator from Italian Atomic Energy Commission.

[2]H. L. Cromroy, *Biol. Div. Semiann, Progr. Rept. Aug. 15, 1961*, ORNL-3201, p 151.

ULTRAVIOLET MICROBEAM IRRADIATION OF NUCLEUS AND CYTOPLASM OF *AMOEBA PROTEUS*

John Jagger D. M. Prescott[3]

Introduction. – Many experiments show that division delay and death following treatment of cells with ionizing radiation is due overwhelmingly to nuclear damage.[4] Among the few experiments with ultraviolet radiation (UV), however, several suggest an important role of cytoplasmic damage.[5] With protozoa, experiments in which irradiated cytoplasm is fused to irradiated cells of *Pelomyxa illinoisensis*,[6] as well as experiments on nucleate and enucleate halves of *Amoeba proteus*,[7] illustrate the importance of cytoplasmic damage and suggest a role of intact cytoplasm in recovery of the cell. These studies were, however, done with surgically altered cells and under conditions where some cytoplasmic shielding of the nucleus probably occurred. The experiments, therefore, do not permit an unequivocal decision on the importance of the cytoplasm in damage to intact protozoa by UV.

We have irradiated individual starved cells of *A. proteus* (flattened to 20-μ thickness under a quartz cover slip) with microbeams[8] of polychromatic UV, which could be used to irradiate the nucleus, the cytoplasm, or both. In our experiments, negligible amounts of cytoplasm remained above or below the nucleus, and the cells were normal and intact except for flattening and irradiation. Thus, we can clearly distinguish between nuclear and cytoplasmic damage and can assess their relative roles in UV division delay and killing with precision and certainty.

Results and Conclusions. – Nuclei were irradiated with a microbeam of 25-μ diameter, which is slightly smaller than the diameter of the flattened nucleus. Results are shown in the first line of Table 28. Each number in the table is derived from data on about 150 individual cells. Cytoplasm was irradiated with four nonoverlapping fields of a microbeam of 100-μ diameter, so that about 60% of the total cytoplasmic area was irradiated. In some experiments, the nucleus was included in one of the four large fields. These results are shown in the second and third lines of Table 28. For equal incident energy per unit area delivered to nucleus and cytoplasm, the cytoplasm will receive a much larger total energy because of its larger area. Relative measurements of energy per unit area were made with a photovoltaic UV meter[9] placed on the microscope stage, where it intercepted the entire microbeam.

The data show that irradiation with the same energy per unit area of either the whole nucleus or 60% of the cytoplasm has a roughly equal effect, measured as either division delay or killing, although in this experiment the cytoplasm received about 50 times as much total energy as the nucleus. This means that

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[3] Cell Growth and Reproduction Group leader.

[4] L. H. Gray, pp 255–70 in *Ciba Foundation Symposium on Ionizing Radiation and Cell Metabolism*, ed. by G. E. W. Wolstenholme and M. C. O'Connor, Little, Brown, Boston, 1956.

[5] R. E. Zirkle, *Adv. Biol. Med. Phys.* 5, 103–46 (1957).

[6] E. W. Daniels, *Ann. N.Y. Acad. Sci.* 78, 662 (1959).

[7] D. Mazia and H. I. Hirshfield, *Exptl. Cell Research* 2, 58 (1951).

[8] R. B. Uretz and R. P. Perry, *Rev. Sci. Instr.* 28, 861 (1957).

[9] J. Jagger, *Radiation Research* 14, 394 (1961).

Table 28. Relative Ultraviolet Incident Energies upon Nucleus and Cytoplasm Required for Effects in *A. proteus*

Part Irradiated	Incident Energy for Division Delay[a]		Incident Energy for Killing[b]	
	Per Unit Area	Total	Per Unit Area	Total
Nucleus	8.1	8.1	12.5	12.5
Cytoplasm	7.9	470.0	10.0	600.0
Nucleus plus cytoplasm	1.5	90.0	3.7	220.0

[a]Doubling of the time required to reach the second division.
[b]50% lysis at 14 days.

in whole-cell irradiation, in which the nucleus and cytoplasm are subjected to the same incident energy per unit area, the cell suffers at least as much measurable damage (division delay and killing) through effects on the cytoplasm as it does through effects on the nucleus. These irradiations were done with a mercury spectrum extending from 2200 to 3400 A, and different results might be obtained with single wavelengths. The data further show that when both nucleus and cytoplasm are irradiated, the effect is greater than would be expected on the basis of simple additivity of the nuclear and cytoplasmic damages.

We have begun studies on the biochemical basis of UV damage and have found that, with sublethal irradiation of the nucleus alone (sufficient to cause moderate division delay), incorporation, during the first 12 hr, of tritiated uridine and cytidine into RNA of the nucleus and cytoplasm is reduced to 1% or less of controls.

RECALCULATION OF FAST NEUTRON DOSE TO FLUX RATIOS

M. L. Randolph

Because our original calculation of the ratio of the flux to the absorbed dose of 14-Mev neutrons[10] was made five years ago and has since become something of a standard calculation,[11] it seemed appropriate to bring the calculations up to date with the latest available nuclear data. The results of these recalculations have reproduced the original values of dose contributed per atom of H, C, and O to within less than the previously estimated errors. For nitrogen, the new value is 30% lower than the previous value – just within the previously estimated error. Calculations are being extended to somewhat higher and lower neutron energies.

[10]M. L. Randolph, *Radiation Research* 7, 47 (1957).

[11]*Measurement of Absorbed Dose of Neutrons and of Mixtures of Neutrons and Gamma Rays*, NBS Handbook 75, 1961.

ELECTRON SPIN RESONANCE STUDIES

M. L. Randolph J. S. Kirby-Smith

Introduction. – As a part of our general group purpose to study radiobiological effects rather immediately related to the initiating physical interaction of radiation with matter, for several years we have centered a series of studies about electron spin resonance (ESR) measurements. Partly as a result of improved instrumentation in the last six months, we have been extending the range of our studies to include visible and ultraviolet (UV) radiation resonances induced during ESR measurement at room temperature and at liquid-nitrogen temperature, and microwave power saturation of ESR signals.

Methods. – For the low temperature and *in situ* irradiation studies, the transmission cavity of our spectrometer was replaced by a Varian multipurpose reflection cavity, and the microwave plumbing was converted to a bridge design. The resulting sensitivity is probably somewhat less than for the transmission spectrometer, although the same electronics were used.

Results and Discussion. – Polycrystalline amino acids irradiated with visible and UV light and simultaneously analyzed at room temperature showed no clear ESR signals. At liquid-nitrogen temperatures, signals were seen only in the aromatics, with tryptophan seemingly the most sensitive. The formation of free radicals by UV seems less per unit of absorbed energy than by ionizing radiation, although the possibility of localized saturation effects with the ultraviolet could refute this conclusion. With identical UV irradiation conditions more resonances are seen in the proteins, bovine serum albumin, and salmine than in any amino acid. These data suggest that low-energy excitations directly play little part in the production of free radicals in amino acids by ionizing radiation and that disulfide and/or peptide bonds are involved in a major portion of the free radicals produced by UV irradiation of proteins. In collaboration with the Plant Physiology and Photosynthesis Group, preliminary work has been done on natural and light-induced resonances in chloroplasts, chromatophores, and whole leaves, all at room temperature. In collaboration with L. Ehrenberg and G. Löfroth of the University of Stockholm, intercomparisons have been made of our ESR standards (DPPH samples on loan from R. Livingston of the Chemistry Division, ORNL), a pitch sample characterized by Varian, and three sucrose samples which are being measured at several European laboratories. As a part of this work, microwave power saturation studies were begun at detector power of 1 mw; they showed the necessity for correction factors of about 1.1 for DPPH and pitch and about 2 for sucrose. Significant saturation phenomena were also noticed in the amino acid work at low temperature.

THE FORMATION OF THYMINE DIMERS IN POLYNUCLEOTIDES BY ULTRAVIOLET LIGHT

R. B. Setlow R. A. Deering[12] W. L. Carrier

Introduction. – Ultraviolet light affects biological systems. A knowledge of the compounds affected and the detailed molecular changes produced by UV is necessary to understand the biological effects of

[12] Associated with New Mexico Highlands University.

UV. The most important molecular change known is the formation of dimers of thymine in polynucleotide chains under the action of UV. The formation of dimers changes the reactions between DNA and polymerizing or degradative enzymes.[13]

Results and Discussion. – The formation of dimers may be detected by chemical procedures[13] or by the fact that dimer formation results in an absorbance decrease at 2700 A. We used the latter criterion to detect dimer formation in model polynucleotides, dithymidylic acid, polythymidylic acid, an alternating A,T polymer, and in native and denatured DNA's of different base compositions. The formation of dimers takes place only between *adjacent* thymines in polynucleotide chains. The dimers are formed in chains which are either single or in double helices. The number of dimers formed is proportional to the number of T,T sequences in the polynucleotide. Dimer formation is reversible by light. The reaction in

$$T,T \rightleftharpoons TT$$

polynucleotides goes to the right with long-wavelength irradiation, and as for the free dimer,[14] to the left with short wavelengths. Dimer formation has an action spectrum similar to thymidine absorption. The quantum yield, about 0.02, is large enough so that, in the biological dose range, dimers are formed in most of the DNA molecules of the system. Dimer breakage is important only at wavelengths below 2500 A. The quantum yield for breakage is 1.

NUCLEAR MAGNETIC RESONANCE

H. G. Jones J. S. Kirby-Smith

Introduction. – The nuclear magnetic resonance (NMR) technique offers a powerful method of attack on a variety of problems in analytical and structural chemistry, in tracer studies, and in a number of more purely biophysical problems, such as the physical state of biologically bound water. In collaboration with other groups in the laboratory, the NMR approach is being used in problems of nucleotide structure and analysis as well as in polypeptide and protein chemistry. Work in the Biophysics Group has centered around the NMR spectroscopy of the radiation-protective compounds and in studies of the physical state of water in plant seeds.

Results and Discussion. – (*a*) Radiation-protective compounds: High resolution NMR spectra of AET and its various hydrolysis products and related compounds have been obtained. With the determination of the spectra of the various pure compounds it has been possible to follow easily the hydrolysis reactions of AET at different pH's by examining the changing NMR spectra as the reactions proceed. This approach has been extended to studies of the formation of complexes of AET and derivatives with serum proteins. Preliminary studies indicate that, although AET does not readily complex, its hydrolysis product at normal

[13]See the section "Radiation Effects on 'Primer' DNA," this report.

[14]R. B. Setlow, *Biochim. et Biophys. Acta* **49**, 237 (1961).

blood pH (MEG) does form a complex with serum protein. This observation thus supports the idea that the radiation-protective mechanism may involve transfer of energy or uncoupled electrons from a protein to the associated MEG molecule where they can then be trapped.

(*b*) Water determination in seeds: Electron spin resonance studies of radiation-induced free radicals in seeds require quantitative knowledge of the amounts of water present. By studies of the low-resolution protein NMR spectra of intact and ground seeds, it has been possible to make a rapid and nondestructive determination. For wheat and barley seeds, these calibration studies show that in the range of water content from 20% by weight to approximately 2%, the water is liquid; that is, a sharp NMR line is observed, indicating that the water molecules are free to rotate and are in an essentially liquid or unbound state. Below 2%, the removable water (by heat or dissociation) is "bound" in some loose chemical or physical manner, for in this range the NMR spectrum shows only the very broad resonance characteristic of the solid state.

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