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SEMIANNUAL PROGRESS REPORT

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BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

For Period Ending January 31, 1966

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MAY 1966

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Publications and Lectures

PUBLICATIONS

During this report period (August 1, 1965–January 31, 1966), 435 papers, abstracts, and book reviews by members of the Biology Division have been published or are in press. This total compares with 343 for the February 16–July 31, 1965, reporting period and with 348 for the August 16, 1964–February 15, 1965, period. Listed below are the 90 published papers, reports, and books edited by Division members for the current period. The 43 published abstracts, 4 published book reviews, and 299 items in press are not listed.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Albright, J. F., and T. W. Evans	Influence of antigen dosage on kinetics of hemagglutinating antibody production	*J. Immunol.* **95**, 368–77 (1965)
Anderson, N. G., and G. B. Cline	Semiannual Contract Progress Report to the National Institute of Allergy and Infectious Diseases, March 1 to September 15, 1965	ORNL-3883
Barber, A. A., W. W. Harris, and G. M. Padilla	Studies of native glycogen isolated from synchronized *Tetrahymena pyriformis* (HSM)	*J. Cell Biol.* **27**, 281–92 (1965)
Baum, R. H., and M. I. Dolin	Isolation of 2-solanesyl-1,4-naphthoquinone from *Streptococcus faecalis*, 10 C1	*J. Biol. Chem.* **240**, 3425–33 (1965)
Berg, Claire M.	Biased distribution and polarized segregation in asci of *Sordaria brevicollis*	*Genetics* **53**, 117–29 (1966)
Bollum, F. J., and R. B. Setlow	Ultraviolet inactivation of DNA primer activity. I. Effects of different wavelengths and doses	Pp. 468–69 in *The Year Book of Cancer* (1964–1965 Series) (ed. by R. L. Clark and R. W. Cumley), Year Book Medical Publishers, Inc., Chicago, 1965
Brewen, J. G.	Cell-cycle and radiosensitivity of the chromosomes of human leukocytes	*Intern. J. Radiation Biol.* **9**, 391–97 (1965)
Burger, C. L., W. W. Harris, N. G. Anderson, T. W. Bartlett, and R. M. Kniseley	Virus-like particles in human leukemic plasma	Pp. 519–20 in *The Year Book of Cancer* (1964–1965 Series) (ed. by R. L. Clark and R. W. Cumley), Year Book Medical Publishers, Inc., Chicago, 1965
Cameron, I. L., and E. E. Guile, Jr.	Nucleolar and biochemical changes during unbalanced growth of *Tetrahymena pyriformis*	*J. Cell Biol.* **26**, 845–55 (1965)
Cammarano, Piero, and Marialuisa Melli	Poly-U stimulated incorporation. A comparison of normal and regenerating liver	*Biochim. Biophys. Acta* **108**, 329–32 (1965)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Chu, E. H. Y.	Effects of ultraviolet radiation on mammalian cells. II. Differential UV and X-ray sensitivity of chromosomes to breakage in 5-aminouracil synchronized cell populations	*Genetics* **52**, 1279–94 (1965)
Cosgrove, G. E.	The radiosensitivity of snakes and box turtles	*Radiation Res.* **25**, 706–12 (1965)
Cosgrove, G. E., M. L. Davis, and Masahide Asano	Oncogenic effects of 4-(*p*-dimethylamino-styryl)quinoline (4M20) in mice	*Cancer Res.* **25**, 938–45 (1965)
Cosgrove, G. E., and T. P. O'Farrell	Papillomas and other lesions in the stomachs of pine mice	*J. Mammal.* **46**, 510–13 (1965)
Cosgrove, G. E., and A. C. Upton	Pathology of the reticuloendothelial system	Pp. 21–45 in *The Pathology of Laboratory Animals* (ed. by W. E. Ribelin and J. R. McCoy), Thomas, Springfield, Ill., 1965
Cosgrove, G. E., A. C. Upton, L. H. Smith, and J. N. Dent	Radiation glomerulosclerosis and other late effects: Influence of radiological factors and AET	*Radiation Res.* **25**, 725–35 (1965)
Cudkowicz, Gustavo	Hybrid resistance to parental hematopoietic cell grafts: Implications for bone marrow chimeras	Pp. 207–19 in *La Greffe des Cellules Hématopoiétiques Allogéniques, Colloques Internationaux du Centre National de la Recherche Scientifique*, No. 147, Centre National de la Recherche Scientifique, Paris, 1965
	The immunogenetic basis of hybrid resistance to parental marrow grafts	Pp. 37–52 in *Isoantigens and Cell Interactions*, Wistar Institute Symposium Monograph No. 3 (ed. by Joy Palm), Wistar Institute Press, Philadelphia, 1965
Curtiss, Roy, III, L. J. Charamella, Claire M. Berg, and Paula E. Harris	Kinetic and genetic analyses of D-cycloserine inhibition and resistance in *Escherichia coli*	*J. Bacteriol.* **90**, 1238–50 (1965)
Davidson, Douglas	Chromosome breakage and reunion: Action of azaserine and x rays	*Z. Vererbungslehre* **96**, 217–27 (1965)
Davidson, J. N., and Waldo E. Cohn (co-editors)	Progress in Nucleic Acid Research and Molecular Biology, vol. 4 (294 pp.)	Academic, New York and London, 1965
de Serres, F. J.	Impaired complementation between nonallelic mutations in *Neurosopora*	Pp. 33–52 in *Genes and Chromosomes – Structure and Function (International Symposium Held at Buenos Aires, Argentina, November 30–December 4, 1964)* (ed. by Juan I. Valencia and Rhoda F. Grell, with the cooperation of Ruby Marie Valencia), National Cancer Institute Monograph No. 18, U.S. Department of Health, Education, and Welfare; Public Health Service, National Cancer Institute, Bethesda, Md., 1965

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Diaz, Manuel, and Crodowaldo Pavan	Changes in chromosomes induced by microorganism infection	*Proc. Natl. Acad. Sci. U.S.* **54**, 1321–27 (1965)
Dolin, M. I., E. F. Phares, and M. V. Long	Bound pyridine nucleotide of malic-lactic transhydrogenase	*Biochem. Biophys. Res. Commun.* **21**, 303–10 (1965)
Ginsberg, D. M., and John Jagger	Evidence that initial ultraviolet lethal damage in *Escherichia coli* strain 15 T^- A^- U^- is independent of growth phase	*J. Gen. Microbiol.* **40**, 171–84 (1965)
	Radiation sensitivity and growth characteristics of an arginine- and uracil-starved culture of *Escherichia coli* strain 15 T^- A^- U^-	*J. Gen. Microbiol.* **40**, 185–90 (1965)
Goodman, Joan Wright	Concerning the capacity of lymphocytes to give rise to erythropoiesis	Pp. 31–36 in *La Greffe des Cellules Hématopoiétiques Allogéniques, Colloques Internationaux du Centre National de la Recherche Scientifique*, No. 147, Centre National de la Recherche Scientifique, Paris, 1965
Greenman, D. L., W. D. Wicks, and F. T. Kenney	Stimulation of ribonucleic acid synthesis by steroid hormones. II. High molecular weight components	*J. Biol. Chem.* **240**, 4420–26 (1965)
Grell, Rhoda F.	Chromosome pairing, crossing-over, and segregation in *Drosophila melanogaster*	Pp. 215–42 in *Genes and Chromosomes – Structure and Function (International Symposium Held at Buenos Aires, Argentina, November 30–December 4, 1964* (ed. by Juan I. Valencia and Rhoda F. Grell, with the cooperation of Ruby Marie Valencia), National Cancer Institute Monograph No. 18, U.S. Department of Health, Education, and Welfare; Public Health Service, National Cancer Institute, Bethesda, Md., 1965
Gross, J. D., and L. G. Caro	Genetic transfer in bacterial mating	*Science* **150**, 1679–84 (1965)
Heddle, John A.	Randomness in the formation of radiation-induced chromosome aberrations	*Genetics* **52**, 1329–34 (1965)
Hollaender, Alexander	Introduction	Pp. xiii–xv in *Genes and Chromosomes – Structure and Function (International Symposium Held at Buenos Aires, Argentina, November 30–December 4, 1964)* (ed. by Juan I. Valencia and Rhoda F. Grell, with the cooperation of Ruby Marie Valencia), National Cancer Institute Monograph No. 18, U.S. Department of Health, Education, and Welfare; Public Health Service, National Cancer Institute, Bethesda, Md., 1965
	Symposium on Hormonal Control of Protein Biosynthesis (Introduction)	*J. Cellular Comp. Physiol.* **66** (suppl. 1) (1965) (189 pp.)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Hollaender, Alexander, S. F. Carson, and J. L. Liverman	Biology Division semiannual progress report for period ending July 31, 1965	ORNL-3853
Inglis, W. G., and G. E. Cosgrove	The pin-worm parasites (Nematoda: Oxyuridae) of the Hapalidae (Mammalia: Primates)	*Parasitology* **55**, 731–37 (1965)
Jakob, K. M., and J. E. Trosko	The relation between 5-amino uracil-induced mitotic synchronization and DNA synthesis	*Exptl. Cell Res.* **40**, 56–67 (1965)
Karasaki, Shuichi	Electron microscopic examination of the sites of nuclear RNA synthesis during amphibian embryogenesis	*J. Cell Biol.* **26**, 937–58 (1965)
Kelmers, A. D., G. D. Novelli, and M. P. Stulberg	Separation of transfer ribonucleic acids by reverse phase chromatography	*J. Biol. Chem.* **240**, 3979–83 (1965)
Kenney, F. T., and W. L. Albritton	Repression of enzyme synthesis at the translational level and its hormonal control	*Proc. Natl. Acad. Sci. U.S.* **54**, 1693–98 (1965)
Kenney, F. T., D. L. Greenman, W. D. Wicks, and W. L. Albritton	RNA synthesis and enzyme induction by hydrocortisone	Pp. 1–10 in *Advances in Enzyme Regulation*, vol. 3 (ed. by G. Weber), Symposium Publications Division, Pergamon, Oxford, 1965
Kenney, F. T., W. D. Wicks, and D. L. Greenman	Hydrocortisone stimulation of RNA synthesis in induction of hepatic enzymes	*J. Cellular Comp. Physiol.* **66** (suppl. 1), 125–36 (1965)
Kimball, R. F.	The induction of reparable premutational damage in *Paramecium aurelia* by the alkylating agent triethylene melamine	*Mutation Res.* **2**, 413–25 (1965)
	Relation of the studies on mutagenesis in Paramecium to the dose-rate problem	*Japan. J. Genetics* (suppl.) **40**, 264–74 (1965)
Kretchmar, A. L., W. H. McArthur, and C. C Congdon	Shift in the relative distribution of body nitrogen between skin and carcass in mouse radiation chimeras	*J. Nutr.* **87**, 261–66 (1965)
Lindsley, D. L.	Chromosome function at the supragenic level	Pp. 275–90 in *Genes and Chromosomes – Structure and Function (International Symposium Held at Buenos Aires, Argentina, November 30–December 4, 1964)* (ed. by Juan I. Valencia and Rhoda F. Grell, with the cooperation of Ruby Marie Valencia), National Cancer Institute Monograph No. 18, U.S. Department of Health, Education, and Welfare; Public Health Service, National Cancer Institute, Bethesda, Md., 1965
Ma, Te Hsiu, and Sheldon Wolff	Far-red-induced mitotic delay and the apparent increase of X-ray induced chromatid aberrations in *Tradescantia* microspores	*Radiation Botany* **5**, 293–98 (1965)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Makinodan, Takashi, J. F. Albright, E. H. Perkins, and Paul Nettesheim	Suppression of immunologic responses	*Med. Clin. N. Am.* **49**, 1569–96 (1965)
Makinodan, Takashi, Immo Hoppe, Toshihiko Sado, E. E. Capalbo, and Martha R. Leonard	The suppressive effect of supraoptimum doses of antigen on the secondary antibody-forming response of spleen cells cultured in cell-impermeable diffusion chambers	*J. Immunol.* **95**, 466–79 (1965)
Malling, H. V.	Identification of the genetic alterations in nitrous acid-induced *ad-3* mutants of *Neurospora crassa*	*Mutation Res.* **2**, 320–27 (1965)
Mazur, Peter	The role of cell membranes in the freezing of yeast and other single cells	*Ann. N.Y. Acad. Sci.* **125**, 658–76 (1965)
Miller, O. L., Jr.	Fine structure of lampbrush chromosomes	Pp. 79–99 in *Genes and Chromosomes – Structure and Function (International Symposium Held at Buenos Aires, Argentina, November 30–December 4, 1964)* (ed. by Juan I. Valencia and Rhoda F. Grell, with the cooperation of Ruby Marie Valencia), National Cancer Institute Monograph No. 18, U.S. Department of Health, Education, and Welfare; Public Health Service, National Cancer Institute, Bethesda, Md., 1965
Moore, B. G., and A. P. Harrison, Jr.	Benzo[a]pyrene uptake by bacteria and yeast	*J. Bacteriol.* **90**, 989–1000 (1965)
Oakberg, E. F.	The effects of dose, dose rate and quality of radiation on the dynamics and survival of the spermatogonial population of the mouse	*Japan. J. Genetics* (suppl.) **40**, 119–27 (1965)
Oaks, Ann	The regulation of nitrogen loss from maize endosperm	*Can. J. Botany* **43**, 1077–82 (1965)
	The synthesis of leucine in maize embryos	*Biochim. Biophys. Acta* **111**, 79–89 (1965)
Odell, T. T., Jr., C. W. Jackson, and D. G. Gosslee	Maturation of rat megakaryocytes studied by microspectrophotometric measurement of DNA	*Proc. Soc. Exptl. Biol. Med.* **119**, 1194–99 (1965)
Pavan, Crodowaldo	Chromosomal differentiation	Pp. 309–23 in *Genes and Chromosomes – Structure and Function (International Symposium Held at Buenos Aires, Argentina, November 30–December 4, 1964)* (ed. by Juan I. Valencia and Rhoda F. Grell, with the cooperation of Ruby Marie Valencia), National Cancer Institute Monograph No. 18, U.S. Department of Health, Education, and Welfare; Public Health Service, National Cancer Institute, Bethesda, Md., 1965

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Pfuderer, Peter, Piero Cammarano, D. R. Holladay, and G. David Novelli	A helical polysome model	*Biochim. Biophys. Acta* **109**, 595–606 (1965)
Popp, R. A.	Hemoglobin variants in mice	*Federation Proc.* **24**, 1252–57 (1965)
Popp, R. A., C. C Congdon, and Joan Wright Goodman	Spleen weight as a measure of marrow cell growth in irradiated mice	*Proc. Soc. Exptl. Biol. Med.* **120**, 395–98 (1965)
Roberts, P. A.	Asymmetrical exchange in adjacent tandem duplications	*Genetics* **52**, 1017–22 (1965)
Russell, W. L.	Effect of the interval between irradiation and conception on mutation frequency in female mice	*Proc. Natl. Acad. Sci. U.S.* **54**, 1552–57 (1965)
	The nature of the dose-rate effect of radiation on mutation in mice	*Japan. J. Genetics* (suppl.) **40**, 128–40 (1965)
Saul, G. B., 2nd, P. W. Whiting, S. W. Saul, and C. A. Heidner	Wild-type and mutants stocks of Mormoniella	*Genetics* **52**, 1317–27 (1965)
Schwartz, Drew, Lucy Fuchsman, and Kathrine H. McGrath	Allelic isozymes of the pH 7.5 esterase in maize	*Genetics* **52**, 1265–68 (1965)
Setlow, Jane K., and M. E. Boling	The resistance of *Micrococcus radiodurans* to ultraviolet radiation. II. Action spectra for killing, delay in DNA synthesis, and thymine dimerization	*Biochim. Biophys. Acta* **108**, 259–65 (1965)
Setlow, R. B., and Jane K. Setlow	The proper use of short-wavelength reversal as a criterion of the importance of pyrimidine dimers in biological inactivation	*Photochem. Photobiol.* (research note) **4**, 939–40 (1965)
Setlow, R. B., P. A. Swenson, and W. L. Carrier	Thymine dimers and inhibition of DNA synthesis by ultraviolet irradiation of cells	Pp. 482–83 in *The Year Book of Cancer* (1964–1965 Series) (ed. by R. L. Clark and R. W. Cumley), Year Book Medical Publishers, Inc., Chicago, 1965
Shaffer, C. R., and R. A. McGrath	The effects of X-irradiation on the DNA of *Escherichia coli:* An autoradiographic study	*Exptl. Cell Res.* **39**, 604–6 (1965)
Simmons, M. L., and J. A. Franklin	Bacteriological studies on irradiated mice	*ASPCA Lab. Animal Care*, 1–4 (1965)
Simmons, M. L., H. E. Williams, and Everline B. Wright	Therapeutic value of the organic phosphate Trichlorfon against *Syphacia obvelata* in inbred mice	*Lab. Animal Care* **15**, 382–85 (1965)
Smith, L. H., and T. W. McKinley, Jr.	Erythropoietic capacity of short-term mouse marrow cultures	*J. Natl. Cancer Inst.* **35**, 573–77 (1965)
Speicher, B. R., Kathryn G. Speicher, and F. L. Roberts	Genetic segregation in the unisexual wasp Devorgilla	*Genetics* **52**, 1035–41 (1965)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Swartzendruber, D. C.	Desmosomes in germinal centers of mouse spleen	*Exptl. Cell Res.* **40**, 429–32 (1965)
Tyndall, R. L., K. Bruce Jacobson, and Ernestine Teeter	Characterization of HeLa cell and viral ribonucleic acid by oligonucleotide analysis	*Biochim. Biophys. Acta* **108**, 11–17 (1965)
Upton, A. C.	Current trends in cancer research	6th IBM Medical Symposium, IBM 320-0918-0, 591–600 (1965)
Uziel, Mayo, and Waldo E. Cohn	Desalting of nucleotides by gel filtration	*Biochim. Biophys. Acta* **103**, 539–41 (1965)
Valencia, Juan I., and Rhoda F. Grell (eds.), with the cooperation of Ruby Marie Valencia	Genes and Chromosomes – Structure and Function (International Symposium Held at Buenos Aires, Argentina, November 30–December 4, 1964) (354 pp.)	National Cancer Institute Monograph No. 18, U.S. Department of Health, Education, and Welfare; Public Health Service, National Cancer Institute, Bethesda, Md., 1965
Volkin, Elliott	Genetic recombination in bacteriophage	Pp. 51–80 in *Progress in Nucleic Acid Research and Molecular Biology*, vol. IV (ed. by J. N. Davidson and Waldo E. Cohn), Academic, New York and London, 1965
Warner, A. H., and F. J. Finamore	Isolation, purification, and characterization of P^1,P^4-diguanosine 5′-tetraphosphate *asymmetrical*-pyrophosphohydrolase from brine shrimp eggs	*Biochemistry* **4**, 1568–75 (1965)
	Isolation, purification, and characterization of P^1,P^3-diguanosine 5′-triphosphate from brine shrimp eggs	*Biochim. Biophys. Acta* **108**, 525–30 (1965)
Welshons, W. J.	Analysis of a gene in Drosophila	*Science* **150**, 1122–29 (1965)
Whiting, Anna R.	The complex locus *R* in *Mormoniella vitripennis* (Walker)	*Advan. Genet.* **13**, 341–58 (1965)
Whitson, G. L.	The effects of actinomycin D and ribonuclease on oral regeneration in *Stentor coeruleus*	*J. Exptl. Zool.* **160**, 207–14 (1965)
Wicks, W. D., D. L. Greenman, and F. T. Kenney	Stimulation of ribonucleic acid synthesis by steroid hormones. I. Transfer ribonucleic acid	*J. Biol. Chem.* **240**, 4414–19 (1965)
Willard, Henrianne G., and L. H. Smith	Relative injection efficiency of homografted erythropoietic cells in mice by isologous immunocompetent cells	*Transplantation* **4**, 56–65 (1965)
Wolff, Sheldon	Mechanisms of dose-rate effects: Insights obtained from intensity and fractionation studies on chromosome aberration induction	*Japan. J. Genetics* (suppl.) **40**, 38–48 (1965)
	On the chemistry of chromosome continuity	Pp. 155–79 in *Genes and Chromosomes – Structure and Function (International Symposium Held in Buenos Aires, Argentina, November 30–December 4, 1964)* (ed. by Juan I. Valencia and Rhoda F. Grell, with the cooperation of Ruby Marie Valencia), U.S. Department of Health, Education, and Welfare; Public Health Service, National Cancer Institute, Bethesda, Md., 1965

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Wolff, Sheldon, and H. E. Luippold	Mitotic delay and the apparent synergism of far-red radiation and X-rays in the production of chromosomal aberrations	*Photochem. Photobiol.* **4**, 439–45 (1965)
Yoneda, Masahiko, and F. J. Bollum	Deoxynucleotide-polymerizing enzymes of calf thymus gland. I. Large scale purification of terminal and replicative deoxynucleotidyl transferases	*J. Biol. Chem.* **240**, 3385–91 (1965)

LECTURES

During the period August 1, 1965, to January 31, 1966, members of the Biology Division presented 199 lectures and speeches. This figure compares with 242 for the corresponding period of 1964–65 and with 247 for the February 1 to July 31, 1965, reporting period. Of the 199 lectures and speeches, 84 were given at meetings and professional societies, and 96 were given at the invitation of universities, laboratories, and scientific institutions in the United States and abroad. A total of 19 was presented as part of the Traveling Lecture Program.

For details on speaking activities outside the continental United States, see the section "Foreign Travel." Educational activities are described in the section of that name (see Contents).

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Adler, H. I.	Radiation biology (discussion)	ORINS-ORNL-UT-AEC Radiation Biology Conference, Oak Ridge, Tenn.
	Repair of radiation-induced damage to the cell division mechanism of *Escherichia coli*	Southwest Center for Advanced Studies, Dallas
Adler, H. I. (and Alice A. Hardigree)	Recovery of irradiated cells promoted by unirradiated bacteria	Am. Soc. Microbiol., Kentucky-Tennessee Branch, Lexington, Ky.
Allen, R. C.	Quantitation of proteins and enzymes in acrylamide gel electrophoresis	Research Conference on Instrumentation Science, Geneva, N.Y.
Allen, R. C. (R. T. Jones and L. J. Serrano)	Quantitative relationships of intestinal bacteria in specifically contaminated mice	Animal Care Panel, Philadelphia
Anderson, N. G.	Cell fractionation	Biology Class, Oak Ridge High School, Oak Ridge, Tenn.
	The development of zonal centrifuges	Argonne National Laboratory, Argonne, Ill.
	The flame ionization analyzer	Institute for Defense Analysis, Washington, D.C.
	Medical research programs in Hadassah-supported institutions	Hadassah Group, Temple Beth-El, Oak Ridge, Tenn.
	Molecular analytical studies on cancer tissues	Rotary Club, Chattanooga, Tenn.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Anderson, N. G.	Molecular anatomy	Kingston Lions Club, Kingston, Tenn.
	1. The Molecular Anatomy Program (The MAN Program) I. Background of the proposal 2. The Molecular Anatomy Program (The MAN Program) II. Implementation	Senior Staff Seminar, ORNL
	1. Origin of life 2. Structure of viruses	University of Tennessee, Knoxville
	Zonal centrifugation	Communicable Diseases Center, Atlanta, Ga.
	Zonal centrifuge development	Conference on Analytical Chemistry in Nuclear Technology, Gatlinburg, Tenn.
	Zonal Centrifuges	Analytical Chemistry Division, ORNL
Anderson, N. G. (G. B. Cline and W. W. Harris)	The isolation of virus particles from large fluid volume	Symposium on Transmission of Viruses by the Water Route, Cincinnati, Ohio
Barnett, W. E.	Multiple synthetase--multiple tRNA systems	Univ. of Texas, Austin
Bender, M. A	Chromosome aberrations in irradiated humans	Brookhaven National Laboratory, Upton, N.Y.
	Radiation-induced chromosomal abnormalities	American College of Obstetricians and Gynecologists, Gatlinburg, Tenn.
	S-4, radiation, and zero *g* on blood	Symposium on Manned Spaceflight Experiments, Washington, D.C.
Bender, M. A (and P. Carolyn Gooch)	Chromosome aberrations in irradiated humans	XIth International Congress of Radiology, Rome
Berg, Claire M.	Chromosome replication during vegetative growth in coisogenic strains of *Escherichia coli* [*Genetics* **52**, 428–29 (1965)]	Genetics Society of America, Fort Collins, Colo.
	Inversions and other transpositions of the *Escherichia coli* chromosome	Am. Soc. Microbiol., Kentucky-Tennessee Branch, Lexington, Ky.
Brahma, S. K. (Piero Cammarano and Marialuisa Melli)	Morphogenetic effects of different RNA fractions of rat liver and their hydrolysates on the newt ectoderm [*J. Cell Biol.* **27**, 14A (1965)]	Am. Soc. Cell Biol., Philadelphia
Brewen, J. G.	Radiation effects at the cellular level	ORINS-ORNL-UT-AEC Radiation Biology Conference, Oak Ridge, Tenn.
Cameron, I. L. (and W. D. Fisher)	The separation of acid-soluble nucleotides and related compounds from yeast cells during different growth conditions [*J. Cell Biol.* **27**, 17A–18A (1965)]	Am. Soc. Cell Biol., Philadelphia
Cameron, I. L. (G. L. Whitson and G. M. Padilla)	Physiological and structural changes to the cell cycle of synchronized *Tetrahymena*	Society of General Physiologists, Woods Hole, Mass.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Caro, L. G.	1. Chromosome synthesis and recombination 2. DNA transfer in bacterial conjugation	College of Medicine, Univ. of Cincinnati, Cincinnati, Ohio
	DNA transfer in bacterial conjugation	1. Univ. of California, Berkeley 2. Stanford Univ., Palo Alto, Calif. 3. Univ. of Oregon, Eugene
Charamella, L. J. (Roy Curtiss III, Janet Renshaw, and Claire M. Berg)	The Hfr-strains and chromosome map of *Escherichia coli* K-12	Am. Soc. Microbiol., Kentucky-Tennessee Branch, Lexington, Ky.
Cline, G. B. (Helen Coates, W. W. Harris, Frances Ball, and N. G. Anderson)	Respiratory syncytial virus isolation by combined continuous flow—isopycnic banding centrifugation	Annual Meeting of Vaccine Development Program Contractors, NIH, Bethesda, Md.
Cohn, Waldo E.	The characterization of transfer RNA's	Los Alamos Scientific Laboratory, Los Alamos, N.M.
Cohn, Waldo E. (and Mayo Uziel)	A one-hour quantitation of nucleic acid components at nanomole levels by elution ion-exchange chromatography [Abstr. of papers, 150th Meeting, Am. Chem. Soc., 1965, p. 64C]	1. Am. Chem. Soc., 150th National Meeting, Atlantic City, N.J. 2. Am. Chem. Soc., Winter Meeting, Phoenix, Ariz.
Congdon, C. C	The destructive effect of radiation on lymphatic tissue	ACS-NCI Conference on Obstacles to the Control of Hodgkin's Disease, Rye, N.Y.
Congdon, C. C (M. A. Kastenbaum and D. A. Gardiner)	Modification of secondary disease mortality [*Exptl. Hematol.*, No. 9, 65 (1965)]	Bone Marrow Transplantation and Radiation Recovery Conference, San Francisco
Cudkowicz, Gustavo	A sex-associated influence on histocompatibility in F_1 hybrid mice [*Genetics* **52**, 438 (1965)]	Genetics Society of America, Fort Collins, Colo.
Curtiss, Roy, III (and L. J. Charamella)	The role of the F^- parent during bacterial conjugation in *Escherichia coli*	Am. Soc. Microbiol., Kentucky-Tennessee Branch, Lexington, Ky.
Curtiss, Roy, III (and Janet Renshaw)	Fertility factor attachment in F^+ strains of *Escherichia coli* K-12 [*Genetics* **52**, 438–39 (1965)]	Genetics Society of America, Fort Collins, Colo.
de Serres, F. J.	Genetic effects of heavy ion irradiations on *Neurospora*	Workshop Conference on Space Radiation Biology, Berkeley, Calif.
	1. The influence of genetic constitution on recombination and interference 2. The nature of X-ray-induced gene mutations	Cornell Univ., Ithaca, N.Y.
	Mutation induction and characterization in *Neurospora*	Pathobiology Conference, Univ. of Colorado Medical Center, Aspen
Doherty, D. G.	Chemical structure and radiation protection	Am. Chem. Soc., Decatur Section, Decatur, Ill.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Edwards, C. L. (C. C Congdon, N. Gengozian, H. Vodopick, and G. A. Andrews)	Treatment of acute leukemia: An experimental immunologic approach [*Exptl. Hematol.*, No. 9, 23 (1965)]	Bone Marrow Transplantation and Radiation Recovery Conference, San Francisco
Ehling, U. H.	Dominant skeletal mutations induced by X-irradiation of mouse spermatogonia [*Genetics* **52**, 441–42 (1965)]	Genetics Society of America, Fort Collins, Colo.
Eisenberg, Sara (and Tuneo Yamada)	1. DNA synthesis in the cells of the lens regenerating system (by title) [*Am. Zool.* **5**, 717 (1965)] 2. A study of the cell cycle in the regenerating lens (by title) [*Am. Zool.* **5**, 718 (1965)]	AAAS (Am. Soc. Zool.), Berkeley, Calif.
Epler, J. L.	Fractionation of sRNA's in *Neurospora*	Florida State Univ., Tallahassee
Foard, D. E.	Anatomic features of radiation-induced mitotic inhibition in plants	UT-AEC Agricultural Research Laboratory, Oak Ridge, Tenn.
	Cell division and organ initiation	Univ. of Tennessee, Knoxville
Goncalves, J. M.	Inhibition of muscle phosphorylases by 2,4-dinitrophenol and its physiological implications	1. Univ. of Wisconsin, Madison 2. Univ. of Washington Medical School, Seattle 3. Western Reserve Univ., Cleveland, Ohio
	Structure of crotamine and a brewer yeast carboxypeptidase as a new tool for c-terminal group determination	1. Univ. of Wisconsin, Madison 2. Univ. of Washington Medical School, Seattle 3. Western Reserve Univ., Cleveland, Ohio
Goodman, Joan Wright (and Gayle C. Bosma)	Some unusual effects of preimmunization with living parental cells [*Exptl. Hematol.*, No. 9, 70 (1965)]	Bone Marrow Transplantation and Radiation Recovery Conference, San Francisco
Gude, W. D.	Autoradiography	Medical Division, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn.
	Biological effects of radiation on mammals (2 lectures)	Tennessee Academy of Sciences Visiting Scientist Program, Alcoa High School, Alcoa, Tenn.
Haber, A. H.	Radiation effects in plants	ORINS-ORNL-UT-AEC Radiation Biology Conference, Oak Ridge, Tenn.
	Radiation-induced and endogenous electron spin resonance signals in lettuce seeds	UT-AEC Agricultural Research Laboratory, Oak Ridge, Tenn.
Hanna, M. G., Jr.	Cancer in the immune system	Baldwin-Wallace College, Berea, Ohio
	Lymphatic tissue changes during antibody production	New England Medical Research Center, Ridgefield, Conn.
Hanna, M. G., Jr. (D. C. Swartzendruber and C. C Congdon)	Morphologic changes in spleen lymphatic tissue during antibody production [*Exptl. Hematol.*, No. 9, 31 (1965)]	Bone Marrow Transplantation and Radiation Recovery Conference, San Francisco

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Harrison, A. P., Jr.	Benzpyrene uptake by bacteria	Syracuse Univ., Syracuse, N.Y.
	Comparison of thymine metabolism and thymine incorporation by various thymine-deficient bacteria	Soc. Exptl. Biol. Med., Southeastern Branch, Winston-Salem, N.C.
Hollaender, Alexander	Studies on radiation biology at the international level	ORINS-ORNL-UT-AEC Radiation Biology Conference, Oak Ridge, Tenn.
Hosszu, J. L.	Relative response of NaI(Tl) and anthracene to ^{32}P β-rays under conditions of partial absorption	Am. Physical Soc., Southeastern Section, Charlottesville, Va.
Huffstetler, J. T. (L. J. Serrano and M. L. Simmons)	Cost comparison of disposable and reusable mouse cages	Animal Care Panel, Philadelphia
Jacobson, K. Bruce	Isozymes of alcohol dehydrogenase in *Drosophila*	1. Univ. of Massachusetts, Amherst 2. Tufts Univ., Medford, Mass. 3. State Univ. of New York, Buffalo 4. Vanderbilt Univ., Nashville, Tenn. 5. Isozyme Conference, Williamsburg, Va.
Jagger, John (and R. S. Stafford)	Evidence that indirect photoreactivation does not split thymine dimers	Pacific Slope Biochemical Conference, Los Angeles
Kenney, F. T. (W. D. Wicks, D. D. Holten, and W. L. Albritton)	Mechanisms involved in the induction of hepatic enzymes by hydrocortisone	AAAS Symposium on Mode of Action of Steroid Hormones, Berkeley, Calif.
Kilbey, B. J.	Ultraviolet mutagenesis in *Neurospora crassa*	Univ. of North Carolina, Chapel Hill
	Ultraviolet mutagenesis and photoreactivation in *Neurospora crassa*	Southwest Center for Advanced Studies, Dallas
Kimball, R. F.	Further studies on mutagenesis by triethylene melamine in *Paramecium aurelia* [*Genetics* **52**, 452 (1965)]	Genetics Society of America, Fort Collins, Colo.
	Radiation genetics (2 lectures)	Saint Augustine's College, Raleigh, N.C.
Leibo, S. P. (and Peter Mazur)	Studies on the mechanism of freezing damage in bacteriophage $T4B_1$ [*Cryobiology* **2**, 17–18 (1965)]	Society for Cryobiology, 2d Annual Meeting, Madison, Wis.
Lion, M. B.	Radiosensitivity of bromouracil-substituted DNA	Karlsruhe Institute for Radiobiology, Karlsruhe, Germany
Liverman, J. L.	Future implications of analytical chemistry in the activities of the biology program in Oak Ridge	Analytical Chemistry Division Annual Meeting, Oak Ridge, Tenn.
Longworth, J. W.	Protein and nucleic acid luminescence	Ontario Cancer Institute, Toronto, Canada
Makinodan, Takashi	Growth and senescence of the immune mechanism	1. Yale Univ., New Haven, Conn. 2. Duke Univ. Medical Center, Durham, N.C.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Mattingly, Ellen	The relationship of the nucleus and cytoplasm in problems of differentiation	1. Univ. of Scranton, Scranton, Pa. 2. Western Illinois Univ., Macomb, Ill. 3. Tuskegee Institute, Tuskegee Institute, Ala. 4. Univ. of Georgia, Athens
Mazur, Peter	Physical factors determining freezing injury in microorganisms	Univ. of Wisconsin, Madison
	Theoretical and experimental effects of cooling and warming velocity on the survival of frozen and thawed cells	Society for Cryobiology Symposium on Measurement and Interpretation of Freezing Rates, Madison, Wis.
Mazur, Peter (and Janice J. Schmidt)	Effect of cooling and warming velocity on the survival of frozen and thawed yeast [*Cryobiology* **2**, 18 (1965)]	Society for Cryobiology, 2d Annual Meeting, Madison, Wis.
McGrath, R. A.	Cells, DNA, and X rays	Univ. of Tennessee, Knoxville
Miller, O. L., Jr.	Amphibian oocyte nucleoli	Univ. of La Plata, La Plata, Argentina
	Chromosomes and nucleoli, structure and function	Univ. of Chile, Santiago
	1. Fine structure of chromosomes 2. Fine structure of chromosomes and nucleoli	Univ. of Buenos Aires, Buenos Aires
	A membranous component in amphibian oocyte nucleoli [*J. Cell Biol.* **27**, 66A (1965)]	Am. Soc. Cell Biol., Philadelphia
	Structure and composition of peripheral nucleoli from salamander oocytes	Symposium on the Nucleolus, Its Structure and Function, Montevideo, Uruguay
Morris, D. M.	Plasma LDH activity during development of a transplantable leukemia in two strains of rats	Tenn. Acad. Sci., Oak Ridge
Novelli, G. David	Protein synthesis during liver regeneration	Topics in Molecular Biology of Nucleic Acids, Temple Univ. School of Medicine, Philadelphia
Oakberg, E. F.	Characterization of type A spermatogonia surviving high radiation doses in the mouse [*Genetics* **52**, 463–64 (1965)]	Genetics Society of America, Fort Collins, Colo.
	Effectiveness of different radiations as measured by spermatogonial killing	Panel Discussion on Agents Adversely Affecting the Gonads, Pacific Coast Fertility Society, Las Vegas, Nev.
	The mammalian oocyte	WHO Meeting on Chemistry and Physiology of the Gametes, Geneva
	Radiation response of spermatogonia and oocytes	Pacific Coast Fertility Society, Las Vegas, Nev.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Odell, T. T., Jr.	Biology in the Oak Ridge National Laboratory	Biology Club, Maryville College, Maryville, Tenn.
	Microspectrophotometry of the DNA of megakaryocytes	Tennessee Blood Club, Paris, Tenn.
Odell, T. T., Jr. (C. W. Jackson and R. S. Reiter)	Depression of megakaryocytes and platelets in rats by transfusion of platelets (by title)	Am. Soc. Hematology, Philadelphia
Pavan, Crodowaldo	Changes in chromosomes induced by microorganism infection	Univ. of Texas Southwest Medical School, Dallas
	Chromosomal changes induced by infection in Rhynchosciara	Frances Delafield Hospital, Columbia Univ., New York City
	Chromosomal differentiation	Southwest Center for Advanced Studies, Dallas
Perkins, E. H. (Paul Nettesheim, Toshiteru Morita, and H. E. Walburg, Jr.)	Effect of gelatin stimulation and the germ-free state on the engulfing potential of peritoneal phagocytes	Reticuloendothelial Society, Salt Lake City, Utah
Popp, Diana M.	Non-H-2 blood group factors on mouse erythrocytes	Tennessee Blood Club, Paris, Tenn.
Popp, R. A.	Hemoglobin variants in mice: Structural changes in the alpha-chain	Tennessee Blood Club, Paris, Tenn.
Rahn, R. O.	UV-induced excited states in DNA	Workshop in Space Radiation Biology, Berkeley, Calif.
Randolph, M. L.	Conversion of derivative ESR data to area under absorption curve	Am. Physical Soc., Southeastern Section, Charlottesville, Va.
Regan, J. D.	Alkaline phosphatases of human cell cultures	Univ. of Michigan, Ann Arbor
Renshaw, Janet (and Roy Curtiss III)	Mechanisms of chromosome transfer by F^+ donors of *Escherichia coli*	Am. Soc. Microbiol., Kentucky-Tennessee Branch, Lexington, Ky.
Roberts, P. A.	Crossover-suppressing translocations in *Drosophila melanogaster* [*Genetics* **52**, 469 (1965)]	Genetics Society of America, Fort Collins, Colo.
	Some effects of X rays on recombination in *Drosophila*	Rutgers Univ., New Brunswick, N.J.
	Studies of chromosomal puffs as an expression of genetic activity in Diptera	West Virginia Univ., Morgantown
Rogers, Stanfield	Some metabolic approaches to tumor chemotherapy	American College of Obstetricians and Gynecologists, Gatlinburg, Tenn.
Russell, Liane B.	Effects of radiation on embryo and fetus	Tennessee Valley Industrial Health Conference, Gatlinburg, Tenn.
	The X chromosome of the mammalian female	American College of Obstetricians and Gynecologists, Gatlinburg, Tenn.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Russell, Liane B. (E. H. Y. Chu and Liane B. Russell)	Pattern of DNA synthesis in X-autosome translocations in the mouse [*Genetics* **52**, 435 (1965)]	Genetics Society of America, Fort Collins, Colo.
Russell, Liane B. (and Clyde S. Montgomery)	The use of X-autosome translocations in locating the X-chromosome inactivation center [*Genetics* **52**, 470–71 (1965)]	Genetics Society of America, Fort Collins, Colo.
Russell, W. L.	A brief review of genetic effects of radiation exposure	Tennessee Valley Industrial Health Conference, Gatlinburg, Tenn.
	Genetics effects of radiation	ORINS-ORNL Health Physics Course, Oak Ridge, Tenn.
	Mammalian genetics program in Oak Ridge	Division of Biology and Medicine, U.S. Atomic Energy Commission, Washington, D.C.
	Mammalian radiation genetics	Univ. of Bern, Bern, Switzerland
Russell, W. L. (and Elizabeth M. Kelly)	Mutation frequency in female mice exposed to a small X-ray dose at high dose rate [*Genetics* **52**, 471 (1965)]	Genetics Society of America, Fort Collins, Colo.
Serrano, L. J. (T. T. Odell, Jr., R. C. Allen, H. E. Walburg, Jr., and R. L. Tyndall)	Techniques for maintaining defined mice in a large facility	Animal Care Panel, Philadelphia
Setlow, R. B.	Radiation cellular repair mechanism	Presbyterian Hospital, Columbia-Presbyterian Medical Center, New York City
	Summary of the conference	Conference on Radiation Microbiology, Chicago
Sheeler, Phillip (and A. A. Barber)	Liver glycogen metabolism in starved and refed rats [*Am. Zool.* **5**, 669 (1965)]	AAAS (Sect. on Comparative Physiology), Berkeley, Calif.
Stapleton, G. E.	Protection and recovery from radiation damage in living cells	1. Shorter College, Rome, Ga. 2. Bucknell Univ., Lewisburg, Pa. 3. Virginia Military Institute, Lexington
Stapleton, G. E. (and W. D. Fisher)	Ribosome profiles and macromolecular synthesis in radiation resistant and sensitive *Escherichia coli*	Am. Soc. Microbiol., Kentucky-Tennessee Branch, Lexington, Ky.
Terry, C. E.	Photoreactivation in *Neurospora crassa*	Am. Soc. Microbiol., Kentucky-Tennessee Branch, Lexington, Ky.
Tyndall, R. L.	Men, mice, and malignancy	Auburn Univ., Auburn, Ala.
	1. Molecular aspects of virus infection 2. Viruses and cancer 3. Virus structure as related to function	Univ. of Puerto Rico, Rio Piedras
	Viruses: A continuing challenge	Florida State Univ., Tallahassee

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Upton, A. C.	Mechanisms of radiation carcinogenesis	World Health Organization, Washington, D.C.
	Radiation effects in mammalian tissues	ORINS-ORNL-UT-AEC Radiation Biology Conference, Oak Ridge, Tenn.
	Radiation Injury: Principles and Perspectives 1. Carcinogenesis 2. Growth, development, and longevity 3. Prevention and control 4. Radiation sickness	Univ. of Chicago, Chicago
	Radiobiologic aspects of the supersonic transport	UT-AEC Agricultural Research Laboratory, Oak Ridge, Tenn.
	Recent observations on radiation carcinogenesis, with particular reference to the fetus	American College of Obstetricians and Gynecologists, Gatlinburg, Tenn.
	Summarization of the conference	Gordon Research Conference on Cancer, New London, N.H.
Upton, A. C. (V. K. Jenkins, H. E. Walburg, Jr., R. L. Tyndall, J. W. Conklin, and Niel Wald)	Observations on viral, chemical, and radiation-induced myeloid and lymphoid leukemias in RF mice	International Conference on Murine Leukemia, Philadelphia
Uziel, Mayo	The chemistry of living systems (2 lectures)	Univ. of Tennessee, Knoxville
Vissers, D. R. (N. G. Anderson, James Attrill, and J. G. Green)	Automated nucleotide analyzer	Ninth Conference on Analytical Chemistry in Nuclear Technology, Gatlinburg, Tenn.
von Borstel, R. C.	1. Genetics of sex determination in Hymenoptera 2. Space biology	University of São Paulo, Ribeirão Prêto, Brazil
	P^{32} and P^{33} suicide experiments in bacteriophage T4	University of Brazil, Rio de Janeiro
	1. RNA and DNA polymerase action on chromosomes and the problem of chromosome puffing 2. Super-suppressors as addition-deletion mutations	Upstate Medical Center, Syracuse, N.Y.
	RNA polymerase and the problem of chromosome puffing	University of São Paulo, São Paulo, Brazil
	Space biology	Peruvian University "Cayetano Heredia," Lima, Peru
	Super-suppressors as addition-deletion mutations	1. University of São Paulo, Piracicaba, Brazil 2. University of Buenos Aires, Buenos Aires

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Walburg, H. E., Jr.	The gnotobiotics laboratory at ORNL: Studies on X-irradiated germ-free mice	UT-AEC Agricultural Research Laboratory, Oak Ridge, Tenn.
Wicks, W. D.	Hormonal control of tyrosine transaminase activity in adult and fetal rat liver	Purdue Univ., Lafayette, Ind.
Wolff, Sheldon	Dose independence of tritiated thymidine-induced chromosome aberrations in *Vicia faba* [*Genetics* **52**, 484–85 (1965)]	Genetics Society of America, Fort Collins, Colo.
	On the chemistry of chromosome continuity	1. Univ. of Michigan, Ann Arbor 2. Univ. of Tennessee, Knoxville 3. Summer Course in Mammalian Chromosome Cytology, Providence, R.I.
	Radiation and chemical effects on chromosomes	Summer Course in Mammalian Chromosome Cytology, Providence, R.I.
Wolff, Sheldon (J. E. Trosko and Sheldon Wolff)	Multistranded mitotic chromosomes of *Vicia faba* as revealed by enzyme digestion studies [*Genetics* **52**, 480–81 (1965)]	Genetics Society of America, Fort Collins, Colo.
Wust, C. J.	Antibody synthesis	1. Goucher College, Towson, Md. 2. Indiana Univ., Indianapolis 3. Randolph-Macon Woman's College, Lynchburg, Va. 4. Trinity College, Washington, D.C.
	Immunoglobulins	1. Goucher College, Towson, Md. 2. Randolph-Macon Woman's College, Lynchburg, Va. 3. Trinity College, Washington, D.C.
Yamada, Tuneo	Control of tissue specificity	AIBS Refresher Course on Recent Advances in Analysis of Differentiation, Urbana, Ill.
	1. Problems of embryonic induction 2. Studies of tissue transformation I 3. Studies of tissue transformation II	Centro de Investigacion y de Estudios Avanzados, Instituto Politecnico Nacional, Mexico City, Mexico
Yamada, Tuneo (and Chinami Takata)	An immunofluorescence autoradiographic study on temporal sequence of final S phase and synthesis of X-crystallins in a lens regenerating system [*J. Cell Biol.* **27**, 114A–115A (1965)]	Am. Soc. Cell Biol., Philadelphia
Zeigel, R. F. (and R. L. Tyndall)	Electron microscopic observations on the proliferation of a murine leukemia virus (Rauscher) in tissue culture [*J. Appl. Phys.* **36**, 2610 (1965)]	Electron Microscopy Society of America, 23d Annual Meeting, New York City
Zeigel, R. F. (R. L. Tyndall and Ernestine Teeter)	Observations on the morphology of a murine leukemia virus (Rauscher) propagated in tissue culture	International Conference on Murine Leukemia, Symposium on Morphology and Development of Murine Leukemia Viruses, Philadelphia

VISITING LECTURERS

During the period July 31, 1965–January 31, 1966, 71 lectures were given on the Biology Division Seminar Program by guest speakers from scientific institutions and universities in this country and abroad. Visiting lecturers from foreign countries included scientists from Brazil, Canada, England, France, Germany, Italy, Japan, The Netherlands, Scotland, Sweden, and Switzerland.

SPEAKER	AFFILIATION	SUBJECT
Gerald E. Adams	Research Unit in Radiobiology, Mt. Vernon Hospital, Middlesex, England	Pulsed methods in radiation chemistry – their relevance to radiobiology
D. Bernard Amos	Duke University Medical Center, Durham, N.C.	Isoantigens in man
Bruce Anderson	The University of Tennessee, Knoxville	Alcohol dehydrogenase mechanism of action
R. D. Birkhoff	Oak Ridge National Laboratory, Oak Ridge, Tenn.	Optical constant measurements in vacuum UV
John F. Brandts	Department of Chemistry, University of Massachusetts, Amherst	Conformational stability of proteins in solution
Robert C. Brown	Geographic Pathology Division, Armed Forces Institute of Pathology, Washington, D.C.	Pattern of DNA replication in chromosomes of the dog
Alain Bussard	Institut Pasteur, Paris	Individual studies of immunocytes from tissue cultures by the method of local hemolysis in gum
C. C. Cavalli-Sforza	Institute of Genetics, Pavia, Italy	Genetics of human populations
Ruggero Ceppellini	Institute of Medical Genetics, University of Turin, Turin, Italy	Genetic control of antibody structure
Kwen-Sheng Chiang	Princeton University, Princeton, N.J.	DNA replication mechanism in the sexual reproduction cycle of *Chlamydomonas reinhardi*
James E. Cleaver	Massachusetts General Hospital, Boston	Some effects of ultraviolet light on DNA synthesis in mammalian cells
M. J. Constantin	UT-AEC Agricultural Research Laboratory, Oak Ridge, Tenn.	RBE of fast neutrons in seed radiosensitivity
Dorothy Skinner Cook	New York University School of Medicine, New York	Studies on the content and turnover of RNA in various tissues during the molt cycle of the land crab, *Gecarcinus lateralis*
John S. Cook	New York University School of Medicine, New York	Cell division delay in echinoderm zygotes following ultraviolet irradiation
Frederick Coulston	Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, Albany, N.Y.	Modern toxicology
Lionel V. Crawford	University of Glasgow, Glasgow, Scotland	Studies on human papilloma DNA
Helen V. Crouse	Institute of Molecular Biophysics, Florida State University, Tallahassee	Recent advances in the cytogenetics of *Sciara*

SPEAKER	AFFILIATION	SUBJECT
A. J. Dalton	Laboratory of Oncology, National Institutes of Health, Bethesda, Md.	Electron microscopic studies on herpes-like viruses
Eugene Day	Duke University Medical Center, Durham, N.C.	Radioantibody localization in tumors
C. J. Dean	Chester Beatty Research Institute, London	Modification of X-ray damage in radio-resistant bacteria
L. J. A. DiDio	Northwestern University Medical School, Chicago	The ultrastructure of the epithelium of the normal ventral lobe of the rat prostate gland
Arthur Z. Eisen	Massachusetts General Hospital, Boston	Remodeling of collagen in amphibian metamorphosis
Duane Englehardt	International Business Machines, Knoxville, Tenn.	How data are stored on IBM cards, magnetic tape, and storage discs. The biologist's point of view
Luciano Fiore-Donati	University of Padua, Padua, Italy	Role of thymus and other host factors in mouse leukemogenesis by Graffi virus
Peter Fong	Utica College, Utica, N.Y.	The unwinding and replication of DNA
Oswaldo Frota-Pessoa	University of São Paulo, São Paulo, Brazil	Mechanism of dosage compensation for sex-linked genes
Felice Gavosto	Università di Torino, Torino, Italy	DNA synthesis in human acute leukemia chromosomes
Karl Habel	National Institute for Allergy and Infectious Diseases, Bethesda, Md.	Specific antigens produced in cells transformed by tumor viruses
Alan Heilman	Department of Botany, The University of Tennessee, Knoxville	Some pollination mechanisms
Ray W. Holton	The University of Tennessee, Knoxville	Cytochromes in algae
Ann B. Jacobson	Botany Department, University of Chicago, Chicago	RNA metabolism in protoplastids of corn
Ken Jones	Institute of Animal Genetics, Edinburgh, Scotland	Ultrastructural modification of amphibian embryonic nuclei by actinomycin D treatment
Masaru Kato	Washington State University, Pullman	Effects of light on the acceleration of the physiological mechanisms of embryonic growth
Harold W. Kohn	Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.	The radiation chemistry of surfaces
Lisbeth Kraft	Bioquest Division, Becton and Dickinson, Hackensack, N.J.	Epidemiology of disease in laboratory animal colonies
William Leach	The University of Tennessee, Knoxville	Retention of H^3 thymidine in grasshopper neuroblasts
Urs Leupold	University of Bern, Institute of General Microbiology, Bern, Switzerland	Studies on recombination, complementation and suppressor mutation in *Schizosaccharomyces pombe*

SPEAKER	AFFILIATION	SUBJECT
Bernice Lipkin	Perinatal Research Branch, National Institute of Neurological Diseases and Blindness, Bethesda, Md.	Psychophysical considerations for computer image processing
Lewis E. Lipkin	Perinatal Research Branch, National Institute of Neurological Diseases and Blindness, Bethesda, Md.	Computer image processing concepts
Stanley Lovett	U.S. Army Biological Laboratories, Fort Detrick, Md.	Some aspects of bacterial permeability
Ross MacIntyre	Cornell University, Ithaca, N.Y.	The role of Drosophila enzyme polymorphisms in population genetics research
J. Heinrich Matthaei	Max-Planck Institute, Gottingen, Germany	Recent studies in protein synthesis
Robert K. Mortimer	University of California, Berkeley	Super-suppressors in yeast
S. K. Niyogi	Johns Hopkins University, Baltimore, Md.	The interaction of oligonucleotides with polynucleotides
Angelo C. Notides	University of Illinois, Urbana	The estrogen induced synthesis of a specific uterine protein
T. S. Osborne	UT-AEC Agricultural Research Laboratory, Oak Ridge, Tenn.	Studies on radiosensitivity of seeds
Robert Perry	Institute for Cancer Research, Philadelphia	The nucleolus and the synthesis of ribosomes
Lennart Philipson	Institute of Medical Microbiology, Department of Cell Biology, University of Uppsala, Uppsala, Sweden	Exclusion chromatography of viruses and nucleic acids on sphere-condensed agarose
Bernard Roizman	University of Chicago, Chicago	Nonpermissiveness in animal infections
Arthur Rörsch	Medisch Biologisch Laboratorium, Rijswijk, The Netherlands	Genes and enzymes involved in recovery from lethal UV damage
Walter Sauerbier	McArdle Laboratory for Cancer Research, University of Wisconsin, Madison	Host modification of bacteriophages
R. W. Schlesinger	Rutgers University School of Medicine, New Brunswick, N.J.	Control mechanisms in replication, latency and tumorigenicity of adenoviruses
Raymond Schofield	Experimental Pathology Branch, U.S. Naval Radiological Defense Laboratory, San Francisco	Spleen colonies in radiation protection
Robert Schrek	Veterans Administration; Edward Hines, Jr., Hospital; Hines, Ill.	Effects of X rays, phytohemagglutinin, and temperature on normal and leukemic lymphocytes
Drew Schwartz	Indiana University, Bloomington	The structure of maize esterases
Fred Sherman	University of Rochester School of Medicine, Rochester, N.Y.	The genetic control of cytochrome c in yeast
Jose Souto	UT-AEC Agricultural Research Laboratory, Oak Ridge, Tenn.	Studies on chemical changes in radiation injury of mammals
Adrian Srb	Cornell University, Ithaca, N.Y.	Genetic components of a developmental system in *Neurospora*

SPEAKER	AFFILIATION	SUBJECT
Audrey Stevens	University of Maryland, College Park	Submethylated ribosomal RNA fractions in bacteria
John B. Storer	Jackson Laboratory, Bar Harbor, Me.	Genetic stability, aging, and radiation resistance in mice
Paolo Strigini	Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.	On the mechanisms of spontaneous reversion recombination in bacteriophage T4
Tsutomu Sugahara	Department of Experimental Radiology, Faculty of Medicine, Kyoto University, Kyoto, Japan	Effects of nucleic acid precursors on the survival of repeatedly irradiated mice
A. L. Tappel	The Rockefeller Institute, New York	Lysosomes: Measurement of enzyme activity
D. C. Tosteson	Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C.	Cyto-differentiation and membrane transport processes in high potassium and low potassium sheep red blood cells
Otto Vos	Radiobiological Institute, Rijswijk, The Netherlands	Radiation sensitivity and repair in various types of mammalian cells
Mitchell Watson	International Business Machines, Knoxville, Tenn.	IBM administrative terminal system
J. Wallace Webster	Neutron Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.	Afterthoughts on Elsasser
Roger Weil	Department of Virology, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland	New results on the biological effects of polyoma virus
H. Brownell Wheeler	Veterans Administration Hospital, West Roxbury, Mass.	The graft-versus-host reaction in lymphoid tissue slice grafts
Frances Womack	Department of Microbiology, Vanderbilt University, Nashville, Tenn.	Recombination in T4 single-burst crosses
Hiroshi Yamazaki	Atomic Energy of Canada, Chalk River, Ontario	Analysis of products of protein synthesis directed by R17 virus RNA

SPEAKERS AT PROFESSIONAL MEETINGS, SPRING–SUMMER 1966

Southern Section of the American Society of Plant Physiologists – A. H. Haber (coauthor, M. L. Randolph)

Symposium on Modern Applications of Column Chromatography – N. G. Anderson (coauthors, J. G. Green and C. D. Scott)

14th Annual Meeting of the Radiation Research Society – M. A Bender (coauthor, P. Carolyn Gooch), R. S. Bender (coauthor, E. B. Darden, Jr.), N. K. Clapp (coauthor, E. W. Kienholz), G. E. Cosgrove (coauthors, M. L. Davis and E. B. Darden, Jr.), A. E. Sassen (coauthors, Paul Nettesheim and D. J. Kroe), L. H. Smith (coauthor, T. W. McKinley, Jr.), and J. E. Trosko (coauthors, Margaret R. Kasschau, Lana T. Covington, and E. H. Y. Chu)

Tenth Annual Meeting of the Biophysical Society – J. R. Corbett, J. E. Donellan, Jr. (coauthors, R. S. Stafford and R. B. Setlow), A. P. Harrison, Jr., S. P. Leibo (coauthor, Peter Mazur), B. H. Levedahl

(coauthor, A. A. Barber), Peter Mazur (coauthor, Walter Füchtbauer), R. A. McGrath (coauthor, R. W. Williams), Amir Muhammed (coauthor, Jane K. Setlow), R. O. Rahn, M. L. Randolph (coauthor, A. H. Haber), Jane K. Setlow (coauthor, R. B. Setlow), R. B. Setlow (coauthor, W. L. Carrier), P. A. Swenson (coauthor, Barbara Hamkalo), and B. K. Zimmerman (coauthor, G. R. Cassani)

American Crystallographic Association – J. R. Einstein

20th Annual Symposium on Fundamental Cancer Research – A. C. Upton

19th Annual Biology Research Conference – J. F. Albright (coauthor, Takashi Makinodan)

American Association of Anatomists – D. M. Morris

50th Annual Meeting of the Federation of American Societies for Experimental Biology – N. G. Anderson (coauthors, D. A. Waters, C. E. Nunley, and G. B. Cline), A. A. Barber (coauthor, Kenneth Allen), W. E. Barnett (coauthor, J. L. Epler), M. J. Bosma (coauthors, Takashi Makinodan and H. E. Walburg, Jr.), J. R. Corbett (coauthor, Carl Price), John Evans (coauthor, Stanfield Rogers), W. D. Gude (coauthors, C. C Congdon and G. E. Cosgrove), D. D. Holten (coauthor, F. T. Kenney), K. Bruce Jacobson (coauthors, E. H. Grell and J. B. Murphy), A. D. Kelmers (coauthors, M. P. Stulberg and and G. David Novelli), Paul Nettesheim (coauthor, Carol J. Chadwick), Peter Pfuderer, L. R. Shugart (coauthor, M. P. Stulberg), A. C. Upton (coauthors, J. W. Conklin and W. D. Gude), Mayo Uziel (coauthor, Waldo E. Cohn), and C. J. Wust

Association of Southeastern Biologists – P. W. Whiting (coauthor, Doris J. Bush)

66th Annual Meeting of the American Society for Microbiology – Claire M. Berg, L. G. Caro, L. J. Charamella (coauthor, Roy Curtiss III), Roy Curtiss III (coauthor, L. G. Caro), Robert Fujimura (coauthor, Elliot Volkin), Alfred Hellman (coauthors, J. G. Farrelly and D. H. Martin), and R. T. Jones (coauthors, Roy Curtiss III, R. C. Allen, and Paula E. Harris)

57th Annual Meeting of the American Association for Cancer Research – N. K. Clapp (coauthor, A. W. Craig), W. W. Harris (coauthors, E. L. Candler, Jr., N. G. Anderson, and C. T. Rankin, Jr.), and Stanfield Rogers

Foreign Travel

On September 24–27, M. A Bender attended the XIth International Congress of Radiology in Rome and presented a paper entitled "Chromosome aberrations in irradiated humans." While in Italy Dr. Bender conferred with Dr. Guglielmo Marin at the Laboratorio Internazionale di Genetica e Biofisica, Naples, on September 28–29.

Tuneo Yamada traveled to Mexico City, Mexico, October 25–30, to present a series of lectures on developmental biology at the Centro de Investigación y de Estudios Avanzados, Instituto Politecnico Nacional. Titles of lectures presented were: (1) "Problems of embryonic induction," (2) "Studies of tissue transformation I," and (3) "Studies of tissue transformation II."

E. F. Oakberg was in Geneva, Switzerland, November 2–8 to participate in a World Health Organization meeting on "Chemistry and Physiology of the Gametes."

Crodowaldo Pavan and W. L. Russell attended meetings of the United Nations Scientific Committee for the Studies of the Effect of Atomic Radiation in Geneva, Switzerland, November 19–23. Dr. Russell is a scientific advisor to the United States delegation. Following the meetings, Dr. Pavan conferred with Prof. Hans Kalmus, University College, London, England, on November 26. On November 24–25, Dr. Russell was in Bern, Switzerland, to confer with Dr. Hans Cottier at the University of Bern, and presented a seminar on mammalian radiation genetics.

Four Biology Division investigators – Alexander Hollaender, O. L. Miller, Jr., Crodowaldo Pavan, and R. C. von Borstel – attended the Symposium on the Nucleolus, Its Structure and Function (December

5–10, 1965) at the Faculty of Medicine, University of the Republic, Montevideo, Uruguay. This symposium was the fifth in a continuing series of conferences sponsored by various Latin American institutions with the encouragement of the Biology Division, ORNL. (Other details are presented in a description of the symposium on page 26.) In addition to attending the symposium, the four investigators conferred and visited as indicated below:

Dr. Hollaender visited the University of São Paulo, São Paulo, Brazil, December 11–13, and the University of Bahia, Bahia, Brazil, December 14–19; and then traveled to Mexico City, Mexico, for a December 20–22 meeting of the organizing committee of the Symposium on Enzymatic Aspects of Metabolic Regulation to be held in Mexico City, November 28–December 1, 1966.

Dr. Miller visited biological laboratories in Peru, Chile, Uruguay, Argentina, and Brazil to discuss cytological research and related problems of mutual interest in these countries. During the course of these visits, he presented four talks on results of his research at the Biology Division. (Lectures are listed on page 13.)

Following is a summary of his visits: November 22–23, the Laboratory for Investigation of Cancer, Lima, Peru; November 24–26, University of Chile, Santiago, Chile; November 27–December 4, Biological Sciences Institute, Montevideo, Uruguay; December 11–17, (1) Instituto de Investigationes Hematologicas, Academia Nacional de Medicina, Buenos Aires, Argentina; and (2) the Argentine Atomic Energy Commission, Buenos Aires, Argentina; Laboratory of Genetics, University of Buenos Aires, Buenos Aires, Argentina; School of Biological Chemistry, National University of La Plata, La Plata, Argentina; Institute of General Anatomy and Embryology, University of Buenos Aires, Buenos Aires, Argentina; December 18–20, University of São Paulo, São Paulo, Brazil; and December 21–24, Institute of Biophysics, University of Brazil, Rio de Janeiro, Brazil.

After the symposium, Dr. Pavan visited the Department of General Biology at the University of São Paulo, where he conferred with Prof. Antonio Brilō da Cunha, Prof. Oswaldo Frota-Pessoa, Dr. L. E. de Magalhães, and other members of that laboratory.

Dr. von Borstel visited biological institutes in Peru, Chile, Argentina, and Brazil, presenting seminars and discussing problems of mutual interest. On November 22–23, he visited Peruvian University "Cayetano Heredia," Lima; November 29–30, the University of Chile, Santiago; December 2–4, the University of Buenos Aires and the Argentine Atomic Energy Commission, Buenos Aires; December 13–14, the University of São Paulo, São Paulo; December 15, the University of São Paulo at Ribeirão Preto; December 16, (1) the Faculty of Philosophy, Science, and Letters, Rio Claro, Brazil, and (2) the University of São Paulo at Piracicaba, Brazil; and December 17–20 at the University of Brazil, Rio de Janeiro, Brazil. Lectures presented by Dr. von Borstel during this trip are listed on page 16 of this report.

J. W. Longworth was in Toronto, Canada, January 4 and 5 to present a seminar entitled "Protein and nucleic acid luminescence" to the Physics Division of the Ontario Cancer Institute and to discuss fast monochromators and flash photolysis with Dr. H. E. Johns.

Meetings and Conferences

NINETEENTH ANNUAL BIOLOGY RESEARCH CONFERENCE (1966)

The 1966 Research Conference, sponsored by the ORNL Biology Division, will be held April 4–7 at the Riverside Motor Lodge in Gatlinburg, Tennessee. The organizing committee for the symposium program included Takashi Makinodan (chairman), J. F. Albright, F. T. Kenney, R. A. Popp, and Tuneo Yamada.

In addition to the regular symposium program, a preconference round-table discussion on the cytokinetics of the immune response is planned for April 2–3. The program for this discussion was planned by Takashi Makinodan, J. F. Albright, and J. J. Vazquez, a consultant for the Division from the Department of Pathology, Duke University. At this meeting, invited investigators will discuss concepts, techniques, and nomenclature pertaining to antibody formation as the manifestation of a differentiating, self-renewable cell system. It is hoped that such a discussion will help in overcoming problems of communication between investigators representing various disciplines (genetics, cytology, biomathematics).

The programs for the round-table discussion and the symposium follow:

I. PROGRAM: ROUND-TABLE DISCUSSION ON THE CYTOKINETICS OF THE IMMUNE RESPONSE

SATURDAY, APRIL 2

Session I

Initial Events – Moderator, Takashi Makinodan

A. Nature of stimulant

B. Nature of receptor sites (cells)

C. Nature of the stimulation process (differentiation)

Session II

Productive (Synthetic) Events – Moderator, D. W. Talmage

A. Synthesis of antibody

B. Proliferation

SUNDAY, APRIL 3

Session III

Terminal Events – Moderator, J. J. Vazquez

A. Somatic vs functional death

B. Transformation

II. PROGRAM: SYMPOSIUM ON DIFFERENTIATION AND GROWTH OF HEMOGLOBIN- AND IMMUNOGLOBULIN-SYNTHESIZING CELLS

MONDAY, APRIL 4

Opening Remarks – Alexander Hollaender

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

Introduction – Takashi Makinodan
Chairman, Organizing Committee

Morning Session

Structure of Hemoglobins

Chairman – V. M. Ingram, Department of Biology, Massachusetts Institute of Technology, Cambridge

Phylogenetic variation in the primary structure of hemoglobins – Gerhard Braunitzer, Max-Planck-Institut für Biochemie, München, Germany

Tertiary structure of hemoglobins and its functional significance – Makio Murayama, Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Maryland

Afternoon Session

Structure of Immunoglobulins

Chairman – Felix Haurowitz, Department of Chemistry, Indiana University, Bloomington

Primary structure of immunoglobulins and its relationship to antibody specificity – Marian E. Koshland, Department of Molecular Biology, University of California, Berkeley

Subunits of immunoglobulins and their relationship to antibody specificity – R. R. Porter, Department of Immunology, Wright-Fleming Institute, St. Mary's Hospital Medical School, London, England

TUESDAY, APRIL 5

Morning Session

Genetic Regulation of Specific Polypeptide Synthesis

Chairman – George Klein, Institute for Tumor Biology, Karolinska Institutet, Stockholm, Sweden

Genetic regulation of peptide synthesis in hemoglobins – Harvey A. Itano, Laboratory of Molecular Biology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Maryland

Genetic regulation of immunoglobulin synthesis – Jacques Oudin, Service d'Immunochimie Analytique, Institut Pasteur, Paris, France

Afternoon
(Free)

WEDNESDAY, APRIL 6

Morning Session

Cytokinetics

Chairman – J. E. Till, Department of Medical Biophysics, University of Toronto, Toronto, Canada

Cytokinetics of antibody formation – Guy Sainte-Marie, Cancer Research Laboratory, The University of Western Ontario, London, Canada

Discussant – Miroslav Simić, Institute "Boris Kidrich," Belgrade, Yugoslavia

Cytokinetics and regulation of progenitor cells – L. G. Lajtha, Paterson Research Laboratories, Christie Hospital & Holt Radium Institute, Withington, Manchester, England

Afternoon Session

Development

Chairman – Edward C. Horn, Department of Zoology, Duke University, Durham, North Carolina

The thymus and the ontogenesis of the immune system – Donald Metcalf, Walter & Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia

Ontogenesis of erythrocytes and hemoglobin formation – Corrado Baglioni, International Laboratory of Genetics and Biophysics, Naples, Italy

THURSDAY, APRIL 7

Morning Session

Growth and Senescence: Conference Appraisal and Conclusions

Chairman – Edwin S. Lennox, The Salk Institute for Biological Studies, San Diego, California

Growth and senescence of antibody-forming cells – J. F. Albright and Takashi Makinodan, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Concluding remarks: the regulation of hemoglobin and immunoglobulin synthesis – Ray D. Owen, Division of Biology, California Institute of Technology, Pasadena, and J. Claude Bennett, Department of Medicine, University of Alabama Medical Center, University

SYMPOSIUM ON THE NUCLEOLUS, ITS STRUCTURE AND FUNCTION

Four Biology Division investigators – Alexander Hollaender, O. L. Miller, Jr., Crodowaldo Pavan, and R. C. von Borstel – attended the symposium on the Nucleolus, Its Structure and Function (December 5–10, 1965) at the Faculty of Medicine, University of the Republic, Montevideo, Uruguay. This symposium was the fifth in a continuing series of conferences sponsored by various Latin American institutions with the encouragement of the Biology Division, ORNL.

During the conference, it was announced that Dr. Hollaender had been elected an honorary member of the Biology Society of Montevideo.

Dr. Hollaender served as President of the symposium, and Drs. Miller, Pavan, and von Borstel were on the organizing committee. In addition, Dr. Hollaender gave both opening and closing remarks. Dr. Miller presented a paper "Structure and composition of peripheral nucleoli from salamander oocytes," and is serving as coeditor of the symposium proceedings with Dr. W. S. Vincent, Department of Anatomy and Cell Biology, University of Pittsburgh, and Drs. F. A. Saez and M. E. Drets of the Instituto de Investigación de Ciencias Biologicas, Montevideo. Dr. Pavan was a chairman of the session on Nucleolar Genes, and Dr. von Borstel for that on The Nucleolus in the Cell Cycle and During Development.

Nine scientific sessions were held during the symposium week covering the subjects of nucleolar structure and composition, nucleolar genes, nucleolar activities, and the nucleolus in the cell cycle and during development. The sessions included a total of 31 papers, three motion-picture filmstrips,

and a panel discussion on the role of the nucleolus in the biogenesis of ribosomes. Spanish or English translation was provided through individual headsets during the conference.

A total of 118 participants registered for the symposium. The countries represented and numbers registering from each were as follows:

Argentina	40	France	3	Sweden	1
Belgium	1	Germany	3	United States	25
Brazil	7	Italy	2	Uruguay	15
Canada	1	Peru	1	Venezuela	1
Chile	15	Scotland	3		

In addition to the registered Uruguayan participants, a large number of students from the various faculties of the University of the Republic attended portions of the symposium.

Symposium sponsors were Biology Division, Oak Ridge National Laboratory; Departmental Council of Montevideo; Institute for Cancer Research, Philadelphia, Pennsylvania; National Council of Scientific and Technical Research (Uruguay); Organization of American States; United States Atomic Energy Commission; United States National Science Foundation; University of the Republic (Montevideo); University of Pittsburgh, Pittsburgh, Pennsylvania; Faculty of Medicine of Montevideo; Faculty of Humanities and Sciences of Montevideo; Faculty of Veterinary Science of Montevideo; and National Commission of Tourism (Uruguay).

ANNUAL INFORMATION MEETING

The Oak Ridge National Laboratory Advisory Committee for Biology made its annual visit to the Division November 17, 18, and 19. The committee is composed of C. E. Carter, Western Reserve University Medical School; Erwin Chargaff, Columbia University; Rollin D. Hotchkiss, The Rockefeller Institute; Henry S. Kaplan, Stanford University Medical School; Earl R. Stadtman, National Heart Institute; and Curt Stern, University of California.

During this visit, Division investigators summarized their work for the visitors at a conference in the Division, and the committee members visited individual laboratories and participated in informal discussions.

BONE MARROW CONFERENCES

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

December Conference Held. – The latest in the series of bone marrow and chemical protection conferences was held December 2–3 at the United States Naval Radiological Defense Laboratory, Hunter Point, San Francisco. This conference was organized by Joan Wright Goodman and L. J. Cole.

Session topics included the following: (1) chemical protection against radiation injury and injury from toxic chemicals, (2) bone marrow transplantation, (3) immunotherapy of cancer, (4) cell separation (hemopoietic and lymphatic tissues), (5) stem cells (hemopoietic and lymphatic tissues), and (6) experimental hematology.

Papers presented by Division investigators include: "Modification of secondary disease mortality," by C. C Congdon (coauthored by M. A. Kastenbaum and D. A. Gardiner); "Some unusual effects of preimmunization with living parental cells," by Joan Wright Goodman (coauthored by Gayle C. Bosma); and "Morphologic changes in spleen lymphatic tissue during antibody production," by M. G. Hanna, Jr. (coauthored by D. C. Swartzendruber and C. C Congdon).

Other Meetings Scheduled. – Plans for future meetings include an evening session at the time of the Federation meetings in Atlantic City, New Jersey, April 12, 1966, and a special conference on Germinal Centers of Lymphatic Tissue to take place in Bern, Switzerland, June 22–24, 1966, just prior to the Third International Congress of Radiation Research in Cortina d'Ampezzo, Italy. Hans Cottier, Universität Bern, is arranging the germinal center meeting.

Experimental Hematology. – The ninth issue of *Experimental Hematology* has been published. The abstract booklet covers the December 1965 conference in San Francisco and also includes, for the first time, additional (i.e., not presented at the conference) invited papers on related subject matter. *Experimental Hematology* is edited and organized in the Division, with the assistance of the Technical Publications Department of the ORNL Technical Information Division.

FALL MEETING OF BIOMEDICAL PROGRAM DIRECTORS

S. F. Carson attended the fall meeting of the Biomedical Program Directors of the United States Atomic Energy Commission on October 14–15 in Los Alamos, New Mexico. These meetings are held three times each year to review research in the various biomedical programs at Commission laboratories.

Educational Activities

THE UNIVERSITY OF TENNESSEE–OAK RIDGE GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

A project designed to develop biomedical scientists capable of original research in biology and fundamental medical sciences has been jointly undertaken by the Biology Division and the University of Tennessee.

Based on a special report prepared after a five-month study by a UT- and ORNL-appointed committee, a plan to establish the Oak Ridge Graduate School of Biomedical Sciences was unanimously approved by the UT Board of Trustees on November 5, 1965. The committee had recommended that the proposed educational unit be classified as a school and be authorized to grant the Ph.D. degree.

R. Clinton Fuller, a former chairman of the Microbiology Department at Dartmouth Medical School and presently a visiting professor at the Department of Life Sciences at the University of California, Riverside, has been named the first director of the new school. He will assume his new position in September 1966, and the first students will be accepted in September 1967.

J. L. Liverman, associate director of the Biology Division, is serving as interim director of the biomedical school. Members of the faculty (including some Division investigators who will teach on a part-time basis) are to be selected.

ORNL members of the study committee were: J. L. Liverman, Chairman, H. I. Adler, S. F. Carson, C. C Congdon, R. F. Kimball, G. David Novelli, and R. B. Setlow, all of the Biology Division; and ex-officio members of the committee were Alexander Hollaender and M. L. Nelson, Director of Education and University Relations, ORNL.

This cooperative effort by the University and the Laboratory should prove mutually beneficial, and will help fulfill a need for institutions offering a strong program in subjects such as biochemistry, biophysics, genetics, immunology, and carcinogenesis studies. Teaching emphasis will be placed on basic rather than clinical approaches to disease problems.

This activity has the support of Glenn T. Seaborg, Chairman of the U.S. Atomic Energy Commission, and of James Shannon, Director of the National Institutes of Health. These two agencies, AEC and NIH, are the principal supporters of research activities in the Laboratory.

STUDENT TRAINEE PROGRAM

Applications for the Student Trainee Program have been reviewed, and 34 undergraduates will be selected to participate in 1966.

Participants are selected on a competitive basis from applicants throughout the United States for temporary summer appointments in the Biology Division. This Summer Student Trainee Program is administered for the U.S. Atomic Energy Commission by the Oak Ridge Associated Universities and is carried out in cooperation with the Oak Ridge National Laboratory.

Through this Program, especially selected juniors from small colleges are assigned to and trained within the Biology Division. They are provided the atmosphere and opportunity to develop a better comprehension of research methods and their significance by carrying out research activities under the guidance of Biology Division scientists.

Each student selects, plans, and executes a research project during his stay. Upon the completion of his project, he prepares a paper and gives an oral report describing the work and results.

In addition, a small group of students who have just received a bachelor's degree and have been accepted by a graduate school will be selected to work in the Division for the summer.

UNIVERSITY COOPERATIVE PROGRAM

H. I. Adler and A. H. Haber are continuing their association with the University of Tennessee in part-time teaching and advisory capacities under the joint UT-ORNL Cooperative Research Program sponsored by the Ford Foundation.

During the academic year, Dr. Adler has been lecturing on bacterial genetics for the University's Department of Bacteriology. Dr. Haber taught a winter quarter course on Developmental Plant Physiology in the UT Botany Department.

Ellen Mattingly is teaching a course on Cytogenetics during the spring quarter in the UT Botany Department under a modified Ford Foundation program.

COURSE ON PRINCIPLES OF BIOCHEMISTRY

A course on the Principles of Biochemistry was offered this fall and winter at the Special Training Building of the Oak Ridge Institute of Nuclear Studies, a unit of Oak Ridge Associated Universities, under the University of Tennessee Graduate Training Program.

The lecture course was open to applicants with the B.A. degree, its equivalent, or an advanced degree. The course covered carbohydrates, proteins, nucleic acids, intermediary metabolism, enzyme kinetics, protein biosynthesis, biochemical aspects of genetics, and topics in physical and medical biochemistry. The Division's F. J. Finamore directed the course.

INTRODUCTORY COURSE IN MODERN ZOOLOGY

Biology Division members are continuing to contribute their services in the Introductory Course in Modern Zoology, now being taught at the Oak Ridge Institute of Nuclear Studies, a unit of Oak Ridge Associated Universities, in cooperation with the Division and the University of Tennessee. The course was organized by the Division's G. L. Whitson and N. G. Anderson, and is now being taught in its second year as a UT course.

In general, the course schedule was planned for college graduates in nonbiological fields and with no previous courses in zoology. Subject matter includes the following: molecular biology, intermediary metabolism, cell substructure, organization of multicellular systems, cellular and organismal control systems, zoological taxonomy, structure and function of animal systems, morphological and chemical embryology, and principles of genetics and evolution.

Biology Division members who have given lectures and conducted lab sessions thus far are N. G. Anderson, W. A. Arnold, W. E. Barnett, Roy Curtiss III, D. G. Doherty, L. H. Elrod, F. J. Finamore, W. D. Fisher, Tom Grizzard, W. D. Gude, T. R. Manney, Peter Mazur, R. A. Popp, A. C. Upton, Mayo Uziel, R. C. von Borstel, and G. L. Whitson.

Tentative plans call for the series to be offered again in 1966–67.

PARTICIPATION IN OTHER COURSES

On August 17 and 18, R. F. Kimball presented two lectures on "Radiation genetics" as part of a summer training course in Radiation Biology given at St. Augustine's College, Raleigh, North Carolina.

Sheldon Wolff was in Providence, Rhode Island, August 26–27, to participate in the 1965 Summer Course in Mammalian Chromosome Cytology. His subjects were "Radiation and chemical effects on chromosomes" and "On the chemistry of chromosome continuity."

During the period October 18 to 25, A. C. Upton gave a series of lectures on Radiation Injury: Principles and Perspectives at the University of Chicago, Chicago, Illinois. Titles of his lectures were: "Radiation sickness," "Growth, development, and longevity," "Carcinogenesis," and "Prevention and control."

L. H. Elrod attended the Amino Acid Analyzer Training Course at the Technicon Chromatography Corporation, Chauncey, New York, October 25–29.

W. D. Gude spoke on "Autoradiography" at the Oak Ridge Institute of Nuclear Studies Medical Radioisotope Course on October 7. On January 18, 1966, he presented two lectures entitled "Biological effects of radiation on mammals" at Alcoa High School, Alcoa, Tennessee, under the Tennessee Academy of Sciences Visiting Scientist Program.

G. David Novelli was a guest lecturer at a special course, Topics in Molecular Biology of Nucleic Acids, at Temple University School of Medicine, Philadelphia, Pennsylvania, on January 19. He spoke on "Protein synthesis during liver regeneration."

W. E. Barnett is serving as a visiting lecturer in zoology during the fall, winter, and spring quarters of UT's Oak Ridge Resident Graduate Program held in the Special Training Building of the Oak Ridge Institute of Nuclear Studies, a unit of Oak Ridge Associated Universities. The course presented by Dr. Barnett is entitled Molecular Biology, and Jane K. Setlow is participating as guest lecturer.

Two other Division investigators will teach spring courses under the UT graduate program. O. L. Miller, Jr., will teach Zoology 4310, a four-hour course on Animal Cytology; and W. D. Gude will teach Zoology 3320, a four-hour course on Histology.

J. D. Regan has been appointed to the faculty of the University of Tennessee and will teach a course on Human Genetics in the Department of Zoology during the spring quarter.

Division Members on Leave of Absence

W. F. Bertsch is at Cambridge University, England, where he is working in the Biochemistry Department with Dr. Robert Hill. Dr. Bertsch's one-year stay in Cambridge is being jointly supported by a National Institutes of Health Special Fellowship and by the Oak Ridge National Laboratory. He left the Division in October 1965 and will return in early November 1966.

Audrey N. Best is on leave of absence from the Enzymology Group while completing her thesis research for the Ph.D. degree under an Oak Ridge Associated Universities Predoctoral Fellowship. Miss Best expects to complete her work during 1966 and will be awarded the degree by the University of Georgia, Athens.

E. H. Y. Chu was awarded a National Science Foundation Senior Postdoctoral Fellowship to spend one year at the Karolinska Institute, Stockholm, Sweden, where he is working with Dr. George Klein. Dr. Chu's stay in Sweden, which is being jointly sponsored by ORNL and NSF, began in September 1965 and will end in September 1966.

Joan Wright Goodman is on leave of absence from the Mammalian Recovery Group to spend six months in the Weizmann Institute of Science, Rehovoth, Israel, under the sponsorship of ORNL and a Louis Lipsky Fellowship. Dr. Goodman will return to the Division in August of 1966.

V. K. Jenkins has been awarded a U.S. Public Health Service Predoctoral Fellowship to carry on graduate study at the University of Tennessee. He will be on leave from the Pathology and Physiology Group while continuing his study under this fellowship.

D. L. Lindsley is spending a year in Rome, Italy, at the Istituto di Genetica in the laboratory of Professor Giuseppi Montalenti, under the joint sponsorship of a National Science Foundation Senior Postdoctoral Fellowship and the Oak Ridge National Laboratory. Dr. Lindsley will return to the Laboratory in September of 1966.

Cytology and Genetics

R. F. Kimball[a]

Effects of Radiation on Paramecium (1.1–1.5)

R. F. Kimball[a]
D. Q. Brown[b]
G. L. Whitson
D. B. Williams[c]
E. G. Bailiff[a]
Barbara R. Bussell[a]
Etheleen L. Cooper
Stella W. Perdue
Carrie L. Wells
Esther L. Williams[c]

Phage Genetics (1.6–1.9)

Jane K. Setlow
C. M. Steinberg
Amir Muhammed[d]
M. E. Boling

Mammalian Genetic Cytology (1.10–1.13)

E. H. Y. Chu[a,e]
J. E. Trosko[a]
J. D. Regan[f]
Lana T. Covington
Margaret R. Kasschau[a]
Nina S. Hammer[a]

Mammalian Biochemical Genetics (1.14, 1.15)

R. A. Popp
W. S. McEwen
Judith G. Heddle
Diana M. Popp

***Rhynchosciara* Genetics (1.16–1.20)**

Crodowaldo Pavan[d]
Renato Basile[d]
Günter Weirich[d]
Shirley P. Ogle
L. C. G. Simões

Participation in Cooperative Programs:

AEC-NIH Chemical Carcinogenesis (23.6, 23.7)

[a]Dual assignments.
[b]Research participant.
[c]Loanee.
[d]Visiting investigator from abroad.
[e]Leave of absence, Karolinska Institute, Stockholm.
[f]USPHS Fellow.

1.1 MITOSIS AND MUTATION INDUCTION BY X RAYS

R. F. Kimball
E. G. Bailiff
Barbara R. Bussell
Stella W. Perdue

Elsewhere in this report[1] evidence was given that an alkylating agent, triethylene melamine (TEM), induces a single kind of premutational damage that is subject to repair at a constant rate throughout the cell cycle, including mitosis, and is fixed as mutation only at DNA synthesis, not at any other time. Our earlier work[2] showed that x rays produced premutational damage that is reparable throughout the preduplication interphase (G_1) period and is converted to final mutation at the DNA synthesis (S) period, accounting for between 50 and 75% of the mutation yield. The remaining 25 to 40% appears to be formed by processes, rejoining of broken chromosomes for

example, that are independent of the S period. Almost all premutational damage, whatever the kind, seems to be repaired when it is produced by irradiation in the postduplication interphase (G_2) period. The present studies were designed to find out what effect mitosis might have upon these various types of damage and their repair.

In one set of experiments, groups of synchronized cells were irradiated at known, closely spaced stages from late G_2 through mitosis to early G_1. A sample of each group was fixed at the time of irradiation to determine the stage. The results show a very rapid increase during mid- to late prophase from the low yields of G_2 to high yields in midmitosis. The yields reach a high point in midmitosis and decline somewhat by very early G_1 and then rise again. Previous work has shown that the mutation yield increases nearly linearly throughout G_1, reaching a maximum at S, presumably because of the decreasing time for repair. Neither the high yield in midmitosis nor the low yield in G_2 can be explained by an extrapolation backward of the G_1 curve.

The low G_2 yield with TEM can be explained by the greater time available for repair before fixation at S.[1] No change in the rate of repair is required. The G_2 yield with x rays is so much lower that this explanation cannot be applied, and a change in the rate of repair seems the most reasonable explanation. This would imply that x-ray-induced premutational damage of the kind that is fixed at S has different repair properties from the otherwise similar premutational damage induced by TEM. Consequently there would have to be at least two different reparable lesions with somewhat different repair properties.

There are several possible explanations for the high yield in midmitosis: (1) There may be a short time for repair of some of the damage because of some mutation fixation at mitosis. (2) There may be an especially high frequency of events, such as chromosome aberrations, that are formed rapidly during this stage. (3) There may be a greater indirect effect and, consequently, more initial damage. These possibilities are not necessarily mutually exclusive. Tests of some of them are in progress.

With TEM,[1] premutational damage induced in G_2 has been shown to persist in reparable form through mitosis by treatment with a mutation-reducing agent, streptomycin, after mitosis. The vanishingly small G_2 yields make this test impossible for x rays, but it is possible to irradiate in midmitosis and then give a streptomycin treatment after division to check for reparability. The result was clear. Streptomycin treatment after division reduces the yield of mutations produced by irradiating in midmitosis. However, the reduction seemed to be less than with cells irradiated in G_1, suggesting that some premutational damage may have been fixed between midmitosis and the postdivision G_1 period. Thus there is no question that some premutational damage produced in midmitosis is not fixed until the next cell cycle, but some of it may be fixed before completion of division. Further tests are being carried out to test this matter more thoroughly.

References

[1]R. F. Kimball *et al.*, "Mitosis and Mutation Induction by TEM," this report.

[2]R. F. Kimball, pp. 167–78 in *Repair from Genetic Radiation Damage* (F. H. Sobels, ed.), Pergamon, London and New York, 1963.

1.2 FURTHER STUDIES ON INTRACLONAL VARIATIONS IN CELL MASS AND GROWTH RATE IN *PARAMECIUM AURELIA*

R. F. Kimball Stella W. Perdue
E. G. Bailiff

Previously we have reported[1,2] that different clonal and subclonal lines of descent within a genetically homogeneous stock of *Paramecium aurelia* differ significantly from one another in dry weight as measured by scanning microinterferometry of recently divided cells. This variation should provide information about the control of cell growth and division processes and in any case must be understood before studies on the effect of various agents on growth and division can be evaluated for possible long-term effects upon the descendants of treated cells. The previous studies have shown that the characteristic size of a given line of descent is a temporary

feature, persisting in some cases for several days and for a number of cell generations.

One possible explanation for these size differences is that they result from variations in the amount of DNA per cell. Most of the DNA of this species is in the macronucleus, and this nucleus divides amitotically into two parts that are only roughly equal. Thus the two daughter cells could have different DNA contents because of unequal division, and these inequalities might persist if there were no mechanism for readjusting the macronuclear size. This explanation is made plausible by the previously reported[2] observation that the macronuclear DNA content, as measured by Feulgen microspectrophotometry, is highly correlated with the dry weight of the cell.

A further study of this relation was made in a strain of *P. aurelia* that is homozygous for an allele, *am*, that greatly increases the frequency of grossly unequal divisions of the macronucleus. Cytoplasmic division is also sometimes unequal but not to the same extent as nuclear division. The cells of this strain are much more variable in dry weight and in DNA content than those of the normal; and, just as in the normal, there was a high correlation between the DNA content and dry weight of the whole cell. The variation in DNA content was about the same at the beginning and the end of the cell cycle, showing that there was little if any regulation back to a "normal" amount during this period. This finding supports the hypothesis that variations in DNA content arising through unequal division persist over at least one and quite probably many cell generations. The data from the *am* homozygote confirm, with a wider range of values than previously available, that the ratio of the DNA content to total cell dry weight is not a constant but increases as the cell weight increases.

Cell size is determined by the rate of increase in cell weight and the frequency with which the cell divides. It was previously reported[2] that dry weight and division rate are positively correlated. Thus the larger cells tend to divide somewhat more frequently than the smaller ones. Further work has confirmed this, but the correlation, though statistically significant, is very small. These findings suggest that variations in the rate of increase in cell mass rather than in the frequency of cell division are the main reason for the variations in cell size.

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1.3 DIVISION DELAY IN *SPATHIDIUM SPATHULA* INDUCED BY X RAYS AND CHEMICAL INHIBITORS

D. B. Williams

Introduction. – *Spathidium spathula* is 10 to 20 times more sensitive than other ciliates to x-ray-induced division delay, but unlike other ciliates it continues to feed and increase in cell size.[1] The reasons for the high sensitivity are not known, but spathidia are not unusually sensitive to radiation-produced hydrogen peroxide, nor is the macronucleus markedly less polyploid than that of other ciliates.[1] The present study is concerned with the division delay phenomenon as it related to *Spathidium*'s high radiosensitivity. Division delay induced by 5 kr of x rays over a period of five cell divisions was studied as a function of time of exposure during the cell cycle. Dry mass measurements were made on individual cells in order to establish more precisely the effect of x irradiation on cell growth and generation time. Spathidia were exposed to a variety of inhibitory agents, including antibiotics and alkylating agents, to determine if any of these simulate the effect of x rays, in turn suggesting what cell systems are primarily responsible for the x-ray sensitivity.

Methods. – Spathidia were synchronized by selecting dividing cells over a 10-min period from one-day-old log-phase cultures. In most experiments the cells were treated with inhibitory agents or irradiated at a standard time of 30 to 60 min after division. [One group of experiments was performed to test the effect of 5 kr on cells of different ages (5 to 40%) in the cycle.] Exposure to antibiotics was either continuous or for 0.5, 1.5, 3, 6, 12, or 24 hr. Exposure to the alkylating agent triethylene melamine (TEM) was for 10 min followed by three rapid washings in culture fluid,

giving a dilution of greater than 1/1000 in a period of 3 min. The effect of 5 kr of x rays on cell mass was studied by drying cells on slides immediately after division. A scanning microinterferometer was used to measure the dry mass of individual cells.

Results and Discussion. – The effectiveness of 5 kr in delaying the first and later divisions was different for cells irradiated at different times in the cell cycle. The first division showed the greatest delay. Cells irradiated when 5% into the cycle (45 min after division) showed a 70% increase over the control in the first division, whereas cells irradiated when 20% into the cycle showed a 135% increase. Cells aged 30 to 40%, however, gave variable results, showing a 68 to 103% increase. Spathidia are more resistant to x irradiation in early G_1 than in later periods of the cycle.

Microinterferometric measurements of total cell mass, at division, of control cells and of cells exposed to 5 kr when 5% into the cell cycle revealed that although the irradiation caused an 80% increase in generation time (17.3 hr compared to 9.5 hr), it did not interrupt growth in cell mass. At division the irradiated cells had twice the average mass of the controls.

Chloramphenicol and puromycin, two protein inhibitors, in a range of doses, did not delay cell division or cause any other abnormality observed in x-irradiated spathidia. It was concluded that *Spathidium*'s high radiosensitivity is not related to interference with protein synthesis, a conclusion supported by the dry mass measurements after irradiation.

Actinomycin D, an inhibitor of RNA synthesis, did cause an increase in generation time (10 μg/ml completely inhibited division), but the cells were smaller rather than larger when division occurred. No other abnormality was observed, and the cells recovered to form normal clones.

It was established by using mitomycin C (10 μg/ml) and TEM (1 mg/ml) – both agents which cause either degradation of DNA or interfere with DNA synthesis – that the damage caused by x rays was of this same nature. Both, like x rays, cause division delay with no interference to feeding and continued growth of the cell. Also, as after x irradiation, the cells had enlarged macronuclei and Feulgen-positive fragments in the cytoplasm, and the majority were without micronuclei.

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1.4 GROWTH OF *TETRAHYMENA* AFTER REMOVAL AND ADDITION OF INORGANIC PHOSPHATE

G. L. Whitson Etheleen L. Cooper
W. D. Fisher[1]

Introduction. – Isotope labeling experiments using ^{32}P have shown that *Tetrahymena* grown in proteose-peptone–liver-extract medium incorporate very little of this label into acid-insoluble RNA.[2] We assumed that this failure to incorporate ^{32}P was due to the presence of large amounts of excess inorganic phosphate in the medium. We have, therefore, decided to study the effects of both the removal and addition of inorganic phosphate on the growth of *Tetrahymena*.

Methods. – The medium used for growing *Tetrahymena* (1% proteose peptone with 0.1% liver extract) was dephosphorylated according to the procedures of Countryman and Volkin.[3] Such medium, after treatment, contains only 2 to 3 μg/ml of inorganic phosphate. For supplementation of inorganic phosphate, a solution containing 2 g of NaCl, 1 g of potassium phosphate (dibasic), and 1 g of potassium phosphate (monobasic) was made up to 1 liter in triple-distilled water. Several different concentrations of this solution were added to flasks containing 250 ml of the dephosphorylated medium. Each flask was then inoculated with *T. pyriformis* strain GL-C, and cell counts were made with a Coulter counter.

Results. – As shown in Fig. 1.4.1, removal of most of the inorganic phosphate from the proteose-peptone–liver-extract medium allowed for nearly normal growth, as indicated by a generation time and final cell density comparable to those of control cells. Although addition of known amounts of inorganic phosphate produced somewhat shorter generation times for those cells, there appears to be a limit after which further addition of phosphate to the medium fails to promote better growth.

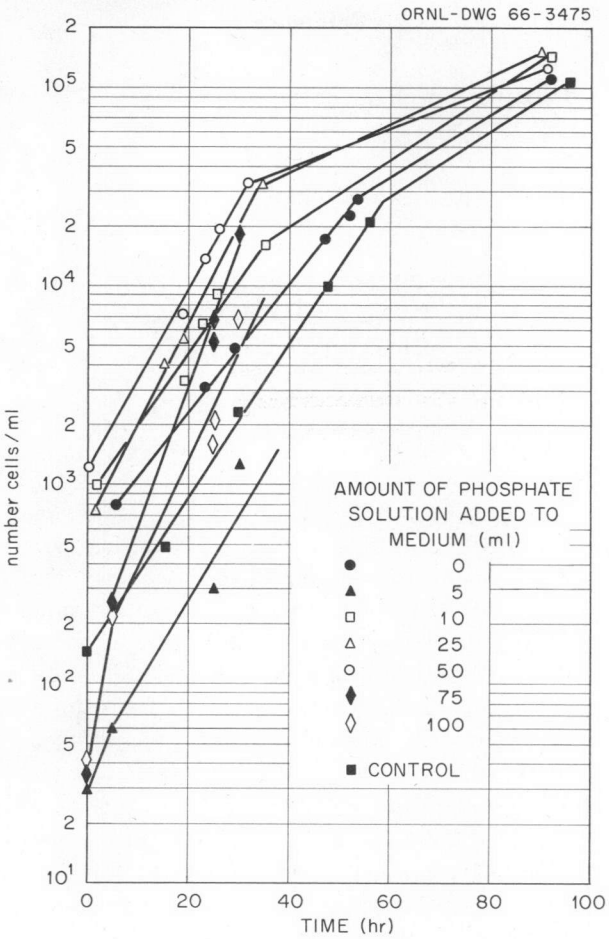

Fig. 1.4.1. Effect of Various Concentrations of Inorganic Phosphate on Growth of *Tetrahymena*.

It appears then that cells will grow well under conditions of low phosphate and that this should allow for a greater incorporation of ^{32}P into acid-insoluble RNA and DNA when this isotope is added to cells grown in this medium.

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1.5 CHANGES IN SOLUBLE CARBOHYDRATES OF SYNCHRONIZED *TETRAHYMENA*

G. L. Whitson J. G. Green[1]

Introduction. – Changes in soluble carbohydrates of synchronized *Tetrahymena* are being investigated by the use of a prototype carbohydrate analyzer.[2] The availability of large quantities of heat-shock-synchronized *Tetrahymena* and the use of this analyzer permit the examination of the total content of soluble sugars with a single analysis, whereas previous investigations have been restricted to the study of only limited numbers of soluble components.[3]

Methods. – Several liters of log cells of *T. pyriformis* strain GL were synchronized using the heat-shock method described by Scherbaum and Zeuthen.[4] About 85% of the cells are in the process of cell division 90 min after the last heat shock. Cells used in this study were sampled at EH (end of last heat shock), at 75 min after EH (just prior to cell division), at 90 min after EH (during peak of cell division), and at 135 min after EH (after cell division has occurred). Cells were pelleted by centrifugation, and 1-ml aliquots of packed cells were lysed with 5 ml of saturated indole contained in Tris buffer pH 7.0. The soluble sugars were then extracted with cold 80% ethanol. The supernatant was then flash evaporated at room temperature and reconstituted in 2 ml of borate elution buffer. One milliliter of this sample was analyzed for the soluble sugar content. In addition, 150 ml of medium in which the cells were grown was flash evaporated to dryness and reconstituted in 4 ml of borate buffer, and 1 ml of this material was also analyzed for sugar content.

Results and Discussion. – The first chromatograms obtained from the cell extracts are shown in Fig. 1.5.1 and from the medium in Fig. 1.5.2. Additional experimentation is required to improve the resolution of the compounds and to identify them. Some preliminary observations, however, can be made. For instance, 14 compounds appear in the cell extract during the predivision period, diminish, and then reappear after division. Changes also occur in the growth medium during the synchronized cell cycle, but the complexity of the chromatograms indicates that they will not be easily resolved.

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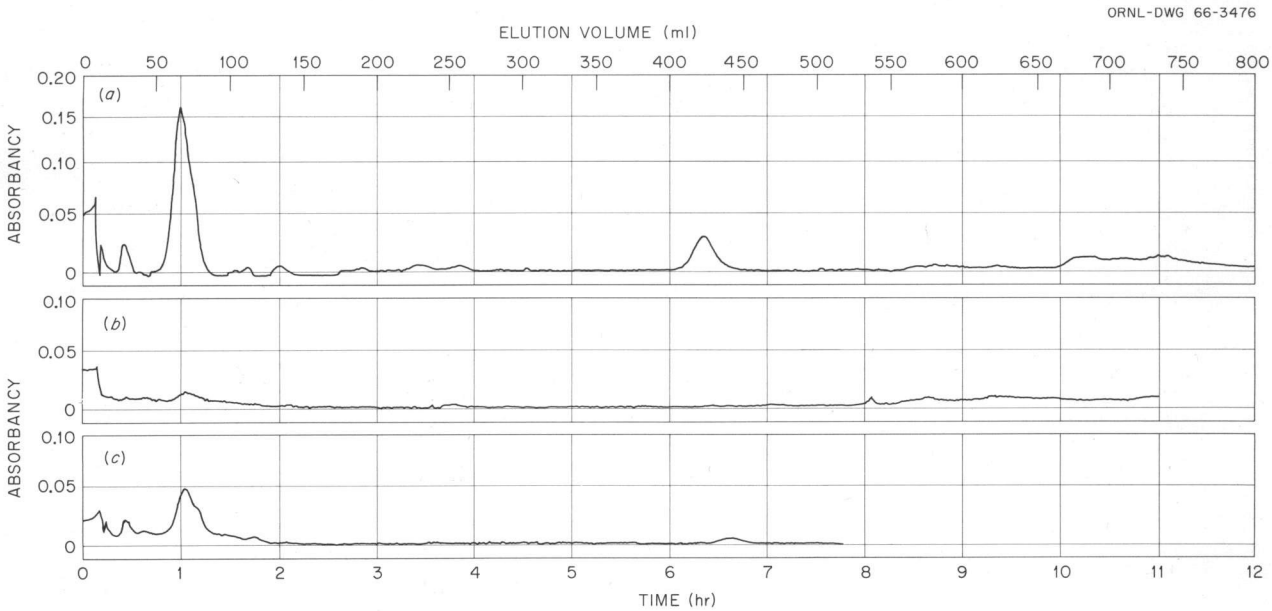

Fig. 1.5.1. Soluble Sugars Extracted from Synchronized *Tetrahymena pyriformis*. Samples containing equal numbers of cells were taken at (*a*) the end of the heat period, (*b*) 90 min after the heat period, and (*c*) 135 min after the heat period.

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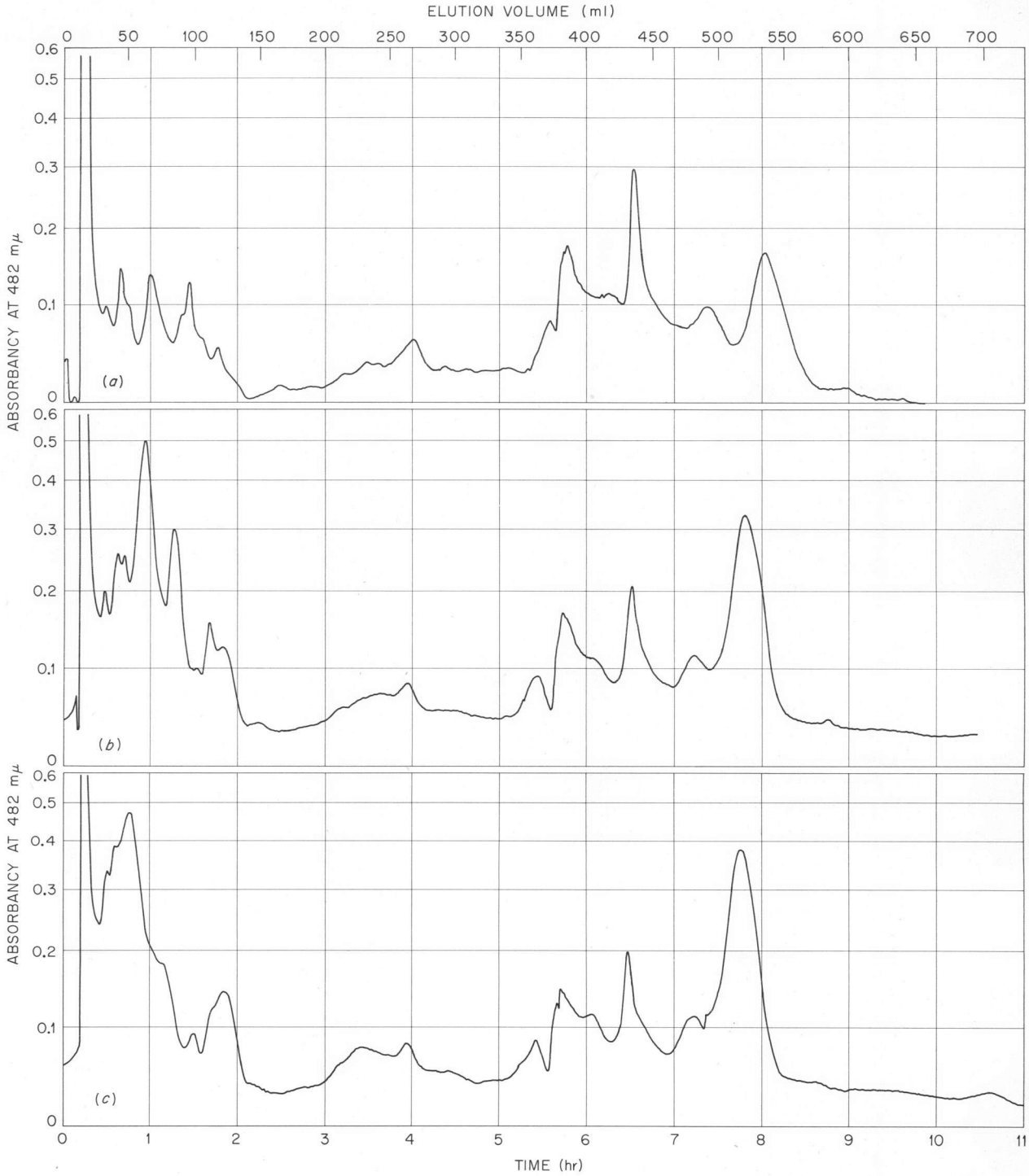

Fig. 1.5.2. Chromatograms Obtained from the Analysis of the Growth Medium of Synchronized Cultures of *Tetrahymena pyriformis*. The samples were taken at (*a*) before the inoculation of the culture vessel, (*b*) at the end of the heat period, and (*c*) 135 min after the end of the heat period.

1.6 EVIDENCE FOR THE CONTRIBUTION OF CYTOSINE-CONTAINING DIMERS TO UV INACTIVATION OF TRANSFORMING DNA

Jane K. Setlow R. B. Setlow[1]

Introduction. – The significance of pyrimidine dimers in biological inactivation was earlier shown by decreasing the amount of these dimers relative to other uv lesions in *Hemophilus influenzae* transforming DNA and observing the biological reactivation that accompanied this decrease.[2] Although most of the pyrimidine dimers in this DNA are thymine-thymine dimers, the biological significance of cytosine-containing dimers may be shown by changing the number of these dimers relative to that of thymine-thymine dimers and observing the effect on transforming activity. Such a selective change in the types of dimers is possible following a large dose at long wavelength by further irradiation in the presence of proflavine. Proflavine inhibits dimer formation[3] but not dimer splitting, and because the absorbance of cytosine-containing dimers is higher than that of thymine-thymine dimers, many more cytosine-thymine dimers are split.[4]

Results and Discussion. – Following 2805-A irradiation, 14 to 33% of the biological damage may be removed by further irradiation in the presence of proflavine at the same wavelength. Approximately 20% of the total dimers are eliminated under these conditions, of which 4% are thymine-thymine and 15% are cytosine-thymine dimers. Therefore, cytosine-containing dimers appear to be biologically equivalent to thymine-thymine dimers in their relative contribution to inactivation. The wavelength dependence for the biological reactivation of DNA and elimination of dimers in the presence of proflavine indicates that the energy for these processes is absorbed in the dimers rather than in the proflavine.

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1.7 PURIFICATION OF THE YEAST PHOTOREACTIVATING ENZYME

Amir Muhammed Jane K. Setlow
M. E. Boling

The purification procedure used previously,[1] while achieving high specific activity, did not yield sufficiently large amounts of material. Successful attempts have been made to scale up the procedure, starting with 150 lb of fresh yeast, by using more batchwise adsorption. The dry yeast is extracted with *M*/15 K_2HPO_4 containing 30% $(NH_4)_2SO_4$, and the enzyme is precipitated from the supernatant fluid at 55% $(NH_4)_2SO_4$. The cake is dissolved in a very large volume (100 liters/kg) of 0.05 *M* phosphate buffer, pH 6.5, containing sufficiently large amounts of phosphocellulose to adsorb all the enzyme. The mixture is stirred overnight, and the enzyme is eluted batchwise. The preparation is now at a sufficient degree of purity to be put through a phosphocellulose column at a reasonable rate of flow.

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1.8 MULTIPLICITY REACTIVATION AND STIMULATION OF RECOMBINATION IN T4 BACTERIOPHAGE COMPLEXES

Amir Muhammed Jane K. Setlow

Introduction. – Multiplicity reactivation is the production of viable phage progeny from cells multiply infected with phage which have been inactivated so that they are incapable of producing viable progeny at low multiplicity of infection. Ultraviolet action spectra for killing of singly and multiply infected cells were obtained to determine whether the types of damage are identical in the two cases. These action spectra were compared with the action spectrum for uv-induced stimulation of recombination in an attempt to assess the role of stimulation of recombination in the survival of multiply infected cells.

Results and Discussion. – For inactivation of multicomplexes, 2805 A is about as effective as 2652 A, whereas 2652 A is considerably more effective in the case of monocomplexes. It is concluded that the lethal damages in multicomplexes are in some respects different from those in monocomplexes. Stimulation of recombination in two pairs of rII mutants is also about as effective at 2805 A as at 2652 A. The data are compatible with the hypothesis that the photochemical events in uv-induced stimulation of recombination and lethality in multicomplexes are the same.

1.9 WAVELENGTH DEPENDENCE OF CROSS-REACTIVATION IN VARIOUS AMBER MUTANTS OF BACTERIOPHAGE T4D

Amir Muhammed C. M. Steinberg

Introduction. – When a bacterium is mixedly infected with phage which have been inactivated by exposure to ultraviolet (uv) light and with unirradiated phage, genetic markers from the irradiated phage may appear among the progeny. This phenomenon is termed cross-reactivation. The frequency of cross-reactivation after a given uv dose varies for different markers. It was recently reported[1] that marker sensitivity shows a smooth variation with map position in T4. The genome of phage T4 was shown to consist of several areas of relatively high or low sensitivity with transition regions of intermediate sensitivity. The above results were obtained with a germicidal lamp (2537 A). It was thought that a determination of the action spectrum for cross-reactivation of markers with different sensitivities would help to clarify the reason for the variation.

Methods and Results. – Wild-type phage T4D was irradiated at various wavelengths from 2300 to 2900 A using a monochromator. *Escherichia coli* strain CR63 was mixedly infected with the irradiated wild-type and an unirradiated amber mutant. The infected cells were then plated on *E. coli* strain B. Amber mutants grow normally in strain CR63 but fail to grow in strain B, while wild-type phage grow equally well in both strains. Three highly cross-reactivable (B25, S52, and N131) and four poorly cross-reactivable (H39, N54, N69, and A453) amber mutants were tested. No differences in the shape of the action spectra for the various mutants were found.

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1.10 UV INDUCTION OF PYRIMIDINE DIMERS DURING DIFFERENT PHASES OF THE CELL CYCLE OF MAMMALIAN CELLS

J. E. Trosko Margaret R. Kasschau
E. H. Y. Chu Lana T. Covington

Introduction. – It has been previously shown that uv-induced pyrimidine dimers seem to be responsible for some uv-induced biological damages. The dimers are formed linearly with dose within the biological dose range; therefore they can be used as a measure of the internal dose absorbed by DNA in the cell nucleus. When synchronized *E. coli* are irradiated with uv, more thymine dimers are formed during the more biologically uv-sensitive part of the cell cycle.[1] However, while the difference in killing was about a factor of 2, the thymine dimer change was only about 12%.

Ultraviolet-induced cell death and chromosome aberrations in mammalian cells exhibit a decreasing gradient of sensitivity from S to G_2 of the cell cycle.[2–4] We wanted to find out if the stage sensitivity differences in mammalian cells were just due to differential nuclear absorption of the uv.

Methods. – Experiments were designed to test the production of thymine pyrimidine dimers at the various stages of the cell cycle in Chinese hamster tissue culture cells. We used 5-aminouracil to synchronize cells (about 95% of cells in S at the time of removal from a 24-hr 5AU block). The cultures were staggered, so that at one uv-radiation time we had three or four different stages of the cycle.

Results and Discussion. – The results are shown in Table 1.10.1. It is obvious that more pyrimidine dimers are produced in early S than G_2. It is almost technically impossible to test G_1 cells. There appears to be about a 20% reduction in pyrimidine dimer production in G_2 as compared to early S. It appears that the difference in absorbed dose is not sufficient to explain the approximate tenfold difference in biological damage in uv cells between S and G_2 if the uv-induced lesions are

Table 1.10.1. Uv-Induced Pyrimidine Dimers During Cell Cycle of Mammalian Cells

Dimer formation, TT/T(%), at 2650 A

Experiment No.	Dose (ergs/mm^2)	Early S	Mid S	Late S	G_2
1	~2000	0.67	0.63	0.56	0.56
2	1500	0.46			0.35
3	1500	0.43		0.38	
4	~1500	0.52	0.48		0.41
5[a]	1500		0.51	0.43	

[a]Double synchronized cells.

produced at about the same efficiency as pyrimidine dimers.

The difference in absorbed dose between the S and G_2 phases can be attributed to several factors. There is evidence that the rate of RNA synthesis is at least $2\frac{1}{2}$ times that of DNA synthesis in late S. Also, the RNA and cell mass are essentially doubled in G_2, as is the total DNA.[5–7] The newly synthesized RNA, protein, and DNA would tend to protect the DNA in G_2. Also, as it has been suggested,[3] the shape of the cell changes from a flat to a round condition as the cell approaches mitosis. This would tend to decrease the amount of absorbed uv in the nucleus. At present, we haven't divorced the contributions of any of these factors from each other.

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1.11 STUDY OF DNASE AND DNA POLYMERASE ACTIVITY DURING *DROSOPHILA* DEVELOPMENT

Amir Muhammed J. M. Goncalves[1]
J. E. Trosko

Introduction. – This study was undertaken to delineate the levels of some of the synthetic and degradative enzymes related to DNA during insect embryogenesis and histolysis-histogenesis.

Methods. – Sterile extracts of *Drosophila* were collected throughout the developmental cycle (fertilized egg to adult). The DNA polymerase activity was determined by the incorporation of ^{3}H-ATP into an acid-insoluble DNA product, while DNase activity was determined by a bacterial transformation assay.

Results and Discussion. – Figure 1.11.1 illustrates the pattern of DNase activity during development. The highest titer of DNase is found just as the larval histolysis begins. This is what one might expect if the pupa, which represents a closed system, needed to break down larval DNA in order to synthesize adult DNA. Low titers are observed during embryogenesis and histogenesis of the adult organs. The fact that a small amount of DNase activity is found in the egg might indicate that the reported stored DNA in the egg is broken down and used during early cleavage when DNA precursors are needed.

It was found almost impossible to characterize the DNA polymerase patterns during development since the DNases in the crude extract interfered

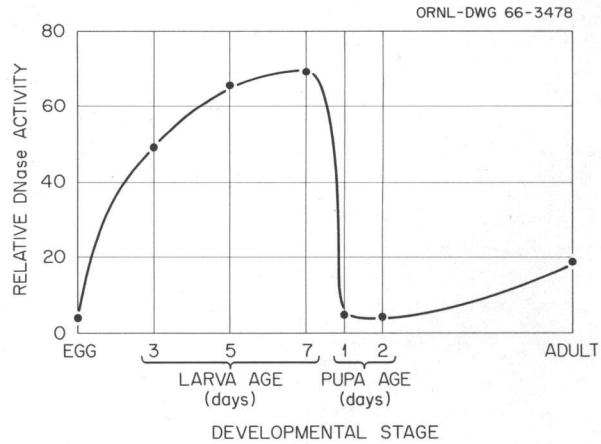

Fig. 1.11.1. Relative DNase Activity in Extracts of Different *Drosophila* Stages.

with the assay. However, DNA polymerase activity in egg extracts indicates that the enzyme could utilize either native or denatured DNA as a primer for DNA synthesis.

Purification and subsequent characterization of these enzymes is under investigation.

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1.12 X-RAY-RESISTANT HUMAN CELLS IN VITRO

J. D. Regan

Comparative survival experiments were performed with RA,[1] a human amnion cell line, and RA X 10, a subline derived from survivors of a single dose of 1000 r of x rays.

Monolayer cultures of RA and RA X 10 were irradiated with 1000 r of x rays and trypsinized, and single cells were planted into culture bottles at a concentration 1×10^5 cells/ml. The medium was replaced weekly, and the number of colonies per bottle was scored after four weeks.

In these experiments RA X 10 showed from 0.5 to 1.4 times greater survival than RA. Survival is defined as the ability to form a macroscopically visible colony in four weeks under these conditions.

Further experiments indicated that the survival of RA X 10 was not enhanced by repeated doses of x rays. The generation time of RA X 10 is somewhat longer than that of the parental cell line. Thus, it is possible that the resistance of RA X 10 is due to an extended G_1 period. Experiments dealing with the generation time, the cell cycle, the karyotype, and the possible existence of enzymatic repair mechanisms in RA and RA X 10 are in progress. X-ray resistance in human cells is of interest intrinsically and also because of the known development of radioresistance in tumors treated with therapeutic x rays.[2]

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1.13 ALKALINE PHOSPHATASE IN SYNCHRONIZED HUMAN CELLS

J. D. Regan

The marked alkaline phosphatase (AP) activity of established human cell lines[1] has been a subject of considerable interest.[2,3] A study has been made of the variation of AP in the cell cycle in human cell cultures. This study was prompted by histochemical observations that rounded-up (presumably dividing) cells displayed an apparent increase in AP and that postmitotic cells showed an apparent increase or concentration of AP around the nuclear membrane.

The method for synchronizing suspension cultures with 5-aminouracil (5AU), measuring DNA synthesis, and following the cell cycle has been described.[4] The AP activity of cell sonicates was measured using *p*-nitrophenyl phosphate.[5]

Figure 1.13.1*a* shows DNA synthesis measured by ^{3}H-thymidine incorporation in control and synchronized cells. Figure 1.13.1*b* shows cell concentration. From these data it appears that in the 5AU-treated cells a major portion of the cell population was synthesizing DNA synchronously and, subsequently, dividing synchronously. The AP activity, however, remained essentially the same throughout this time.

Although from 0 to 9 hr the number of cells in both control and experimental cultures was essentially the same, the AP activity in the 5AU-treated cells appeared consistently somewhat higher. Cells treated with 5AU have a slightly larger volume than control cells; if cells are left for 60 hr in 5AU, giant cells up to 20 times normal size result.[6] Thus, one would expect 5AU-treated cells to possess greater amounts of such cytoplasmic constituents as AP.

The results with *p*-nitrophenyl phosphate indicate that there is not an increased AP activity in dividing and postmitotic cells. Thus, the apparent increased activity seen histochemically in dividing cells would seem to be an effect produced by the rounding up of such cells. The high activity frequently seen around the nuclear membrane of postmitotic cells may well reflect subcellular movement of AP to this site at this time, rather than an increase in total AP. This provides some basis for speculation on the role of AP, the function(s) of which are unknown.[2]

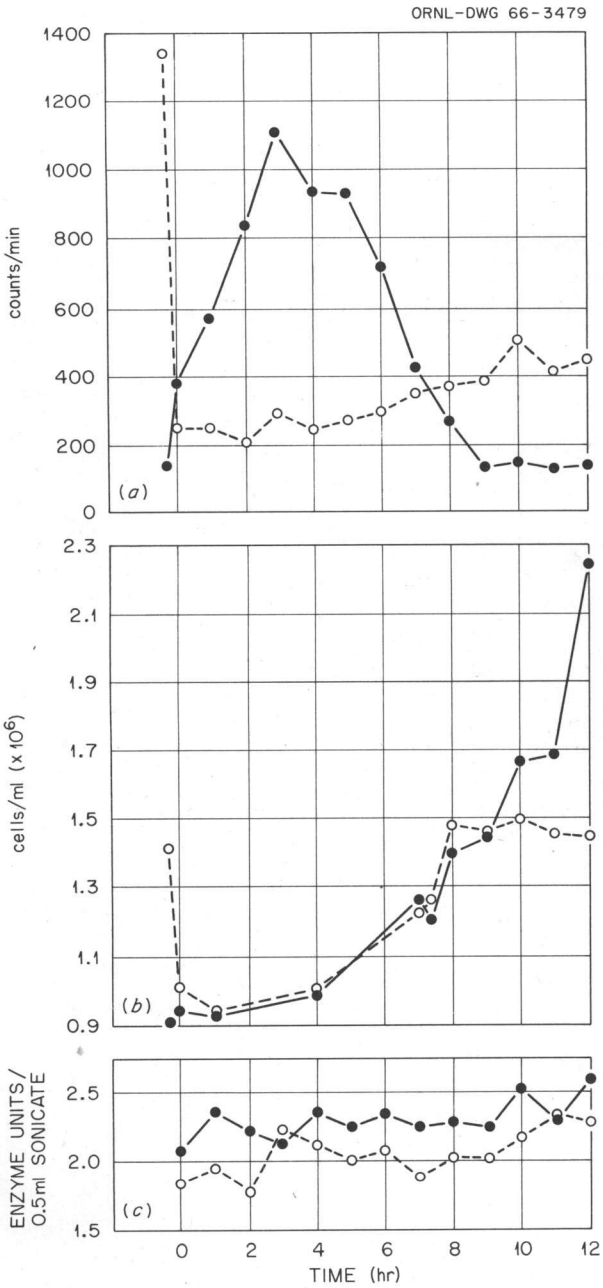

Fig. 1.13.1. (*a*) DNA Synthesis; (*b*) Cell Number; (*c*) AP Activity in Control and 5AU-Treated Cells. 1 enzyme unit = AP activity in 0.5 ml of sonicate sufficient to hydrolyze 1 millimole/ml of *p*-nitrophenol in 20 min at 37°C. •----•, control; o——o, 5AU treated.

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1.14 USE OF ZONAL CENTRIFUGATION TO ISOLATE PARTICLES THAT CONTAIN TRANSPLANTATION ANTIGENS

R. A. Popp N. G. Anderson[1]
Diana M. Popp

Introduction. – Histocompatibility antigens are isoantigens associated with transplantation immunity. The principal transplantation antigens in the mouse are those controlled by the H-2 locus; they are therefore called H-2 antigens. Some studies suggest that the antigens are associated with the cell membranes; others suggest a microsomal or lysosomal localization.[2] This report describes the use of a new zonal centrifuge rotor to isolate particulate material that contains H-2 antigen.

Methods and Results. – Spleens from 60 C57BL/6 mice were homogenized in 25 ml of 8.5% sucrose. The homogenate was layered over a 10-30-55% sucrose gradient in a B-XV rotor while spinning at approximately 3000 rpm in a modified Beckman model L preparative ultracentrifuge. An overlay of 200 ml of Golder-Miller buffer was added to the system. The rotor speed was increased to and maintained at 20,000 rpm for 12 min before it was reduced to approximately 3000 rpm again to unload the rotor. The effluent was collected in 40-ml fractions. The optical density at 280 mμ was recorded. Each fraction was centrifuged for 1 hr at 100,000 × *g* to sediment the particulate material. The pellet was resuspended in 40 ml of 0.85% saline, and the sample was centrifuged again for 1 hr at 100,000 × *g*. The washed pellet was resuspended in 0.6 ml of 0.85% saline; aliquots of the suspension were assayed for H-2 antigen,

acetylesterase, adenosine triphosphatase, and acid phosphatase. The absorption of one run is shown in Fig. 1.14.1. When 10 μl of the suspension from each fraction was incubated in diluted C3H anti C57BL serum, fractions 8 to 12 removed specific H-2 agglutinins from the antiserum such that the absorbed serum failed to agglutinate C57BL erythrocytes; maximum hemagglutination inhibition was observed with particulate material from fraction 10. Hemagglutination inhibition was not observed with other fractions. The activities of the three enzymes studied are shown in Fig. 1.14.2. In fractions 8 to 12, peak activity for ATPase and acetylesterase was found in fraction 9, whereas fraction 8 had the higher acid phosphatase activity.

Discussion. – The new zonal centrifuge rotor B-XV has been shown to be capable of separating the subcellular particles that contain very high concentrations of the H-2 antigen. The morphology of these particles will be determined by electron microscopy; however, the position of these particles in the sucrose gradient suggests that they are endoplasmic reticulum. The capacity of each fraction to induce H-2 antibody is presently being studied. The enzyme assays, which show peak activities in fractions slightly different from that of the highest concentration of H-2 antigen, indicate that three or more particles appear in fractions 8 to 12. Separation of these components should be possible through longer periods of centrifugation and/or recentrifugation of particles in these fractions. The location of non-H-2 transplantation antigens is presently unknown. Attempts will be made to separate the H-2 antigen from other transplantation antigens and proteins. The ultimate goal is to study the chemical nature of the H-2 antigens from several strains of mice, primarily because this information will help us to understand and interpret the extensive knowledge already gathered about the genetics and immunology of the H-2 system. In addition, the H-2 antigens are the principal transplantation antigens in mice,

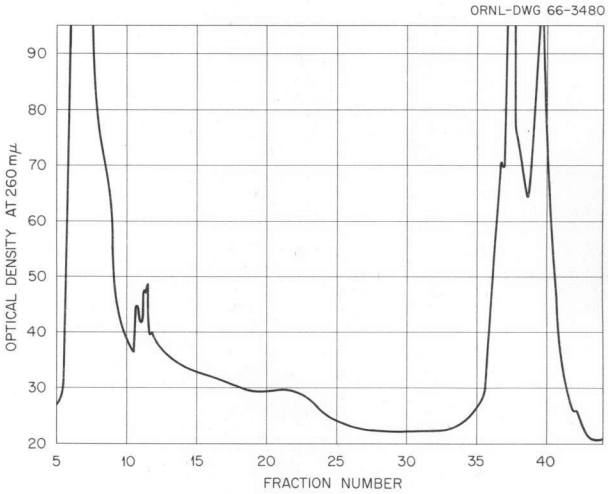

Fig. 1.14.1. Absorption Curve for Density Gradient Fractions of C57BL/6 Spleen.

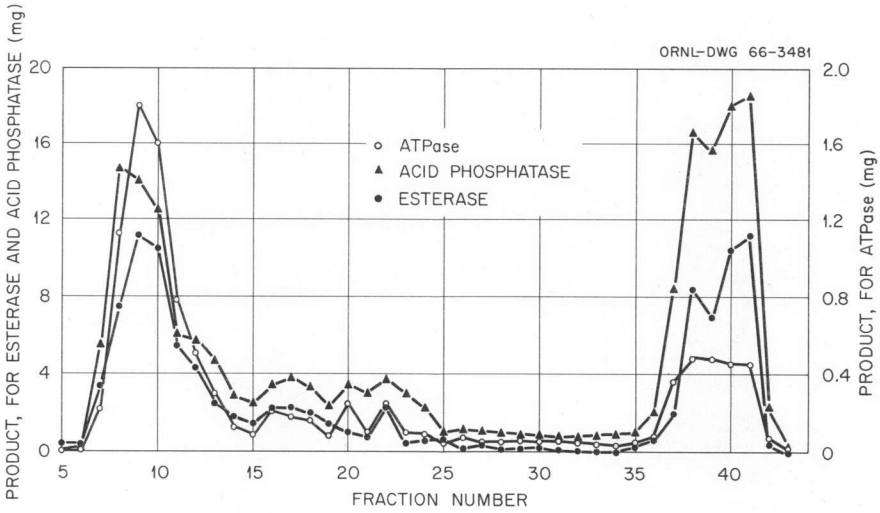

Fig. 1.14.2. Enzyme Activity for Density Gradient Fractions of C57BL/6 Spleen.

and information gained can perhaps be used as a model for future studies on transplantation and tumor antigens of man.

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1.15 RELATIONSHIP OF SOME ESTERASES IN MOUSE ERYTHROCYTE AND KIDNEY EXTRACTS

R. A. Popp W. S. McEwen

The inheritance of a variant esterase in mouse erythrocytes was reported earlier.[1] A similar variation has recently been observed in zymograms of mouse kidney extracts. The esterase variants in the kidney were shown to be identical or allelic to those described by Ruddle and Roderick.[2] The identity of the erythrocyte and kidney esterase was established from analyses of their electrophoretic mobility, strain distribution, substrate affinity, inactivation in 10^{-3} *M* eserine, and heat inactivation at 56°C for 10 min. Genetic tests revealed that the erythrocyte and kidney esterases are controlled by the same locus, as shown in Table 1.15.1. The locus was identified[2] by the symbol *Es-3* prior to the report of Pelzer,[3] who used the symbol *Ee-2* to identify the locus that controlled the erythrocyte esterase variants described. As a rule, gene products such as enzymes are found in many tissues; therefore, the locus symbol should not be restrictive by including an abbreviation of a tissue in its symbol. A nonrestrictive symbol, *Es*, was proposed many years ago to designate all esterase loci of the mouse, with a number following the symbol to identify a particular esterase locus. For these reasons, the symbol *Es-3* is recommended; the alleles are identified by an appropriate superscript as used in Table 1.15.1. The Es-3c phenotype has not been described previously; however, Es-3c is controlled by $Es\text{-}3^c$, which is allelic to $Es\text{-}3^a$ and $Es\text{-}3^b$.

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Table 1.15.1. Backcross and F_2 Progeny Classified for Their *Es-3* Genotypes Based on Analyses of Zymograms of Erythrocyte and Kidney Extracts

Matings	Number and Genotype of Progeny				
	$Es\text{-}3^a/Es\text{-}3^a$	$Es\text{-}3^a/Es\text{-}3^b$	$Es\text{-}3^b/Es\text{-}3^b$	$Es\text{-}3^b/Es\text{-}3^c$	$Es\text{-}3^c/Es\text{-}3^c$
$(A \cdot CA \times RFM)F_1 \times F_1$				7	3
$(DBA/2 \times RFM)F_1 \times DBA/2$				11	9
$(C57BL \times RFM)F_1 \times C37BL$	12	12			

1.16 POLYTENE CHROMOSOME METABOLISM

Crodowaldo Pavan Renato Basile
L. C. G. Simões

As stated in previous reports,[1] we are interested in making a comparative study of the metabolism of the polytene chromosomes of different tissues of *Rhynchosciara angelae*. Partial results were published,[2] and new data are presently being analyzed. The results until now obtained on the metabolism of protein are very inconclusive, on account of which new methods are being developed by Ellen Mattingly, who recently joined our group.

New results on the synthesis of RNA in the salivary gland of *R. angelae* are being obtained in a work done in collaboration with W. D. Gude. In the salivary gland cells of the larvae of *R. angelae* the nucleolar organizer region is located in the base of the X chromosome, where a dispersed nucleolus is normally formed. Besides this nucleolus, however, a great number of other microbodies (nucleotides) are formed in different parts of the chromosomes. We are interested in studying these nucleotides, to know the meaning of their formation and the frequency in which they occur in different phases of larval development. The sectioning method which we are using now to study the larvae as a whole, and the salivary gland in particular, gives very good results, which are being analyzed now. We are applying also other methods to these studies. Among them we are observing the chromosomes by transparency using a phase-contrast microscope in the salivary gland, in isolated cells of that gland, and also in isolated nuclei.

New data are being obtained in the study of the metabolism of DNA which confirm what we already knew about the existence of metabolic DNA in chromosomes of the salivary gland cells, but we still could not decide if something similar occurs also in chromosomes of other tissues of the same larva. Our intention is to induce changes in the pattern of synthesis of DNA, for which we are using physical, chemical, and biological agents in the larvae or in the salivary gland maintained in vitro for several days. We are doing experiments also to see whether there is or is not turnover of DNA in the larvae of *R. angelae*.

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1.17 CYTOLOGY AND CELL METABOLISM OF INFECTED CELLS OF *RHYNCHOSCIARA ANGELAE*

Crodowaldo Pavan Renato Basile

Two infections, one by a protozoan and another by a virus, were recently discovered by Diaz and Pavan,[1] which induce an increase in cell size and changes in the size, shape, and behavior of the chromosomes. We could obtain new cultures infected, and we are now trying to control the transmission of the infections, to study how many times they can be infected, what kind of changes the infection induces, and other related problems.

We could observe that the infection by the protozoan can be transmitted by adding spores of the microorganism in the food of the larvae. We still could not obtain good success with the transmission of the virus infection; we have therefore to study the sporadic cases of larvae or groups of larvae naturally infected.

About the protozoan, we have all indications that it belongs to the family *Microsporidia* and is probably of the genus *Thelohania*. It affects cells of at least six different tissues of the larvae and some of the adult. The infected cells react to the infection by an increase of their size, including the chromosomes. Although the majority of the cells show hypertrophied polytene chromosomes (see figures in ref. 2) which are similar in cells of different tissues, in some cases the entire genome is puffed and each chromosome is represented by a pompon-like structure. In still other cells this structure is presented only by the X chromosome, the other chromosomes having the normal polytene structure. In some cases we could observe that the puffing of the X chromosome starts in the distal end and progresses toward the base of the chromosome. In many infected cells by the

protozoan the X chromosome in a pompon-like structure or as polytene shows a volume relatively larger than the other chromosomes of the same nucleus. When stained by the Feulgen method, it shows a lighter color when compared with the other chromosomes. This suggests that the larger volume is due to a higher activity in other chromosomal components and not in the DNA. This is confirmed by preliminary spectrophotometric measurements (P. A. Roberts *et al.*, not published). The metabolism of RNA is very intense in these chromosomes, as shown by autoradiographic preparation of infected larvae injected with ^{3}H-uridine.

The use of biological agents which change the cytoplasmic balance of live cells, promoting changes in the function and structure of the nucleus, looks to be a very efficient tool to study not only problems of infections but also problems of normal and abnormal differentiation. The use of cells of Diptera for this type of study is very convenient also, because the structure of the polytene chromosomes permits a very detailed analysis of the reaction of specific chromosomal sections to the infective agent, which normally cannot be done in other types of chromosomes.

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1.18 SYNTHESIS OF NUCLEIC ACIDS AND PROTEIN IN FOLLICLE OF *R. ANGELAE*

Renato Basile

In the last report we described the work done on the morphology of the ovary and its development during the life cycle of *R. angelae*.

Now we are studying, by the autoradiographic method, the synthesis of nucleic acids and protein. We are using as precursor ^{3}H-thymidine, ^{3}H-uridine and cytidine, and ^{3}H-leucine for DNA, RNA, and proteins respectively. We performed experiments in different stages of pupae development, during which the chromosomes of nurse cells undergo great changes in shape. The follicle at this phase increases largely in size, mainly due to the increase in the oocyte.

The results can be summarized as follows:

1. DNA is synthesized only in follicular cells, which show mitosis during the growth of the follicle. No synthesis was observed in oocytes. Probably meiosis is completed only in adult flies, after fertilization. In nurse cells DNA synthesis stops after the completion of endomitosis, which is in early pupae.
2. RNA is synthesized in nurse cells and follicular cells, but not in oocytes. However, it comes from these cells to the oocyte, which has a big growth. This conclusion we can take looking at the different times at which larvae were killed after injection. In nurse cells, 30 min after injection, we see labels only over the nucleus; after 1 hr, label can be seen over the nucleus and the cytoplasm; after 6 to 8 hr, label in nurse cells decreases and appears over the oocyte.
3. Protein is made at the same time in nurse cells and in oocytes. We can conclude that, from RNA of nurse cells, proteins are made in the oocyte. The RNA would be a messenger which comes from other cells.

1.19 ISOLATION OF NUCLEI OF THE SALIVARY GLAND OF *RHYNCHOSCIARA ANGELAE*

Günter Weirich

In order to get a useful in vitro system for polytene nuclei of the fly *Rhynchosciara angelae* (Sciaridae), experiments were performed to elucidate the following points:

1. composition of an adequate medium,
2. methods to exclude or dissolve the mucus which is produced by the salivary glands and renders the cell fractionation more difficult,
3. partial digestion of the cell walls in order to release the nuclei without damage due to hard homogenization procedures,
4. homogenization of pretreated glands,
5. fractionation of the homogenate.

During these preliminary experiments, the following method seemed to be most successful. The salivary glands were sampled in a buffered solution of nine amino acids, three sugars, succinic and fumaric acid, and inorganic salts, adjusted to pH 7.2; this was elaborated by F. J. S. Lara, following the composition of *Rhynchosciara* hemolymph. The same solution was used for the following steps. The glands were incubated at 25°C in a solution of 0.05 to 0.1% pronase and, after washing out the enzyme 1 to 5 min, were shaken with glass beads (3 mm in diameter) in ice-cold buffer solution. The homogenate was filtered through cloth of 100 and 50 μ mesh width and then subjected to centrifugation.

1.20 DNA CONTENT OF PUFFED CHROMOSOMES OF *RHYNCHOSCIARA ANGELAE*

P. A. Roberts[1] R. F. Kimball
Crodowaldo Pavan

Salivary gland cells of *R. angelae* that are infected by a microsporidian are often enlarged, with chromosomes several times the size of the corresponding polytene chromosomes found in normal cells. The chromosomes of infected cells can be found in various stages of enlargement in the same salivary gland and can be compared with chromosomes of neighboring uninfected cells in order to study the nuclear response to microsporidial infection. We are now measuring the relative amounts of DNA of individual polytene chromosomes of normal and infected cells.

Methods. – Salivary glands of larvae of *R. angelae* were isolated, fixed and squashed, air dried, and stained by the Feulgen procedure. The DNA content of individual chromosomes was measured with the Zeiss scanning microspectrophotometer.

Results and Discussion. – Although a limited number of nuclei have been measured at this time, several tentative conclusions can be made:

1. In spite of pronounced morphological changes, the individual chromosomes of infected cells maintain the relative proportions of DNA found in chromosomes of uninfected cells.

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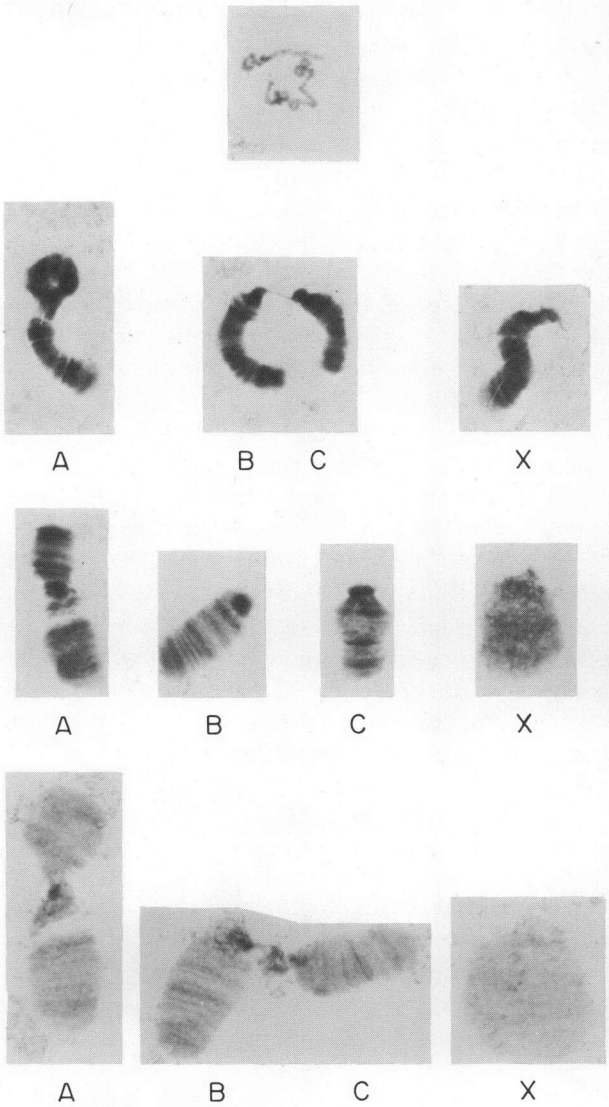

Fig. 1.20.1. Feulgen-Stained Chromosome Complements of an Uninfected (top) and Three Infected Salivary Gland Cells of a Female Larva of *R. angelae* Photographed at the Same Magnification. The chromosomes of the infected cells are (left to right) A, B, C, and X.

2. DNA values of infected cells fall into classes that approximate the geometric series, 2:4:8:16 . . . times the DNA content of uninfected cells.
3. Chromosomes of widely disparate sizes can belong to the same DNA class. Figure 1.20.1 shows chromosomes from three different infected cells

of a female larva and a chromosome (at the top) from a noninfected cell photographed at the same magnification. The three sets of chromosomes from infected cells are of the same DNA class, approximately 16× that of uninfected cells on this slide. This indicates that the chromosomes of at least some cells respond to infection by puffing at a large number of loci on all chromosomes as well as by increases in polyteny.

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Biophysical and Biochemical Cytology

Sheldon Wolff
Ellen Mattingly
Sandra L. Bell[a]
J. A. Heddle[b,c]
H. E. Luippold
C. L. Parker

[a]Consultant.
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[c]James Picker Foundation Fellow.

2.1 EFFECTS OF ULTRAVIOLET RADIATION ON HUMAN LEUKOCYTES

Sheldon Wolff

In order to study the effects of uv on a population of human leukocytes, all in the G1 phase of the cell cycle, it was first necessary to prepare a suspension of unclumped leukocytes that were free from all erythrocytes that could absorb uv. This was accomplished by allowing heparinized, freshly drawn venous blood to settle for 1 hr at room temperature. The upper layer, now containing about equal amounts of white cells and red cells in serum, was removed by pipet and then spun down in a clinical centrifuge. The pellet was resuspended in distilled water for 20 sec to hemolyze all the red cells. Isotonicity was restored by adding an equal volume of Versene in NaCl. The Versene prevents the white cells from agglutinating. Centrifugation now results in a pellet of leukocytes uncontaminated with erythrocytes. This pellet was then washed and resuspended in saline A, and aliquots were placed in quartz cuvettes containing a magnetic stirring bar. Stirred samples were irradiated with uv from a large monochromator at 2500, 2650, 2800, 2900, 3000, and 3100 A.

Control cells that were stirred but received no uv, or cells that were exposed to radiation at 3100 A, grew and underwent mitosis when cultured by standard leukocyte culture methods. There were no chromosome aberrations in these cells. At all other wavelengths, cells would not undergo mitosis. The inhibition is caused by extremely low doses of radiation: 25 ergs/mm^2.

Experiments in which the cells were exposed to tritiated thymidine showed that after uv radiation fewer lymphocytes became transformed, there was an inhibition of incorporation of label into DNA, and some cells became necrotic.

Studies are now under way to determine just when in the cell cycle this uv effect is maximal.

2.2 EFFECTS OF THYMIDINE ON INCORPORATION OF DEOXYCYTIDINE INTO DNA

Sandra L. Bell Sheldon Wolff

When experiments were carried out in which *Vicia faba* root tips were grown in various concentrations of thymidine for 60 min and then transferred to fresh treatment solutions to which ^{3}H-deoxycytidine had been added, grain counts of

autoradiographs showed an inhibition of ^{3}H-deoxycytidine incorporation into the DNA of roots treated with high concentrations (10^{-2} and 10^{-3} *M*) of thymidine. Low concentrations of thymidine (about 10^{-5} *M*) had no effect on ^{3}H-deoxycytidine incorporation. In the presence of the DNA synthesis inhibitor 5-fluorouracil deoxyriboside, the thymidine effect is intensified. Removal of thymidine from the treatment solutions relieves the inhibition.

It is known that in systems in which thymidine inhibits DNA synthesis, both mitotic inhibition and synchronization of cell division occur. Thymidine does not, however, either inhibit mitosis or cause synchronization of cell division in *Vicia* roots. Therefore, because thymidine apparently has no obvious physiological effect on root cells, we do not think it likely that thymidine inhibits DNA synthesis per se in *Vicia*. We then suggest that the presence of a high concentration of exogenous thymidine affects the pathways of pyrimidine synthesis and causes an increase in the size of the deoxycytidine pool within the cell. If this occurs, proportionately less of the pool would consist of exogenous ^{3}H-deoxycytidine, and therefore less of the ^{3}H-deoxycytidine would be incorporated. There would then be no grains over nuclei from roots treated with high concentrations of thymidine.

2.3 DIMINUTION OF X-RAY-INDUCED CHROMOSOME ABERRATIONS BY TREATMENT WITH ACTINOMYCIN D

Ellen Mattingly

The repair of x-radiation-induced damage to chromosomes is known to involve protein synthesis, but very little is known about the type of protein that is formed. It seemed feasible that information on the behavior of RNA synthesis and the results of its inhibition during the period following x radiation of cells might lead to a better understanding of the processes involved in the repair of chromosome breaks. Therefore we began an investigation of the effects of pre- and post-treatment of soaked seed of *Vicia faba* with actinomycin D on the number of chromosome aberrations resulting from exposure of the seeds to 600 r of x radiation.

Preliminary work determined the optimum concentration and period of exposure to actinomycin D. Seeds presoaked in tap water for 18 hr were exposed to actinomycin D (50 μg/cc) for periods of 0.5, 1, 3, 5, and 7 hr. Subsequently, the seeds were thoroughly washed and were held in moist cotton for two days. At the end of this period the length of the primary roots was measured. We found that exposure of seeds to actinomycin D for three or more hours resulted in a 50% reduction in the elongation of the primary root. No significant differences in mitotic index were seen, however, in such roots.

Soaked seeds were then treated in the following ways: x radiation (600 r, 200 r/min), followed by 3 hr immersion in actinomycin D; 3 hr pretreatment with actinomycin D, followed by x radiation (600 r, 200 r/min); and x radiation during 3 hr exposure to actinomycin D (total dose, 600 r). These seeds were held for two days and then fixed. Metaphase cells in the resulting root-tip smears were analyzed for chromosome aberrations. We found that the number of such aberrations seen in actinomycin D–treated roots, regardless of the time of x radiation, was consistently reduced by 75% from the control values.

We are currently investigating this protective effect of actinomycin D to determine if the effect is a result of inhibition of RNA synthesis or is instead related to a specific protective effect by the antibiotic that is unrelated to RNA synthesis.

Fungal Genetics

F. J. de Serres[a]

Radiation Mutagenesis (3.1–3.3)

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Chemical Mutagenesis

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Participation in Cooperative Programs:

AEC-NIH Chemical Carcinogenesis (23.11, 23.12)
AEC-NASA Space Biology (23.26)

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3.1 MODIFICATION OF IMPAIRED COMPLEMENTATION BETWEEN NONALLELIC MUTATIONS IN *NEUROSPORA*

F. J. de Serres

Introduction. – Previous studies on complementation between irreparable mutations in the *ad-3* region have shown that *ad-3A*^{IR} + *ad-3B*^{IR} combinations always grow at less than wild-type rate and may grow ten times more slowly on either minimal or adenine-supplemented media.[1] The lower growth rates of dikaryons involving *ad-3*^{IR} mutations in comparison with those found in dikaryons involving *ad-3*^{R} mutations have been interpreted as resulting from reduced functional activity of nonallelic loci in the genetic regions immediately adjacent to *ad-3*^{IR} mutations as a result of a spreading-type position effect[2] rather than undetected genetic damage. Irreparable mutations in the *ad-3* region (*ad-3*^{IR}) increase as the square of the dose with x irradiation,[3] and all data from this type of experiment or homology tests[4] are consistent with either chromosome deletion or chromosome translocation as the mode of origin of this class of mutations. This is in contrast to *ad-3*^{R} mutations that increase linearly with x-ray dose and result from point mutations.

If the growth rates of forced dikaryons involving *ad-3*^{IR} mutations are due to reduced functional activity of other loci in the immediately adjacent regions, then it should be possible to modify the growth rates of such combinations in specially constructed trikaryons containing reparable mutations at various loci in the *ad-3* region as the third component (Fig. 3.1.1). On the position effect hypothesis, the growth rate of the dikaryon (Fig. 3.1.1) on minimal medium is limited by reduced functional activity of loci to the left and/or to the right of the *ad-3A* locus in component 1 as well as loci to the left and/or to the right of the *ad-3B* locus in component 2. By using an *ad-3A*^{R} *ad-3B*^{R}

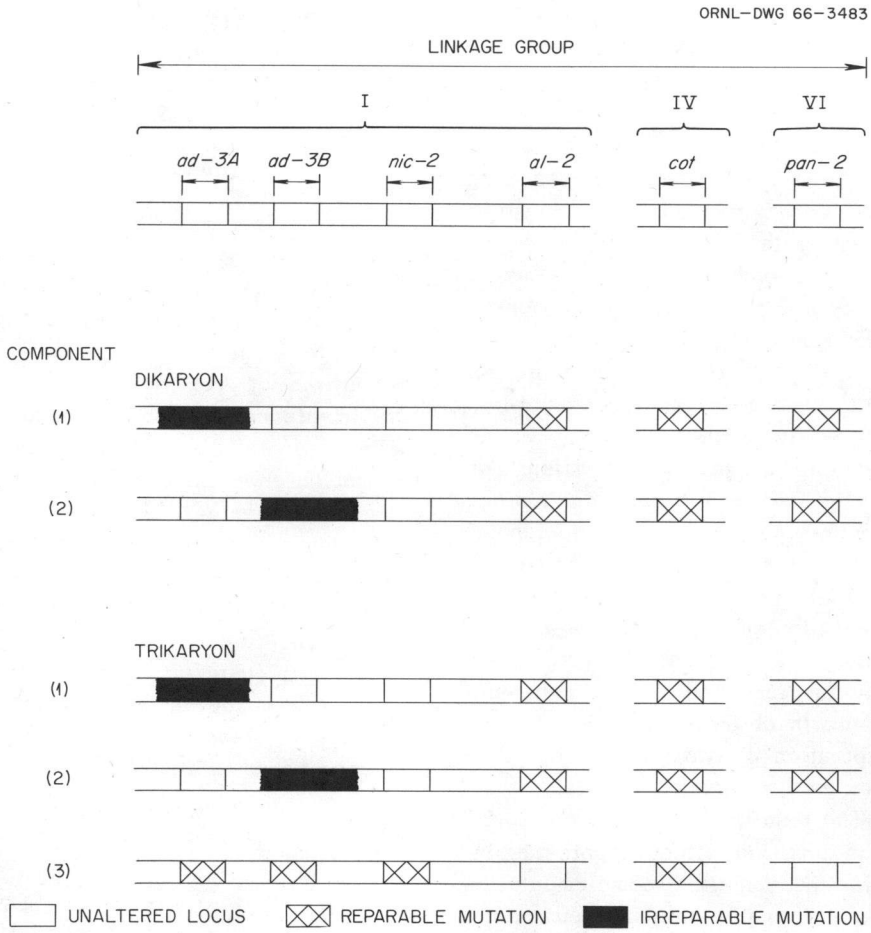

Fig. 3.1.1. Genetic Composition of Strains and Diagrammatic Representation of the Extent of the Functional Inactivation in *ad-3A*^{IR} and *ad-3B*^{IR} Mutations as Forced Dikaryons or Trikaryons in Combination with an *ad-3A*^{R} *ad-3B*^{R} Double Mutant (Strain 74-OR152-7A).

double mutant as component 3 in a trikaryon, the growth rate on minimal medium should be dependent primarily, if not entirely, on the activity of the *ad-3A*$^{+}$ locus in component 2 and the *ad-3B*$^{+}$ locus in component 1. On minimal medium supplemented with high levels of adenine, such trikaryons should grow at wild-type rate since all requirements of the original dikaryons will have been satisfied by the addition of either unaltered genes in component 3 or the addition of adenine to the medium.

Results. – The position effect hypothesis was tested on dikaryons involving three *ad-3A*^{IR} mutations (11-1-6, 11-1-13, and 11-1-122) and one *ad-3A*^{R} mutation (11-1-11) in combination with four *ad-3*^{IR} mutations (11-1-7, 11-1-10, 11-1-121, and 11-1-123) and one *ad-3B*^{R} mutation (11-1-1). The *ad-3*^{IR} + *ad-3*^{R} combinations as well as the *ad-3A*^{R} + *ad-3B*^{R} combination served as controls. The growth rates of the forced dikaryons between these various combinations as well as forced trikaryons in combination with an *ad-3A*^{R} *ad-3B*^{R} double mutant (74-OR152-7A) were determined in quadruplicate in growth tubes on minimal medium. Comparisons were made at 27 rather than 23°C[1] since the higher temperature enhances the reduction in growth rate found with *ad-3*^{IR} mutations.

The results of these tests showed that in all cases the growth rates of the trikaryons involving *ad-3*^{IR} mutations were more rapid than the growth rate of the corresponding dikaryon and in some

cases were at wild-type growth rate. No difference was found in the growth rate of the dikaryon and trikaryons involving the *ad-3A*^{R} + *ad-3B*^{R} combination used as a control.

On the position effect hypothesis the growth rate of trikaryons involving *ad-3*^{IR} mutations showing less than wild-type growth rate on minimal medium is limited primarily by the activity of the *ad-3A*$^+$ locus in component 2 and the *ad-3B*$^+$ locus in component 1. Tests for adenine stimulation of growth rate were made on these trikaryons on medium supplemented with 100 γ of adenine per milliliter (at least 2.5 times higher concentration than that required to give wild-type growth rate of *ad-3A* or *ad-3B* mutants). In this test trikaryons growing at less than wild-type rate on minimal medium grew at wild-type rate on adenine-supplemented medium. The presence of the expected percentages of trikaryotic conidia at the *distal ends* of the growth tubes (determined by plating conidia on minimal medium) showed that the growth observed on adenine-supplemented medium could not be attributed to growth of these heterokaryons as a dikaryotic combination of two of the three components rather than as a trikaryon.

Discussion. – The results of the present experiments support the position effect hypothesis invoked to explain the impaired complementation found between *ad-3*^{IR} mutations in *Neurospora*. Apparently *ad-3*^{R} mutants result only from the inactivation of the *ad-3A* or the *ad-3B* locus. *Ad-3*^{IR} mutations resulting from chromosome deletions (and perhaps chromosome translocation) give not only gene loss (in the former case) but also partial gene inactivation at other loci in the immediately adjacent regions by an unknown mechanism. Experiments proposed[1] to approach this problem at the biochemical level are in progress.

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3.2 QUANTITATIVE AND QUALITATIVE ASPECTS OF PHOTOREACTIVATION OF FORWARD MUTATION IN *NEUROSPORA CRASSA*

B. J. Kilbey F. J. de Serres

Photoreactivation of ultraviolet (uv)-induced lethal and mutagenic damage is a well-documented phenomenon in bacteria. Recently, experiments were described which showed that uv-induced lethal effects were similarly photoreactivated in a wild-type strain of *Neurospora crassa*.[1] These first experiments have been confirmed and extended to include several other strains, and the details of the phenomenon have been further investigated.[2] In an earlier report[3] data were also presented from experiments designed to permit the characterization of uv-induced *ad-3B* mutants in *Neurospora* using specific reversibility and complementation tests. The results of these experiments demonstrated that uv induces several different types of genetic alteration in *Neurospora*. The object of the present experiments was to combine these two approaches to study:

1. the quantitative effects of photoreactivation on forward mutation at the *ad-3A* and *3B* loci in *Neurospora;*
2. the qualitative aspects of this problem, that is, whether this repair is specific for a particular type or types of mutant or whether it is nonspecific and affects all types of mutant similarly.

Quantitative information was obtained from experiments to determine the frequency of uv-induced forward mutation at the *ad-3A* and *ad-3B* loci with and without maximum photoreactivation (based on data obtained earlier concerning photoreactivation of survival) using the Direct Method.[4] The qualitative aspects of the problem were investigated with tests for allelic complementation among *ad-3B* mutants. The percentages of mutants with nonpolarized and polarized complementation patterns were determined, and a detailed study of distribution of mutants on the complementation map and their average complon coverages before and after photoreactivation was made. These tests were chosen not only for their simplicity but also because it has been shown in reversion studies with *ad-3B* mutants induced with nitrous acid,[5] ethyl methanesulfonate,[6] and uv[3] that differences in the spectrum of genetic alterations as a result of

mutagen specificity are reflected in the patterns of complementation observed.

Results. – In the forward-mutation experiments *ad-3* mutants were induced with graded doses of uv, with the highest dose giving a minimum of 36% survival. After each uv treatment a portion of the suspension received an exposure of photoreactivating light sufficient for maximum photoreactivation.[1] Three such experiments were carried out, and the data from one of these are given in Table 3.2.1.

When mutation frequency is expressed in terms of the numbers of conidia treated, photoreactivation also results in a marked reduction in the number of mutations. Mutants were isolated from all uv doses indicated in Table 3.2.1. In most cases more than 200 mutants were selected at random from the samples at each dose. At the lowest dose, however, the numbers of mutants were so small that they were all analyzed. After each mutant had been made homokaryotic, heterokaryon tests were made to determine whether the mutants had been induced at the *ad-3A* or the *ad-3B* locus. In all, approximately 870 *ad-3B* mutants were tested with a set of 19 *ad-3B* mutants to determine their patterns of complementation, as described previously.[7] These data are presented in Table 3.2.2.

The data clearly indicate that no change in the proportions of the different types of complementing mutants takes place either as a result of increasing uv dose or as a result of photoreactivation of mutation. This is true when a comparison

Table 3.2.1. Effect of Photoreactivation on the Induction of Adenine Mutants by UV in *Neurospora crassa*

Incident Energy (ergs/mm^2)	Survival (%)		Mutations per 10^6 Survivors					
			ad-3A		*ad-3B*		Total	
	L	D	L	D	L	D	L	D
Control	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
1000	100.0	100.0	0.9	3.5	3.5	11.5	4.4	15.0
2400	100.0	86.0	13.5	31.5	21.5	61.5	35.0	92.0
3500	92.0	65.0	22.0	48.5	54.0	94.0	76.0	142.5
4800	76.0	36.0	61.0		82.0		142.0	

Table 3.2.2. Analysis of the *ad-3B* Mutants Induced With and Without Photoreactivation

Incident Energy (ergs/mm^2)		Total Mutants			Complementing Mutants		
		Noncomplementing	Complementing	Total	Nonpolarized	Polarized	Total
1000	D	32(54%)	27(46%)	59(100%)	23(85%)	4(15%)	27(100%)
	L	16(50%)	16(50%)	32(100%)	9(56%)	7(43%)	16(100%)
2400	D	88(56%)	68(44%)	156(100%)	43(64%)	25(36%)	68(100%)
	L	72(50%)	73(50%)	145(100%)	54(73%)	19(26%)	73(100%)
3500	D	97(55%)	80(45%)	177(100%)	57(71%)	23(29%)	80(100%)
	L	99(53%)	89(47%)	188(100%)	65(74%)	24(26%)	89(100%)
4800	D						
	L	74(52%)	68(48%)	142(100%)	50(74%)	18(26%)	68(100%)

is made between complementing and noncomplementing mutants and between polarized and nonpolarized complementing mutants. The same is true of the pattern distribution on the complementation map and the total complon coverage. However, it should be noted that this finding only relates to mutants obtained from points on the ascending part of the mutation induction curve.

Discussion. – The simplest interpretation of these findings is that there is but one type of premutational lesion responsible for all the mutants studied here. When this is repaired during photoreactivation, all classes are similarly affected. This also agrees well with the finding that no change in the types of mutant induced occurs with increasing uv dose in this range. An alternative explanation would be that the initial premutational lesions are of more than one type, some of which are photoreactivable. In this case one would have to assume that they are all equivalent in the types of mutant they give rise to. If this were not so, a change in the proportions of mutants would be expected when photoreactivation occurred. Failure to find any differences between populations of mutants obtained with and without photoreactivation raises interesting questions concerning the determination of the size of the fraction of photoreactivable damage which leads to mutation.

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3.3 CARBON DIOXIDE STIMULATION OF THE *ad-3* MUTANTS OF *NEUROSPORA CRASSA*

F. J. de Serres

Introduction. – Studies on purine biosynthesis in *Neurospora crassa* have shown that the purple adenine mutants (*ad-3*) are blocked in the conversion of 5-amino-imidazole ribonucleotide (AIR) to 5-amino-4-imidazole-*N*-succinocarboxamide ribonucleotide (SAICAR),[1,2] which is a two-step reaction with 5-amino-4-imidazole carboxylic acid ribonucleotide (CAIR) as the intermediate.[3] Genetic analyses[4] of the *ad-3* mutants have shown that there are actually two separate genes in the *ad-3* region (designated *ad-3A* and *ad-3B*) rather than a single gene. However, whether these genes control the same or different steps has not as yet been determined.

Since the conversion of AIR to CAIR involves a reaction requiring CO_2, it seemed likely that the enzyme controlling this step would have specific binding sites for CO_2 as well as for AIR and that mutations at the locus controlling the formation of this enzyme could lead to a reduced affinity for either substrate. Thus adenine-requiring mutants at the *ad-3A* or *ad-3B* locus or both might actually be carbon dioxide mutants that would grow on minimal medium in a CO_2-enriched atmosphere.

Charles[5] has shown that CO_2-requiring mutants of *Neurospora* grow vigorously on minimal medium when the gas phase is 70% air + 30% CO_2. In the present experiments this simple test has been used to determine whether the *ad-3A* or *ad-3B* locus or both control the reaction AIR + CO_2 → CAIR in *Neurospora.*

Results. – Tests for CO_2 stimulation were made on leaky and nonleaky *ad-3A* and *ad-3B* mutants[6] by comparing the linear growth made by conidia in a water suspension (filtered through 180-mesh platinum wire gauze and adjusted to 2.5×10^4 conidia per drop) on the surface of agar in petri plates when the gas phase was (1) 70% air + 30% CO_2, (2) 95% air + 5% CO_2, (3) ordinary air, and (4) CO_2-free air.

The results of these tests are summarized in Table 3.3.1. Whereas both *ad-3A* and *ad-3B* mutants show a delay or complete inhibition of growth in CO_2-free air, only leaky *ad-3B* mutants will grow in the absence of adenine in CO_2-enriched air.

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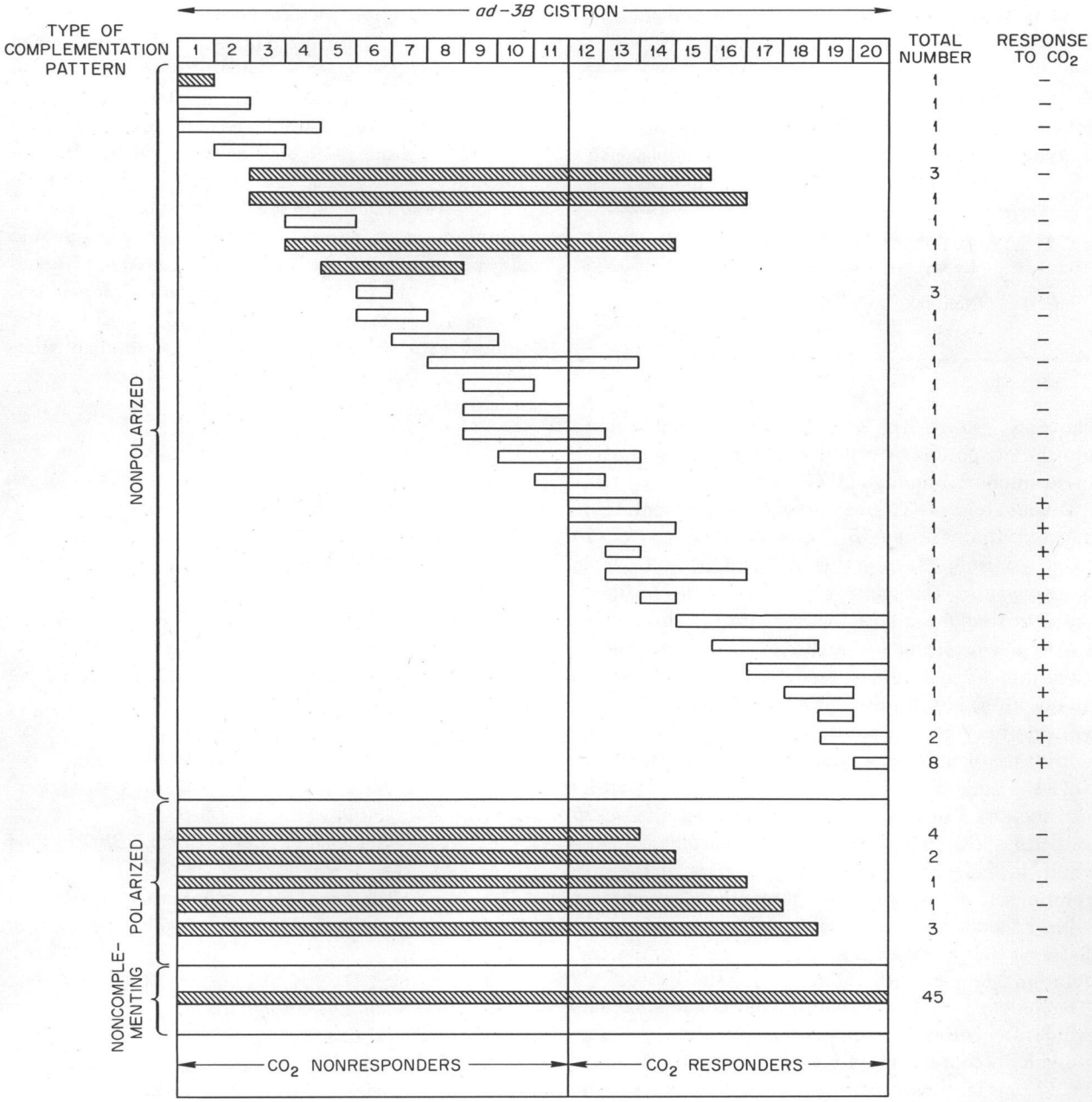

Fig. 3.3.1. Complementation Map of the *ad-3B* Cistron of *Neurospora* Showing the Correlation Between Complementation Pattern Type and Position and Growth on Minimal Medium in an Atmosphere of 70% Air and 30% CO_2. ▨ = nonleaky mutant; □ = leaky mutant.

Tests for allelic complementation[6] among these *ad-3B* mutants have shown that the complementation map on the *ad-3B* cistron has at least 20 complementation subgroups or *complons*. All of the leaky mutants have nonpolarized complementation patterns and are distributed over the entire complementation map. The striking feature of the present experiments is the precise correlation between response to CO_2 and complementation pattern and map position. The only *ad-3B* mutants

Table 3.3.1. Number of Nonleaky and Leaky *ad-3A* and *ad-3B* Mutants Showing Growth on Minimal Medium in an Atmosphere of 70% Air and 30% CO_2

Mutant Type	Adenine Requirement	Total Sample	Total Number Showing Positive Response to CO_2
ad-3A	Nonleaky	36	0
	Leaky	30	0
ad-3B	Nonleaky	63	0
	Leaky	35	20

showing growth in a CO_2-enriched atmosphere are the nonpolarized mutants with patterns starting in complons 12 through 20 (Fig. 3.3.1).

Discussion. – The results of the present tests suggest that the *ad-3B* locus and not the *ad-3A* locus controls the reaction AIR + CO_2 → CAIR in *Neurospora*. One possible implication of these tests is that the *ad-3A* locus controls the reaction CAIR + L-aspartate → SAICAR, and tests for L-aspartate stimulation of *ad-3A* (and *ad-3B* mutants as controls) are in progress, as well as more direct verification from comparative assays of the enzyme activities of wild-type, *ad-3A*, and *ad-3B* mutants.

The remarkable specificity in the type of pattern and locale on the complementation map of the *ad-3B* mutants showing growth in a CO_2-enriched atmosphere is especially interesting in view of the correlations found between the percentages of mutants with nonpolarized and polarized complementation patterns and mutagenic origin[7] as well as the correlation found with specific reversibility.[8–10] The only mutants responding to CO_2 are mutants which are believed to specify a complete polypeptide chain containing only a single erroneous amino acid; the noncomplementing mutants or mutants with polarized patterns are believed to specify either a polypeptide fragment, a polypeptide with a series of erroneous amino acids, or no protein at all (for discussion see refs. 6 and 7).

The fact that only the mutants with nonpolarized patterns located on the right side of the complementation map respond to CO_2 suggests a possible sharp demarcation between the location of the CO_2 binding sites and AIR binding sites on the enzyme molecule. The simplest possible interpretation of these results is that the left side of the complementation map corresponds to that part of the enzyme containing the binding site for AIR, whereas the right side of the complementation map corresponds to that part of the enzyme containing the binding site for CO_2. Even if an oversimplification, this interpretation will provide a useful model to test in future experiments.

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Drosophila Cytology and Genetics

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Radiation Genetics (4.1–4.4)

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Chromosomal Behavior and Biochemical Genetics (4.5–4.7)

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4.1 A TANDEM DUPLICATION THAT LOWERS RECOMBINATION THROUGHOUT A CHROMOSOME ARM OF *DROSOPHILA MELANOGASTER*

P. A. Roberts

One of the crossover suppressors recovered from irradiated females was a long tandem duplication, Dp(2;2)619. This rearrangement was singled out for detailed study because the tandem duplications reported up to now have either had little effect or have increased meiotic recombination in the region of the duplication.

Methods. – Pairing of the duplication was examined in polytene chromosomes, and meiotic recombination was studied in three marked regions of chromosome arm 2L in duplication heterozygotes and homozygotes.

Results and Discussion. – Recombination throughout the chromosome arm of duplication heterozygotes was 1/6 the control value and in homozygotes only 1/3 the control value. Lowered recombination in the duplication homozygote is particularly surprising because one would predict that addition of a considerable segment of the chromosome would increase the genetic length of the arm. The data suggest, however, that the duplication interferes with pairing in meiotic cells. A clue is provided by the pairing relations observed in polytene chromosomes, where the duplication is regularly seen paired with the corresponding region on the *same* chromosome rather than with the corresponding region of the homolog. It is probable that meiotic pairing of hyperploid regions in Drosophila, as in other organisms, is 2X2 although this cannot be verified by cytological studies. The crossover reductions caused by this duplication are attributed to its size and distal location: The great length of the duplication permits pairing of the duplicated region with the adjacent homologous region; pairing between homologs, ordinarily initiated distally, is thereby reduced because pairing affinity is complete throughout a con-

siderable distal region (that which is duplicated) before homologs pair.

4.2 TRANSLOCATIONS AS CROSSOVER SUPPRESSORS

P. A. Roberts Mary C. Stewart

Investigation of the nature of crossover reduction in translocated arms is proceeding through a more detailed examination of some of the 70 translocations detected by their ability to suppress recombination[1] and by studies of recombination in some X-autosomal translocations recovered in studies of male sterility by D. L. Lindsley. Preliminary data on the behavior of one X-autosomal translocation, T(1;2)6, supports the impression that an exchange of chromosomal tips does not lead to profound crossover reductions in the translocated arms.

Studies of recombination in translocation heterozygotes using multiply marked chromosome arms have revealed that in the majority of cases where there is a distal breakpoint in a translocated arm, recombination is lowered to levels comparable to that of inversion heterozygotes. Two translocations have been recovered in which a single, distal breakpoint lowers recombination in one arm to less than 1%. Since there is no obvious way in which crossover chromatids can be selectively eliminated in translocation heterozygotes (as single crossover chromatids of inversion heterozygotes are eliminated in polar nuclei of Drosophila oocytes), it appears that certain translocations are better crossover suppressors than simple two-break inversions. These translocations probably exert their effects on recombination by preventing pairing, which is most likely initiated in distal regions. The failure of translocations such as T(1;2)6 to lower recombination throughout the chromosome arm suggests that pairing may be initiated in distal parts of the chromosome other than the tip (or telomere).

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4.3 COMPARATIVE FREQUENCY OF X-RAY-INDUCED CROSSOVER-SUPPRESSING ABERRATIONS RECOVERED FROM OOCYTES AND SPERM OF *DROSOPHILA MELANOGASTER*

Ruth E. Thomas P. A. Roberts

When oocytes of Drosophila females bearing free X chromosomes are irradiated, the frequency of x-ray-induced translocations is only 1/100 of that recovered from sperm given the same x-ray exposure.[1] It has been reported that intrachromosomal aberrations (inversions) are recovered with a considerably greater frequency from oocytes than are translocations (interchromosomal aberrations), and this finding was interpreted as evidence that lack of proximity of breaks in nonhomologous chromosomes was largely responsible for the low frequency of translocations recovered from oocytes.[2] In this experiment, however, no cytological studies were made of crossover suppressors, which were assumed to be inversions. Recent evidence that translocations instead of inversions are the most frequently recovered crossover suppressors from irradiated sperm[3] called for repetition of the experiment using the same radiation exposure and screening techniques on females (including cytological examination of crossover suppressors) as had been used on sperm.[3]

Results and Discussion. – One thousand chromosome arms from irradiated oocytes (stage 7 and earlier) were screened for crossover-suppressing aberrations; two such aberrations were recovered, but neither was an inversion. One was a translocation; the other, Dp(2;2)619, was the first crossover-suppressing tandem duplication reported. The yield of intrachromosomal rearrangements recovered from oocytes was an order of magnitude below that recovered from sperm given the same radiation exposure. If tight packing of chromosomes in the sperm head is responsible for the higher aberration yield from sperm than from oocytes, the present data indicate that it must increase the probability of intrachromosomal rearrangements as well as interchromosomal rearrangements.

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4.4 SCINTILLATION COUNTING OF TRITIATED THYMIDINE TRANSFERRED TO FEMALES BY LABELED *DROSOPHILA MELANOGASTER* MALES

W. E. Trout III

The daily pattern of label transfer from males fed or injected with tritiated thymidine (^{3}H-T) to females was studied quantitatively using scintillation counting methods.

Adult males injected with ^{3}H-T and mated daily lost 90% of their radioactivity within the first three days. About 1% of the label lost on each of these days was taken up by the females. Dissection indicated that at most only 10% of the female label was received through mating, presumably as unincorporated label in the seminal fluid. Other studies indicated that at least 50%, and probably all, of the remaining female label was received through ingestion of label excreted by the males and as surface contamination on the females. By four days after injection, transfer of label by the male reached the background counting level, and only occasionally were label peaks observed in later matings, which may represent transfer of labeled sperm or seminal fluid.

Male larvae which had been fed ^{3}H-T for several hours and allowed to mate four to eight days later as adults also transferred label to females during the first two or three days of mating. Although some of this transferred label was taken up by the female through ingestion, there are indications that much of the radioactivity was transferred through mating as labeled sperm and seminal fluid.

4.5 A CHEMICALLY INDUCED MUTATION WHICH ALTERS ELECTROPHORETIC MOBILITY OF ALCOHOL DEHYDROGENASE ISOZYMES IN *DROSOPHILA MELANOGASTER*

E. H. Grell K. Bruce Jacobson[1]

Introduction. – Alcohol dehydrogenase (ADH) of Drosophila may be resolved into several isozymes by gel electrophoresis. A fly from a natural population or laboratory stock has one of three patterns of ADH isozymes. Patterns are controlled by two naturally occurring alleles at a locus on the second chromosome. Homozygotes for either allele contain three isozymes. Those in Adh^F homozygotes migrate more rapidly to the anode than the corresponding isozymes in Adh^S homozygotes (Fig. 4.5.1). Adh^S/Adh^F heterozygotes have nine isozymes. In addition to the three of each parental type, there are three intermediate or hybrid enzymes.

To test models of the organization and structure of ADH isozymes, an attempt was made to induce mutations in a gene which specifies the structure of alcohol dehydrogenase. Mutations were sought which would impart to ADH electrophoretic mobilities not found in nature.

Methods. – The search for a mutation was carried out in the following way: Adh^F males were injected with a 0.625% solution of the potent chemical mutagen, ethyl methanesulfonate. The treated males were mated to Adh^F females containing genetically marked chromosomes. The F_1 males were first backcrossed to the marker-bearing females, and then each fertile male was tested for the presence of electrophoretic variants of ADH. The offspring of males with new isozymes were further tested and mated in attempts to establish lines of Drosophila with variant ADH for further study.

Results. – Approximately 10 of 5800 tested F_1 males were observed to have an altered form of the enzyme. Among the progeny of only one male was an altered enzyme found to be inherited. This new ADH (No. 5228) contains isozymes that migrate more rapidly to the anode than the corresponding isozymes in either of the naturally occurring patterns (Fig. 4.5.1).

The new mobilities appear to be controlled by a mutation at the *Adh* locus in the second chromosome and thus constitute a third allele at that locus. Heterozygotes of No. 5228 and Adh^F or Adh^S have the expected parental and hybrid isozymes. Heterozygotes of No. 5228 and a deficiency of the *Adh* locus have only the three isozymes of No. 5228.

Discussion. – The infrequent recovery of *Adh* mutations among progeny of variant F_1 males is most probably the result of mosaicism. Some of the somatic cells of variant males carry a mutation, but the mutation was not included in germ

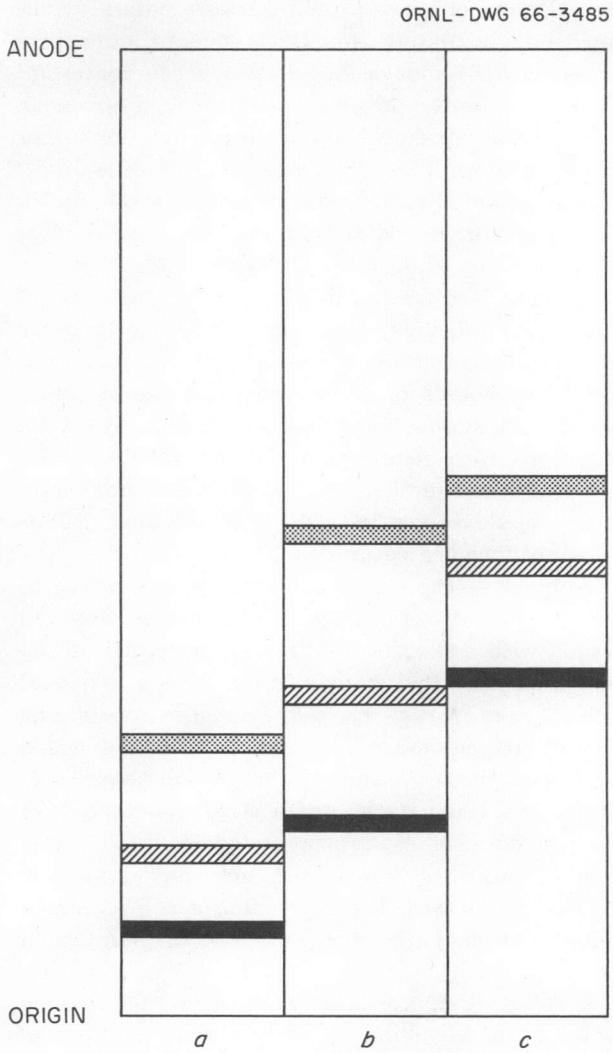

Fig. 4.5.1. Diagrammatic Representation of Drosophila Alcohol Dehydrogenase Isozyme Patterns. (*a*) Adh^S/Adh^S; (*b*) Adh^F/Adh^F; (*c*) 5228/5228.

cells and thus could not be transmitted to progeny. Similar results have been reported in the literature for mutations affecting morphological characteristics.

The isozyme pattern of No. 5228 allows with certainty the elimination of a model of the nature of ADH isozymes that postulates random dimerization of two polypeptide chains from two nonallelic loci. Such a random association of subunits occurs in the formation of the lactate dehydrogenase tetramer in many animals. In No. 5228 homozygotes all three ADH isozymes are more negatively charged than the corresponding isozymes of the ancestral Adh^F (Fig. 4.5.1), yet the newly induced mutation is presumably a single event that affects only one gene and hence only one polypeptide chain. Therefore, all three isozymes must contain polypeptides specified by the *Adh* gene. If the system were analogous to lactate dehydrogenase, one isozyme would not be altered by a mutation in one gene.

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4.6 THE MEIOTIC ORIGIN OF TEMPERATURE-INDUCED CROSSOVERS IN *DROSOPHILA MELANOGASTER*

Rhoda F. Grell

Alteration in the frequency of crossing-over by temperature has been known since the early work of Plough[1] with Drosophila and subsequently has been reported for a variety of organisms including Neurospora, Ascobolus, and Sphaerocarpus. In Drosophila the origin of the temperature-induced recombinants has been variously ascribed to the stem cells,[2] to oogonia,[3] and to the oocyte.[1] The possibility of a stem cell induction seems to be ruled out for the following reasons: (1) The effect is limited in its duration; and (2) heat treatment of stem cells during early development, when gonia and cytes are absent, does not produce the effect. The present experiments are concerned with discriminating between a gonial origin, a cyte origin, or a mixed origin.

Methods and Results. – Two special types of meiotic segregations have been employed in these experiments. In each case the frequency of the segregation is directly related to the amount of *meiotic* crossing-over. When exchange is increased by temperature, the alteration or lack of alteration in the ensuing segregation indicates whether the additional recombination occurred at the gonial stage, the cyte stage, or at both stages.

The first test utilized the phenomenon of secondary nondisjunction. Newly eclosed females of the genotype In(1)65, $y\ f/y^2\ sc\ v\ car{\cdot}y^+/Y^{c\text{-}s}$ were

treated with a temperature of 35°C ± 1° for 8 hr, and the frequencies of crossing-over and secondary nondisjunction among their progeny were compared with control values. Female exceptions were examined for the occurrence of homozygosis for the markers *sc, v, f,* and *car,* and a portion of them were progeny tested to uncover possible crossovers that were not directly detectable.

The expectations are as follows: (1) for a meiotic origin, a reduction in secondary nondisjunction and the occurrence of female exceptions bearing exclusively noncrossover X chromosomes; (2) for a gonial origin, no reduction in secondary nondisjunction and the occurrence of some female exceptions bearing crossover X chromosomes; (3) for a mixed origin, a reduction in secondary nondisjunction and the occurrence of some female exceptions bearing crossover X chromosomes.

The results demonstrated that a reduction in secondary nondisjunction accompanied the increase in crossing-over (Fig. 4.6.1). No evidence for X chromosome crossovers was obtained among 1954 female exceptions as judged by homozygosis for markers and among 208 female exceptions as judged by progeny tests. These results are consistent with an exclusively meiotic origin for temperature-induced recombinants.

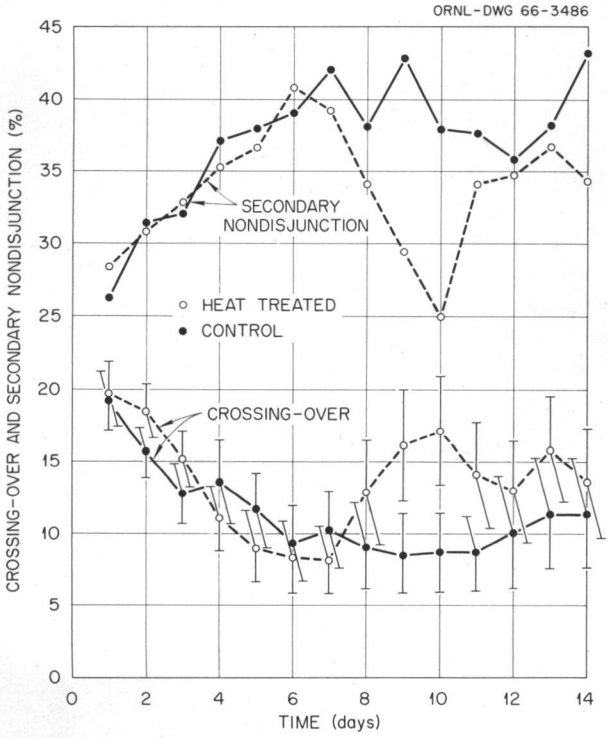

Fig. 4.6.1. Crossing-Over and Secondary Nondisjunction Frequencies for Females of the Genotype In(1)65, *y f/y*^{2} *sc v car·y*$^+$/Y$^{C\text{-}S}$ Treated Within 6 hr of Eclosion with a Temperature of 35 ± 1°C for 8 hr and Their Controls Kept at 25 ± 1°C.

The second test utilized the phenomenon of nonrandom disjunction. Newly eclosed females of the genotype T(1;4)B^S, *B·y*$^+$/*y*^{2} *sc f* were treated with a temperature of 35°C ± 1° for 24 hr and crossovers between *B* and *y*$^+$ were scored among the progeny. Crossing-over in this region leads to the formation of asymmetric dyads at anaphase I and the preferential inclusion of the shorter of the two chromatids into the egg nucleus at the next division. If the temperature-induced crossovers are meiotic in origin, no change in the "c" value (i.e., the coefficient of nonrandomness describing the degree of preference) is expected, whereas if such crossovers are of gonial or mixed origin, a reduction in the "c" value should occur. The observed "c" values were 78.9 ± 2.4 and 78.6 ± 4.0 for the progeny of heat-treated and control females respectively, while corresponding crossover values were 7.9 ± 0.5 and 2.3 ± 0.2. Again the results indicate an exclusively meiotic origin for temperature-induced crossovers.

Discussion. – Recent studies[4] have suggested that the times of crossing-over and DNA replication in the Drosophila oocyte are roughly coincident. This conclusion hinges on the assumptions that the temperature effect on crossing-over is a direct one and that temperature-induced crossovers are predominantly or exclusively meiotic in origin. The latter assumption has now been verified. Should it become possible to demonstrate that the former assumption is also valid, a very strong case for the interdependence of DNA synthesis and crossing-over could be made.

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4.7 THE EFFECTS OF EXCHANGE AND STRUCTURAL HETEROZYGOSITY ON RADIATION-INDUCED NONDISJUNCTION AND LOSS

J. W. Day Rhoda F. Grell

Structural heterozygosity in the Drosophila female leads to a reduction in exchange between the homologs involved, and in the case of the X chromosome it is known to increase the frequency of primary nondisjunction.[1] Further, it has been demonstrated that following irradiation a marked increase in the frequencies of both chromosome nondisjunction and loss occur.[2] The question arises as to whether a crossover between homologs, which presumably ties them together during meiotic prophase, or isosequentiality of homologs, which may enhance their pairing properties at this time, affects the frequency with which they nondisjoin or are lost after treatment with x rays. The problem has been investigated by irradiating two kinds of females, one heterozygous and the other homozygous for a multiply inverted X chromosome.

Materials and Methods. – The chromosome used for these studies was Ins(1)dl-49, B^{M1}, which carries two inversions, one distal and one proximal. It is estimated that the maximum frequency of exchange tetrads in females heterozygous for this chromosome and a normal X is ~24%,[3] whereas the corresponding frequency in females homozygous for the multiply inverted chromosome is at least 90%. Thus, the two types of females are expected to differ with respect to the proportion of their exchange tetrads by a factor of about 4.

Newly eclosed females of both types were exposed to 4000 r of x rays, held for two days, and then mated to appropriate males. The parents were brooded daily for seven days, during which time the temperature was maintained at 25°C ± 1°.

Results and Discussion. – The frequencies of X nondisjunction among the progeny from the two types of mothers are given in Table 4.7.1, columns 4 and 5. The day 1 brood represents oocytes irradiated at stage 7, and later broods represent those irradiated at progressively earlier oocyte stages. No significant difference is observed for any brood; neither are the average values for the seven-day period significantly different.

Table 4.7.1. Effect of 4000 r of X Ray on the Frequencies of X Chromosome Nondisjunction and Loss in the Oocytes of Females Homozygous and Heterozygous for Ins(1)dl-49, B^{M1}

Brood Day	Number		X Nondisjunction (%)		X Loss (%)	
	Ins/Ins	Ins/+	Ins/Ins	Ins/+	Ins/Ins	Ins/+
1	11,377	3,965	1.93	2.23	5.21	6.97
2	3,227	2,216	1.61	1.66	5.55	4.92
3	5,556	2,066	1.03	1.62	3.74	3.34
4	3,148	1,630	1.43	2.29	3.41	4.45
5	1,786	1,267	1.44	0.83	4.18	5.74
6	1,183	1,138	1.34	1.62	4.63	4.21
7	809	1,188	1.13	0.90	5.60	5.17
	27,086	13,470	1.57	2.01	4.66	5.14

The frequencies of X chromosome loss are given in Table 4.7.1, columns 6 and 7. Again no significant difference between the homozygous and heterozygous females is observed. Since the greatest discrepancy in loss appeared in the day 1 brood, an experiment was carried out to determine whether a significant difference might be found in still later oocyte stages. Both types of females were aged for several days to ensure the presence of mature oocytes (stage 14), irradiated with 500 r of x ray, mated, and discarded after 24 hr of egg laying. The two broods that emerged represented predominantly oocytes irradiated at stage 8 to stage 14. Among 12,000 progeny examined, no statistical difference was observed in nondisjunction or loss between the two broods.

The results indicate that neither structural heterozygosity nor the presence of a crossover between homologs modifies the frequencies of radiation-induced nondisjunction or loss.

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Cytochemistry and Cell Reproduction

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Yeast Genetics (5.1, 5.2)

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5.1 SUPER-SUPPRESSORS AS ADDITION-DELETION MUTATIONS

G. E. Magni[1] R. C. von Borstel
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Introduction. – There exists a class of mutations in the yeast *Saccharomyces cerevisiae* which can in general be suppressed by a class of external suppressors. Such mutations are referred to as being "super-suppressible," while the suppressors are called "super-suppressors." It has been found that certain mutants in yeast show a higher spontaneous mutation rate during meiosis than during mitosis.[3] This is called the "meiotic effect" and can be attributed to base addition-deletion mutations which arise in meiosis by unequal genetic exchanges. In genes specifying the structure of proteins, addition-deletion mutations may lead to shifts of reading frame.

Results. – Revertants from a strain carrying the mutations ly_{1-1} and hi_{5-2} were selected by plating (in the same experiment) on media (1) lacking histidine, (2) lacking lysine, and (3) lacking both histidine and lysine and were analyzed for the presence of external suppressors. The double revertants must have come from mutations at the super-suppressor loci, and this was confirmed by the analysis. The single revertants were of two classes: (1) reversions in or near the locus of the original mutation, and (2) outside suppressors which suppress one but not the other of the original mutations. In the second experiment a strain carrying the markers tr_{5-2} and ad_{2-1} was analyzed for the frequency of double revertants. As before, these double revertants must have been due to mutations at super-suppressor loci. Analysis of the reversions at the loci of the original mutations was not carried out in this experiment. The results are presented in Table 5.1.1. There are two conclusions to be drawn: (1) the suppressible mutations do not show a meiotic effect, and (2) super-suppressors do show a meiotic effect.

Table 5.1.1. Mutation Rates: Reversion from Dependence to Independence

Mutant	Locus of Mutation	Rate	
		Mitosis	Meiosis
		$\times 10^{-9}$	$\times 10^{-9}$
ly_{1-1}	ly_1	5.0	7.0
	Super-suppressors[a]	32.0	480.0
hi_{5-2}	hi_5	1.8	3.6
	Super-suppressors[b]	36.0	410.0
tr_{5-2} ad_{2-1}	Super-suppressors[c]	22.4	260.0

[a]Includes super-suppressors which suppress both ly_{1-1} and hi_{5-2} (81%), as well as suppressors which suppress ly_{1-1} but not hi_{5-2} (19%).

[b]Includes super-suppressors which suppress both hi_{5-2} and ly_{1-1} (77%), as well as suppressors which suppress hi_{5-2} but not ly_{1-1} (23%).

[c]Includes only super-suppressors which suppress both tr_{5-2} and ad_{2-1}.

Discussion. – As explained above, the absence of a meiotic effect is interpreted to mean that the super-suppressible mutant is not of the addition-deletion type. It is consistent with the notion that the super-suppressible mutants are nonsense mutants. Similarly, the existence of the meiotic effect is interpreted to mean that the super-suppressor mutations are of the addition-deletion type. The suppressible mutations are thought to be nonsense mutations. In the presence of a super-suppressor, an amino acid is inserted at the position of the nonsense codon. Capecchi and Gussin[4] found that in a cell-free system, with RNA from a suppressible mutant of the phage R17 as messenger, the synthesis of the coat protein occurred in the presence but not in the absence of serine-accepting soluble RNA from the suppressor strain. They left the question open whether the suppressor mutation was a mutation in the sRNA gene or in a gene coding for an enyzme which could modify the sRNA (e.g., a methylating enzyme).

Let us consider the consequences of the latter hypothesis, that is, that a super-suppressor gene codes for an sRNA-modifying enzyme. It is possible that the enzyme is absent in the wild type and that a mutation to the suppressor state restores the activity; this assumption is rather implausible, since there exist a large number of super-suppressor loci. It is also possible that the enzyme is present in the wild type and that an altered enzyme is present in the suppressed strain; this assumption seems unlikely, since the consequences of an addition-deletion mutation would be so drastic that a completely nonfunctional rather than an altered enzyme would be produced. The final possibility, the absence of the enzyme in the suppressed strain, is hardly consistent with the essentially dominant nature of super-suppressors. Thus the above considerations would seem to exclude the possibility that the super-suppressors generally code for enzymes. On the other hand, our observations are eminently consistent with an alteration in the sRNA itself.

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5.2 A FRAGMENT OF TRYPTOPHAN SYNTHETASE IN A SUPER-SUPPRESSIBLE MUTANT OF YEAST

T. R. Manney Margaret D. Schellinger

Introduction. – Super-suppressible mutants are believed to make incomplete proteins as a consequence of a nonsense sequence that terminates translation. By analogy with the properties of tryptophan synthetase in *E. coli*, it is possible to predict the properties of certain fragments that could be formed by nonsense mutants affecting this enzyme in yeast; they should have the properties of a somewhat defective version of the A protein of *E. coli* tryptophan synthetase. We have been able to identify an enzyme activity having these predicted properties in crude extracts of

three mutants that map in a small region near the center of the genetic map of the yeast tryptophan synthetase locus.

Results. – Detection of this activity requires a highly sensitive assay. The specific activity of these mutants is less than 0.1% of the wild-type activity. The assay was made sensitive enough by using ^{14}C-labeled substrate (indole-3-glycerol phosphate) and measuring the ^{14}C-labeled product (indole). The molecular weight of the fragment having this activity has been estimated by gel filtration to be in the neighborhood of 30,000, which is the size of the *E. coli* A protein. Because of this small size this fragment can be separated from the wild-type enzyme, which has a molecular weight of about 140,000. This provides a method for studying the action of the super-suppressors, which act on these mutants. In the one case where the action of a super-suppressor on the enzyme has been studied, both the mutant enzyme (the fragment) and the wild-type-like enzyme were formed. The results were consistent with the suppressor allowing the protein chain to be completed 60 to 70% of the time.

Discussion. – These results provide the first direct evidence that super-suppressible mutants of yeast make prematurely terminated proteins, and are therefore analogous to the *amber* and *ochre* mutants of bacteria and bacteriophage. The results also provide evidence that the tryptophan synthetase of yeast, which appears to be a single protein, contains a region that is very similar to the A protein of the *E. coli* enzyme. By inference, then, it must also have a region similar to the B protein of *E. coli*.

5.3 MASS PRODUCTION OF DIAPAUSE LARVAE IN *MORMONIELLA*

P. W. Whiting

Introduction. – Facultative diapause, marked by cessation of feeding and failure of molting to form a pupa, occurs under certain conditions in the last larval instar of *Mormoniella*. This lack of metamorphosis, often involving a large proportion of the individuals, proved a great liability before it was understood how to avoid diapause and to break it once it was initiated. As a result of suggestions by Drs. Carroll Williams and H. A. Schneiderman, it was avoided by keeping culture temperature high, 28°C, and it was found that refrigeration for at least three months would induce the diapause larvae to pupate and mature normally after being placed in the incubator.

Diapause is now regarded as an asset in maintaining genetical stocks. If the larvae are kept in a refrigerator they will live for two years and may be removed at any time after three months, when they will pupate and can be used for experimental purposes. At room temperature the life span of diapause larvae may be lengthened to three years.

In many species of insects a stimulus applied directly to the young larvae will cause them, after several molts, to go into diapause, but in *Mormoniella* and a few other species this technique is ineffective. The stimulus must act through the mother on her unlaid eggs. Many data have been accumulated and many publications have appeared; but results from one investigator have conflicted with those from another, and explanations advanced have seemingly been inconsistent from year to year.

For five years at the University of Pennsylvania Doris J. Bush maintained a large number of *Mormoniella* stocks, over 150 different mutant genes, while she was making tests for homologies of deleterious factors. Her technique was patterned after the natural fall temperature changes: long cool nights followed by short warm days. Cultures had overnight storage at 12°C, but each day they were transferred to the laboratory, 23 to 25°C, for 5 hr. This alternation of temperatures was continued for three weeks, after which the cultures were placed in the incubator to continue development. The mothers were left in the same vials until death resulted from exhaustion of the food supply by their progeny. High but variable proportions of diapause larvae to adults were obtained. Storage in the refrigerator assured availability of the numerous stocks as needed for the tests.

Methods and Results. – The present tests conducted under carefully controlled conditions in this laboratory have furnished data from settings made between November 22 and December 30, 1965, of about 60 genetically different stocks. Adult females were set in glass shell vials with host *Sarcophaga* pupae and placed in a refrigerator in which 6-hr "days" at 18°C alternated with

Table 5.3.1. Numbers of Vials, with Numbers in Parentheses of "Good" Puparia, Arranged According to Successive Vials Through Which the Mothers Were Transferred and According to "Ratios" of Diapause Larvae to Adults Produced

"Ratios" of Diapause Larvae to Adults	Successive Vials					Totals
	a	*b*	*c*	*d*	*e*	
None	23(184)					23(184)
Few	34(304)	5(43)				39(347)
Several	19(215)	6(45)	2(18)			27(278)
Many	4(28)	10(85)	4(32)		2(14)	20(159)
All	2(14)	41(350)	47(349)	38(330)	14(75)	142(1118)
Total	82(745)	62(523)	53(399)	38(330)	16(89)	251(2086)

18-hr "nights" at 10°C. The "days" were brightened by two fluorescent lights. Complete darkness characterized the "nights" except for the brief periods when the vials were removed for transferring the mothers and supplying fresh host pupae. Transfers of mothers were made through successive vials, *a, b, c,* etc., the later vials indicating increasing age of the mothers and longer periods under experimental conditions. Modal days for vials *a* were 8; for *b,* 7; for *c,* 7; and for *d,* 14. For each of 14 of the 16 transfers to vials *e,* at least one of the mothers had had 33 or more days of adult life at the time of the transfer.

Vials with infected pupae containing eggs or young larvae were set at about 28°C immediately after removal of the mothers.

A very rough estimate of the "ratios" of diapause larvae to adults was made for the contents of each of the 251 vials used, based on an examination of the contents of the 2086 puparia containing viable progeny. These ratios are expressed (Table 5.3.1) as *none, few, several, many,* or *all.* Had records been made for each puparium instead of grouping them in vials, the proportion containing either *no* or *all* diapause would have been much higher.

Table 5.3.1 shows the distribution of the 251 vials (containing the 2086 host puparia examined) with respect to age of mothers (successive vials *a–e*) and "ratios" of diapause to adult offspring from *none* to *all.*

Reference should be made to the papers by Schneiderman and Horwitz[1] and by Saunders.[2] Each contains extensive data and a discussion of the subject.

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Cell Growth and Differentiation

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6.1 GENERAL METHOD FOR THE ISOLATION AND PURIFICATION OF PHOSVITIN FROM VERTEBRATE EGGS

R. A. Wallace

Recent evidence has strongly supported the hypothesis that phosvitin, a highly phosphorylated protein found as a major constituent of vertebrate eggs, is synthesized in the liver and transported to the developing ovum by the circulatory system. In an effort to demonstrate this phenomenon by immunofluorescent techniques, we have experienced considerable difficulty in preparing suitable antibodies against phosvitin. The relative nonantigenicity of this protein has necessitated the development of a method to obtain extremely pure preparations, thus avoiding false positive results provoked by traces of contaminants. Present techniques for the isolation of phosvitin, however, are somewhat laborious, are not generally applicable to the variety of vertebrate materials one may wish to study, and, in our hands, have not always yielded pure preparations. For these reasons, we have devoted considerable effort toward devising a simple and general method for the isolation and purification of phosvitin from vertebrate eggs.

The method developed is based on the realization that, with the exception of most teleosts, virtually all the phosvitin and lipovitellin of vertebrate eggs are complexed together in the form of insoluble granules (in physiological salt solutions). Therefore, by isolating these granules, one then has the relatively simple task of separating the phosvitin from the lipovitellin. This is achieved by an ammonium sulfate precipitation step in which two volumes of saturated ammonium sulfate are added to the dissolved granules (in *M* NaCl), thus precipitating the lipovitellin.

The eggs of most teleosts, however, contain a nongranular fluid yolk, but this difficulty can be circumvented by preparing an artificial phosvitin-lipovitellin complex. We have found that this can be accomplished by diluting a saline extract of eggs with sufficient water.

Finally and in order to obtain phosvitin of the highest purity, this two-step method (granule isolation and precipitation of the lipovitellin contaminant) may be supplemented by a third step involving DEAE-cellulose chromatography of the supernatant from the ammonium sulfate procedure. The sample (in 50 m*M* Tris-Cl buffer, pH 8.0) is applied to the adsorbent and eluted with a linear gradient of NaCl. Phosvitin is eluted at a salt

concentration of about 0.3 *M*. This final step has yielded homogeneous (in the ultracentrifuge) avian and amphibian phosvitins with the lowest phosphorus/nitrogen ratios reported to date. Using these procedures, we have further obtained preparations for the first time from the turtle, trout, gar pike, and lamprey as representatives of the vertebrate phylum.

6.2 EFFECTS OF ALCOHOL TREATMENT ON THE LENS-INDUCING ABILITY OF THE OPTIC CUP IN AN IN VITRO SYSTEM OF *XENOPUS LAEVIS*

S. K. Brahma Tuneo Yamada

It has been shown that the optic vesicle of the embryo of *Xenopus laevis* at stage 24 induces lens in vivo and in vitro from the competent ectoderm either of the same or different species.[1] The induced lens undergoes differentiation according to the stage of the ectoderm. Studies to be reported here are the first series of experiments made in an attempt to identify factors involved in lens induction using an in vitro system of *Xenopus laevis*.

Material and Method. – Eggs were obtained from *Xenopus laevis* according to the technique of Nieuwkoop and Faber,[2] and the eggs were kept in a defined medium[3] containing penicillin and streptomycin. Operation and culture were done in Holtfreter's saline buffered at pH 7.1 with Tris/HCl and containing above-mentioned antibiotics. In the control series the optic vesicles were removed from the stage 24 embryos[2] with the help of fine tungsten needles, and a sandwich explant was prepared by inserting the optic vesicle between two pieces of ectoderm from an early gastrula at stage 10½. The explant was then transferred to a fresh culture medium and cultured for 72 hr at 18°C. In the first and second experimental series, the optic vesicle was removed from the donor at stage 24, put into 70% ethanol either at 5 or 18°C for 24 hr, respectively, washed quickly, and tested for its effects on the ectoderm as in the control series.

Results and Discussion. – In all control experiments, lenses were formed in the reacting ectoderms, and after 72 hr of culture at 18°C they indicated differentiation of lens fibers accompanied by lens epithelium. Development of experimentally induced lenses was closely comparable to that of the normal lens. In the first and second experimental series the optic vesicle treated with ethanol failed to induce any lens in the reacting ectoderms. On the other hand, mesenchyme and neural tissues were formed in the ectoderms in those series. Table 6.2.1 summarizes the results. If we assume that a factor is transferred from the optic vesicle to the ectoderm and causes lens differentiation in the latter, the present results indicate either

Table 6.2.1. Effects of Ethanol Treatments on the Inducing Ability of Optic Vesicle

Treatment of Optic Vesicle	Total Number of Explants	Number of Ectoderms in Which Lens Formed	Number of Ectoderms in Which Mesenchyme Formed	Number of Ectoderms in Which Neural Tissue Formed	Number of Inductions
Untreated; control	16	16	0	0	0
Treated with 5°C ethanol	18	0	3	10	6
Treated with −18°C ethanol	18	0	7	9	3

inhibition of the transfer mechanism or inactivation of the factor by ethanol treatment. A preliminary attempt to obtain lens induction in the explanted ectoderm by a treatment with the homogenate of the optic vesicle made by D. H. Reese and S. K. Brahma has failed to produce positive results so far. In their experiment, as well as in the present one, failure to obtain lens induction might be due to the situation that the optic vesicle at stage 24 still does not contain a sufficient amount of lens-inducing factor but that it synthesizes the factor subsequently in a larger amount. If this is the case, treatment of the ectoderm with the homogenate or extract from the optic cup or the larval retina may give positive results. Another possible reason for the failure of induction might be that the gastrula ectoderm is not suited for this type of experiment. In this case, we should use the head ectoderm of the late gastrula as the reacting system.

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6.3 APPEARANCE AND ACCUMULATION OF A FINE, FIBROUS COMPONENT IN THE NUCLEUS DURING CELLULAR DIFFERENTIATION IN THE LENS REGENERATE, AND ITS POSSIBLE RELATION TO GAMMA CRYSTALLINS

Artur Jurand Tuneo Yamada

Studies with the immunofluorescent technique indicate that, during transformation of the iris epithelium into lens in Wolffian lens regeneration in *Triturus viridescens*, gamma crystallins appear in the phase of fiber differentiation[1] and are accumulated in the nucleus as well as in the cytoplasm.[2] An intensive accumulation of gamma crystallins in the nucleus was suggested in the later phase of fiber differentiation. Some circumstantial evidence is present for synthesis of gamma crystallins in the nucleus.[2] In this report an attempt was made to locate this protein group in the nucleus with the electron microscope. According to a number of earlier electron-microscopic works,[3-6] lens crystallins appear as a fibrous component of low or intermediate density in the cytoplasm of lens fiber cells. However, no report has been made that a similar component appears also in the nucleus of fiber cells, as one would expect from the immunofluorescent data.

Material and Methods. – Lens regenerates of *Triturus viridescens* at stages IV to XII were investigated. The regenerates were dissected during the 4-hr period of fixation at 4°C in the 2.5% glutaraldehyde fixative buffered at pH 7.2. Postfixation with 1% osmic acid solution buffered at pH 7.2 followed a thorough rinse with buffer. After dehydration with graded alcohol the regenerates were embedded in Epon, using a slow rotary shaker designed for embedding in viscous media.[7]

Observations. – The nuclei of control iris cells consist of two distinct spaces: (1) the chromatin space occupied by microfibrillar components appearing homogeneous at lower magnification, and (2) the interchromatin space occupied mainly by loosely scattered dense granules (Fig. 6.3.1). It is quite possible that the latter space contains extended DNA strands. During transformation of iris into lens, the first conspicuous change in the morphology of the nucleus to occur is an increase of the size with simultaneous rounding up of a rather irregular contour (Fig. 6.3.2). This change corresponds in time with an increase in the RNA synthesis in the nucleus.[8,9] After the change the interchromatin space appears significantly increased in extent, and the space is gradually occupied by a larger number of dense granules (Fig. 6.3.3). In the interphase nucleus of multiplication phase the interchromatin space communicates with the cytoplasm through the pores in the nuclear membrane.[10] In the young primary lens fiber cells, the interchromatin space begins to show a fine, fibrous component (Fig. 6.3.4) similar to the lens protein complex described by Resnik and Wanko. The dense interchromatin granules are present side by side with the fine component. A tendency exists that the amount of fine component increases with the progress of lens fiber differentiation. In somewhat older lens fiber cells the nuclear membrane degenerates, and it becomes difficult to distinguish the nucleus from the cytoplasm.

Discussion. – The data seem to suggest that the fine, fibrous component corresponds to or contains gamma crystallins. This is further supported by other experiments.[11] Localization of the fine component in the nucleus is in good conformity with that of gamma crystallins revealed by immunofluorescent studies. However, we need other methods for direct identification of the morphological component with gamma crystallins. It should be added that during the whole phase of cellular alteration the nucleus does not contain granules similar in ultrastructure to cytoplasmic ribosomes. The dense interchromatin granules which appear associated with the fine, fibrous component are larger in size and distinct in ultrastructure from ribosomes or polysomes.

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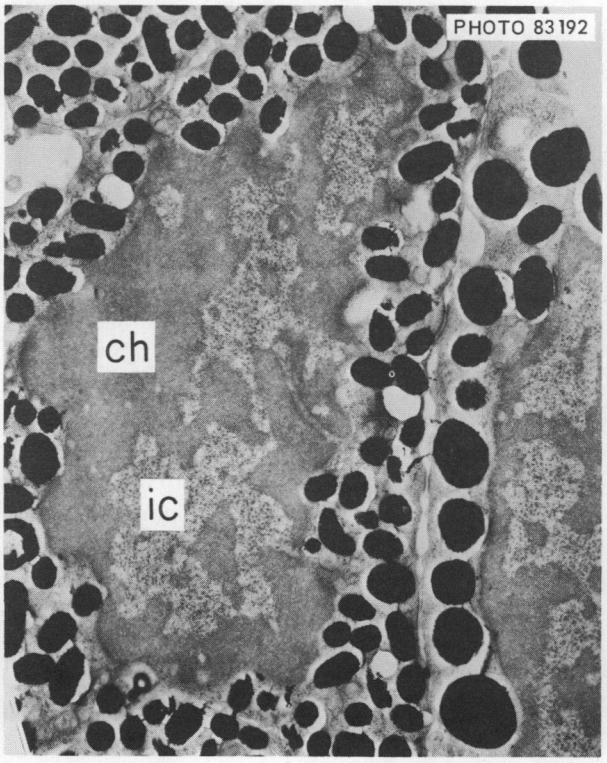

Fig. 6.3.1. Control Iris Cell at Low Magnification with the Nucleus in the Center and Cytoplasm Filled with Pigment Granules. Note the chromatin space (ch) with the interchromatin areas (ic) inside. 10,000×.

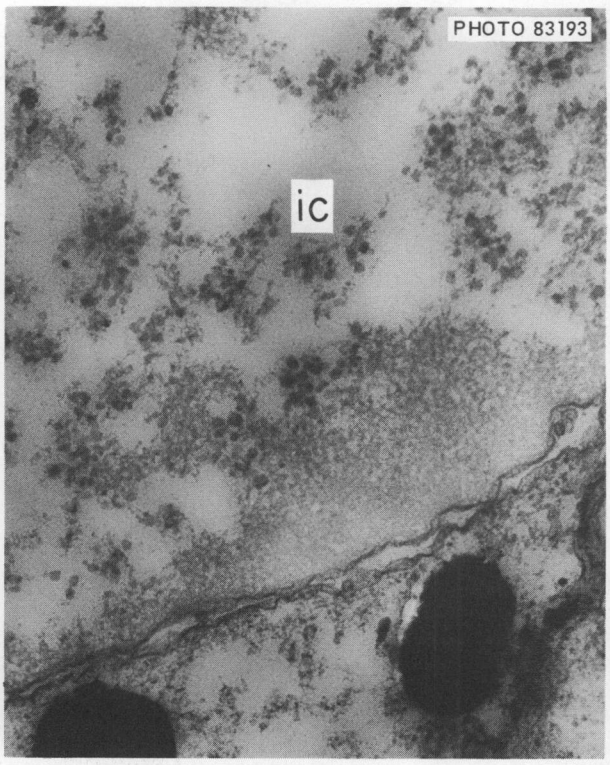

Fig. 6.3.2. Fragment of the Nucleus of an Iris Cell at an Early Stage of Depigmentation (Sixth Day). The interchromatin space (ic) greatly extended. 41,800×.

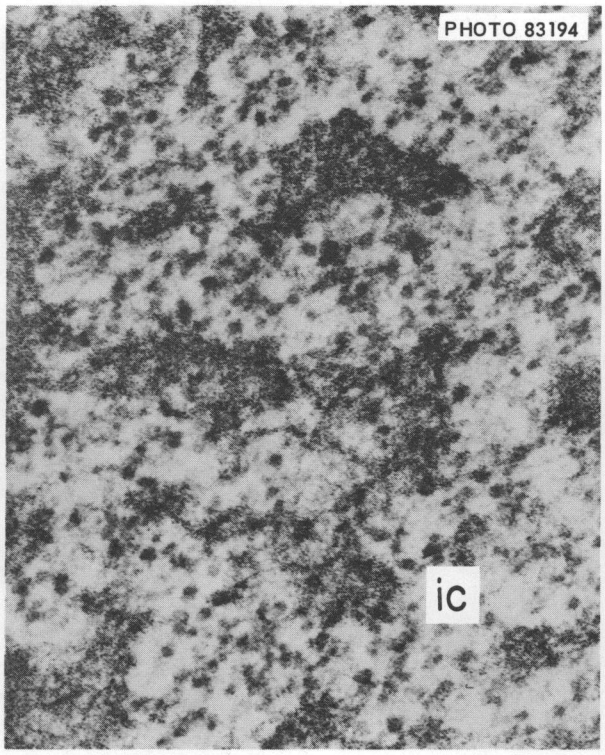

Fig. 6.3.3. Fragment of the Nucleus of a 15th-day Regenerate. The interchromatin space (ic) contains more dense granules. 55,000×.

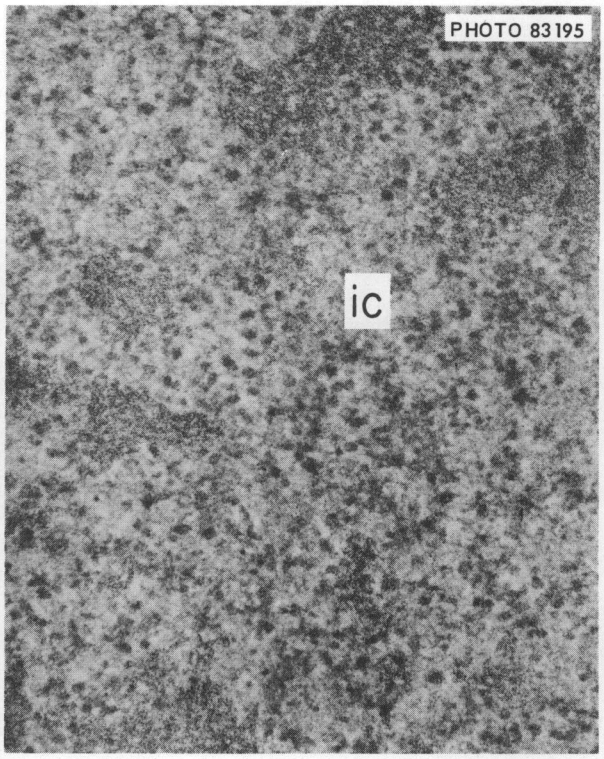

Fig. 6.3.4. Primary Fiber Nucleus. Interchromatin space (ic) contains some fine fibrous material between the dense granules. Chromatin lumps far apart from each other. 41,800×.

6.4 CHANGES IN THE PATTERN OF RIBOSOMES DURING CELLULAR DIFFERENTIATION OF THE LENS REGENERATE

Artur Jurand Tuneo Yamada

Earlier reports indicate that, during transformation of iris into lens in Wolffian lens regeneration, the cells undergo sequential changes which are characterized by the pattern of macromolecular synthesis. In the present report the distribution pattern of ribosomes in the cytoplasm of regenerate cells in various phases of cellular alteration[1] was studied in thin sections with the electron microscope. Special attention was given to the cells in fiber differentiation in which synthesis of gamma crystallins is restricted. For methods and materials, see the preceding paper.[2]

Observations. – In the depigmentation phase of the regenerate cells the majority of the ribosomes are scattered throughout the cytoplasm as monosomes (Fig. 6.4.1). Some of the ribosomes stud the scarce endoplasmic reticulum. In this condition, ribosomes appear in clusters. During the multiplication phase more endoplasmic reticulum is present, which is either provided with clusters of ribosomes or not (Figs. 6.4.2 and 6.4.3). In both cases the remaining ribosomes are in the form of monosomes or of polysomes of various sizes. There is a tendency for an increase in large polysomes with the progress of regeneration in the cells of the multiplication phase. In the early phase of fiber differentiation the majority of the ribosomes are arranged into regular polysomal groups consisting on the average of eight units (Fig. 6.4.4). Otherwise, the cytoplasm is scanty in formed organelles but filled up with a fine, fibrous component mentioned in the preceding paper.[2] Later in the fiber differentiation phase the ribosomes lose their polysomal arrangement

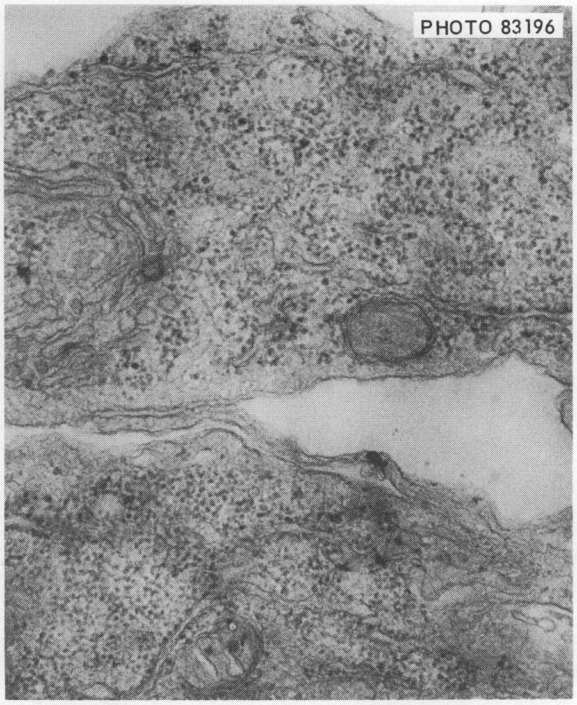

Fig. 6.4.1. Fragments of Two Cells at the Stage of Depigmentation (15th Day). 37,000×.

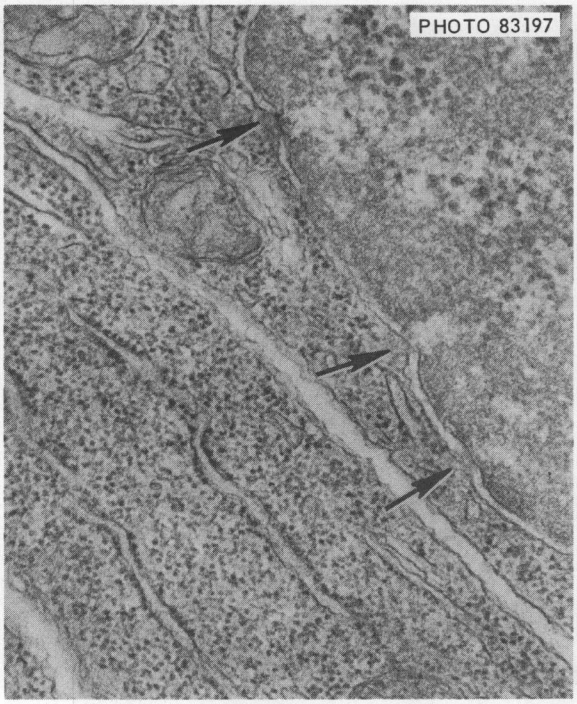

Fig. 6.4.2. Epithelial Cells in a 21-day Regenerate. Ribosomes as monosomes or studding the endoplasmic reticulum. Note nuclear pores (arrows). 37,000×.

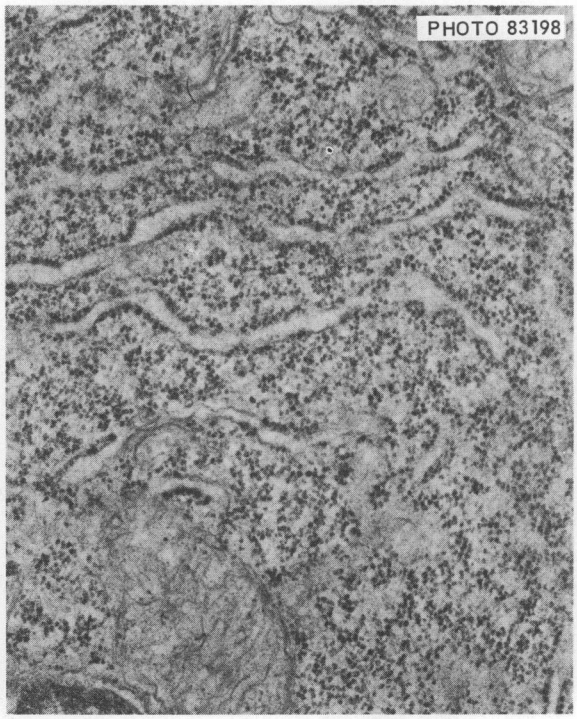

Fig. 6.4.3. Epithelial Cell More Advanced in Differentiation. Ribosomes rather in polysomic condition. 37,000×.

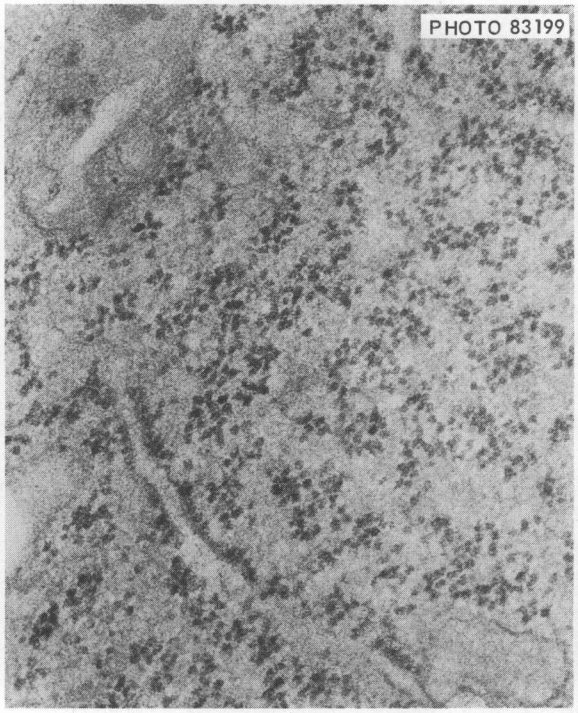

Fig. 6.4.4. Polysomes in a Young Primary Fiber Cell (21 Days). 62,500×.

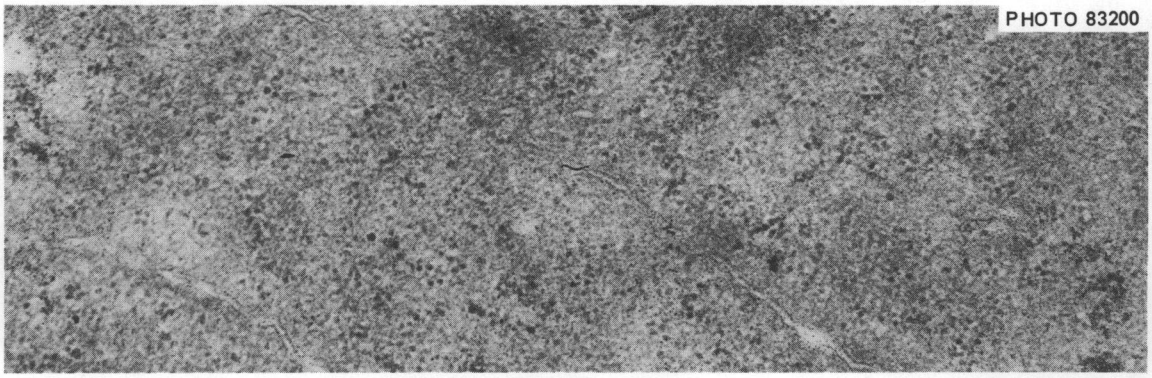

Fig. 6.4.5. Primary Fiber Cell (28 Days). Note that ribosomes are less electron dense than in earlier stages of differentiation. 37,000×.

and become less electron dense than the ribosomes in the earlier phases (Fig. 6.4.5). The fine component predominates the cytoplasm in this phase.

Discussion. – The observations are interesting when considered in connection with earlier results:[3] In the early phase of lens regeneration, depigmenting and multiplying cells are engaged in production of ribosomes and various proteins. However, synthesis of lens specific proteins in large amounts only starts after the cells enter the terminal cell cycle (the fiber differentiation phase), when production of ribosomes ceases. In the latter phase, gamma crystallins should be the predominating proteins to be synthesized. The data obtained in the present study fit in well with the notion that during depigmentation and multiplication phases only part of the ribosomes produced are utilized for synthesis of various proteins and that after entering the fiber differentiation phase most of the ribosomes are engaged in synthesis of lens specific proteins, including gamma crystallins.

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6.5 ENHANCEMENT OF NUCLEIC ACID SYNTHESIS IN THE IRIS EPITHELIUM CAUSED BY LENS REMOVAL IN *AMBLYSTOMA OPACUM*

Marion E. Roesel Tuneo Yamada

Lens regeneration from the dorsal iris margin is a phenomenon which regularly follows lens removal in several salamander species. *Triturus viridescens* has been used in this laboratory for the purpose of studying the cellular events which take place in the iris prior to the formation of a new lens. Of particular interest is an increase in DNA and RNA synthesis which begins to appear in the iris cells soon after lentectomy.[1,2] Whether and how these changes in nucleic acid metabolism are connected with alteration of tissue specificity are two of the problems in which we are interested. Preliminary experiments seem to indicate that in *Amblystoma opacum* no transformation of iris epithelium into lens can occur. We are, therefore, interested to find out if any change in DNA or RNA synthesis is caused by lens removal in the iris epithelium of this species.

Methods. – *Cytidine Series.* – At a stage prior to metamorphosis, ten larvae of *Amblystoma opacum* were unilaterally lentectomized. They were injected with 10 μc of ^{3}H-cytidine per gram of body weight into the body cavity at 4 to 22 days after lentectomy. Eyes were fixed 6 hr after the injection. Three additional larvae not lentectomized were used as controls for the series. Part of the slides were incubated with ribonuclease, and the

remaining slides simply with phosphate buffer. All slides were subsequently extracted with cold trichloroacetic acid (TCA). NTB-3 emulsion was applied, and the slides were exposed for ten days. By subtracting the average grain counts per unit nuclear area of ribonuclease-treated section from those of buffer-treated sections, grain counts due to radioactivity in RNA were estimated. The corrected grain counts of the cells of the marginal iris epithelia of the left operated and right nonoperated eyes were compared. The comparison was made for the dorsal and ventral irises separately.

Thymidine Series. – Twelve larvae of *Amblystoma opacum* prior to metamorphosis were lentectomized. They received injections of 1 μc of ^{3}H-thymidine per gram of body weight into the body cavity at 5 or 11 days after lentectomy. Eyes were fixed at 6 hr following injection. Slides were extracted with cold TCA before being coated with NTB-3 emulsion. The cells of the iris epithelium of each eye were studied for the presence of silver grains over the nuclei. The cells having more than five grains over the nucleus were counted as labeled. The number of labeled cells and that of the total number of cells counted were registered for dorsal and ventral iris epithelia of left operated and right nonoperated eyes.

Results and Discussion. – In the cytidine series no significant difference was observed between the corrected average grain counts per unit nuclear area of the marginal iris epithelia of the left operated and right nonoperated eyes. The same was true for the control series. In the thymidine series a significant increase in the percentage of labeled cells was indicated by a comparison of the iris epithelia of the left operated and right nonoperated eyes in a majority of the cases (Table 6.5.1). Thus our results show that an enhancement of DNA synthesis but no detectable enhancement of RNA synthesis is caused by lens removal in the iris epithelium, which does not regenerate lens. This observation seems to emphasize the importance of enhancement of RNA synthesis sub-

Table 6.5.1. Percentage of Labeled Cells in the Iris Epithelia of the Lentectomized and Control Eyes[a]

Series	Left (Operated)			Right (Control)		
5 day[b] – 1[c] dorsal	6.29	**8.35**	10.41	−0.18	**0.18**	0.55
1 ventral				0.39	1.88	3.38
2 dorsal	14.63	**17.87**	21.11	0.89	**2.04**	3.18
2 ventral				0	0	0
3 dorsal	2.55	**4.65**	6.74	−0.29	**0.76**	1.83
3 ventral	1.30	**2.61**	3.91	1.77	**3.12**	4.46
4 dorsal	0.91	**1.97**	3.03	−0.13	**0.14**	0.42
4 ventral	1.28	**2.66**	4.03	0	**0**	0
5 dorsal	3.17	**5.19**	7.21	1.33	**2.51**	3.68
5 ventral	0.02	**0.98**	1.95	1.30	**3.11**	4.92
11 day[b] – 1 dorsal	2.25	**3.88**	5.50	3.49	**5.64**	7.79
1 ventral	5.11	**7.85**	10.60			
2 dorsal	11.13	**13.41**	15.68	5.97	**8.30**	10.62
2 ventral				1.42	**2.84**	4.26
3 dorsal	6.80	**8.84**	10.88	0	0	0
3 ventral				0.53	**1.53**	2.52
4 dorsal	12.29	**14.39**	16.48	2.64	**4.81**	6.98
5 dorsal	14.41	**17.04**	19.67	0.09	**0.75**	1.40
5 ventral	7.03	**9.09**	11.15	2.13	**3.38**	4.64

[a]The percentage is given with lower and upper limits of confidence at 95%. Values at the left and the right of each bold line should be compared.

[b]After lentectomy.

[c]Each number stands for one animal.

sequent to lens removal in the iris epithelium of *Triturus viridescens* for the mechanism of transformation of the iris epithelium into the lens.

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6.6 CONTINUED GROWTH OF THE REGENERATING LENS IN VITRO

D. H. Reese Tuneo Yamada

Previous experiments on the culture of the iris with its regenerating lens have shown that, if the explant is put into culture before the depigmented cells reach the terminal cell cycle, cell multiplication continues without fiber differentiation.[1] It has now been found that if the whole eye with its regenerating lens is removed from the animal and put into culture, the regenerating lens continues to grow and differentiate.

Method. – In the first experiment, the lens was removed from the left eye of each of ten adult newts (*Triturus viridescens*). After lens removal the animals were maintained at 21°C. At 2 and 11 days after lens removal, five lentectomized eyes were surgically removed from the animals and cultured in a chemically defined medium according to the method described in an earlier report.[1] The five eyes removed at 2 days were cultured for 20 days, and those removed at 11 days were cultured for 11 days. Thus, all eyes were allowed to regenerate for 22 days prior to fixing. All eyes were subcultured every 4 or 5 days.

In the second experiment, bilateral lentectomies were performed on 20 adult newts (*Triturus viridescens*). After lens removals the animals were maintained at 18°C for 50 days. After this period the left eye of each animal was removed and cultured for 8 days, with one subculture at 4 days. The right eye was fixed at the time the left eye was removed from each animal for culturing. This right eye served as a basis for determining the progress of regeneration in vitro by comparing it with the eye that was cultured.

Results and Discussion. – In the first experiment, all the explants which were put into culture 2 days after lentectomy showed completely depigmented cell populations on the dorsal pupillary margin. However, these cell masses did not have the organization characteristic of in vivo regenerates. There were many more depigmented cells present than in the corresponding regenerate in vivo. The regenerates looked much like those reported on earlier[1] in which the depigmented cells stayed in the multiplication phase and failed to enter the terminal cell cycle. Three of the regenerates, however, did show a small number of fiber-like cells in the inner layer of the depigmented iris. In all the regenerates, the outer layer of the iris, which is to become the lens epithelium, was many cell layers thick. This is not characteristic of the normal lens epithelium.

In those eyes which were put into culture 11 days after lens removal the regenerates were quite different from those described above. In every case but one, each showed a large well-formed lens with extensive secondary lens fibers. The epithelia in these were characteristically one cell layer thick.

It would appear that, since in both cases cited above the lenses were removed 22 days prior to fixation (and thus all lenses should be at the same stage), there must be some factor or factors provided in vivo which are responsible for the almost normal development of those eyes put into culture 11 days after lens removal as compared with the retarded development of those put into culture only 2 days after lentectomy. This, however, may not be the case since it became apparent in the process of removing the eyes for culturing that the corneas of the eyes which had their lenses removed only 2 days before were not sufficiently healed. Due to the operation, in each of the five eyes the incision in the cornea was ruptured, allowing the fluid of the eye to leak out. This did not occur in the eyes removed for culturing 11 days after lens removal. If one accepts the working hypothesis that the neural retina produces a substance which is responsible for the initiation of lens regeneration, it can be interpreted that the rupture of the cornea allowed the leaking out of this factor, creating an unfavorable intraocular environment for lens differentiation.

In the second experiment, sufficient time elapsed between removal of the lens and removal of the eye to allow the cornea to heal completely. A comparison of the stage of the regenerate in the cultured eye with that of the regenerate in the

Table 6.6.1. Comparison of the Left Eye, Which Was Cultured for Eight Days, with the Right Eye of the Same Animal, Which Was Fixed at the Time the Left Eye Was Put into Culture

Eye	Regeneration Stages																			
Left[a]	10	10	9	10	11			11	10	10	10	10	10	10	9	10		9	10	10
Right[a]	5	2	6	8	7	4	6	6	6	7	7	6	6	7	6	6	5	6	5	7

[a]Each column represents a pair of eyes from one animal.

right eye (which was fixed at the time of culturing) from the same animal is summarized in Table 6.6.1. It is apparent from the table that considerable development of the regenerating lenses occurred in most cases during the 8-day culture period. The amount of development as determined by the stage attained by the regenerates at the end of the culture period is in close agreement with in vivo data at the same temperature obtained elsewhere.[2]

In both the experiments described above, the histological condition of the tissue components of the eye remained in very good condition. Those eyes cultured for 20 days stayed in as good condition as those cultured for 8 days.

The present culture condition then allows in vitro development and differentiation of the regenerating lens. This technique now provides a means for the analysis and possible elucidation of the factors responsible for tissue transformation in Wolffian lens regeneration.

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6.7 WOLFFIAN LENS REGENERATION: DEPIGMENTATION AND FIBER DIFFERENTIATION IN A CHEMICALLY DEFINED MEDIUM

D. H. Reese Tuneo Yamada

Attempts have been under way for some time in this laboratory in obtaining the in vitro initiation of Wolffian lens regeneration in a chemically defined medium. We have cultured the isolated nonregenerating iris of the adult newt *Triturus viridescens* under different conditions without obtaining the initiation of depigmentation, which is the first reliable morphological manifestation of impending lens regeneration. With the culture method described in a previous report[1] we have recently obtained depigmentation of dorsal iris cells and transformation of these cells into fiber cells by putting the whole eye into culture immediately after lens removal. In many cases, morphological differentiation of these fiber cells is normal. There are, however, some features in this system which deviate from lens regeneration in vivo. One type of deviation is a distortion of the lens epithelium, which in extreme cases interferes with secondary fiber formation although primary fiber formation usually takes place. A certain amount of distortion in the epithelium is tolerated, however, allowing secondary fiber cells to form. Such distortion is due to a collapse of the optic cavity caused by loss of intraocular pressure during lens removal. Another type of deviation from the normal course of lens regeneration is a retardation of the rate of depigmentation in the dorsal iris. In vivo depigmentation is completed by about 10 days, while in vitro depigmentation does not begin until about 15 days. One factor which may have a bearing on this retardation is the availability of amoeboid cells, which have been shown by electron-microscope studies to take up melanin granules from the depigmenting iris cells in vivo.[2] Although it is evident from a histological examination of the explants that some of these cells remain within the explanted eye and do take up pigment granules from the iris, many more may be required for depigmentation to proceed at a rate comparable to in vivo depigmentation.

The inability of the isolated iris to start lens regeneration in our culture conditions, in contrast

to its ability to undergo depigmentation and fiber differentiation when it is cultured within the isolated eyeball, indicates that there is some factor(s) in the eye tissues which induces the iris epithelium to convert into the lens. It is possible that the hypothetical retinal factor[3] is one. We are currently doing a number of experiments in an attempt to identify the factor(s).

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6.8 FURTHER STUDIES ON THE MEMBRANOUS COMPONENT OF AMPHIBIAN OOCYTE NUCLEOLI

O. L. Miller, Jr.

The several hundred peripheral nucleoli in the nucleus of a *Triturus* oocyte can readily be isolated by manual techniques. The coarsely fibrous core and granular cortex components of these nucleoli can be cleanly separated in several types of isolation media. In a previous semiannual report,[1] the finding of a third, membrane-like component attached to the nucleolar cores was described. This component has been further characterized using phase-contrast microscopy.

When an oocyte nucleus is isolated into 0.1 *M* KCl and the nuclear contents subsequently isolated into 0.01 *M* saline, a very rapid separation of the nucleolar core and cortex components occurs. Under these conditions, the membranous components separate from the cores and disperse as spherical vesicles 1 to 4 μ in diameter on the front edge of the circle of dispersing nucleoplasm. A basal portion of the membranous component, however, apparently remains with each core and appears as a small dark spot or thickened region on the core component. The speed at which the core and cortex components separate can be reduced by isolating the nucleus into 0.1 *M* phosphate buffer previous to isolating the nuclear contents into low-molarity saline. Under these conditions, many of the membrane-like structures remain attached to the cores but still maintain a spherical shape.

The effect of the change in saline molarity can be further reduced by adding 0.1 to 0.2 *M* sucrose to the final isolation medium. In this medium most of the cores retain the membranous component, but the shape of this component now varies from a bladder-like structure on a thin stalk to a lone attenuated filament up to 25 μ in length. Treatment of the membrane-like component with acidic solutions (pH 2) or with detergents gives results similar to that obtained with the nuclear envelope, that is, both structures immediately break down and disappear in the medium.

Biochemical studies with this system have shown that the precursor of ribosomal RNA is a 40-S RNA molecule, and concurrent autoradiographic studies demonstrate that the first incorporation of precursor into the 40-S molecule occurs inside the nucleolus at a point eccentric toward the nuclear envelope.[2] This is also the point where the membranous nucleolar component is attached to the core component. The coincidence of the attachment point of the membranous component and the site of initial incorporation suggests that the membrane-like nucleolar component may play a role in the transformation of the ribosomal precursor RNA into the smaller ribosomal RNA's. Combined electron-microscopy–autoradiography experiments designed to answer this question are in progress.

References

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[2]J. G. Gall, unpublished results.

Radiation Immunology

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7.1 SECONDARY ANTIBODY-FORMING POTENTIAL OF MICE IN RELATION TO AGE – ITS SIGNIFICANCE TO SENESCENCE

Takashi Makinodan W. J. Peterson

Introduction. – Our previous studies[1] on the growth and senescence of the primary antibody-forming potential (PAFP) of intact mice and their spleens revealed that growth and senescence of PAFP are due mainly to increase and decrease, respectively, in the number of progenitor cells. The present report is concerned with the secondary antibody-forming potential (SAFP) of individuals of varying age groups and of their tissue.

Results. – When one-month-old mice were primed with an adequate dose of antigen, their SAFP increased 25-fold to a maximum level and then decreased exponentially with a $T_{1/2}$ of about 6 months until at least 27 months of age. The growth of the SAFP per spleen was more spectacular. It increased 100-fold to a maximum level and then decreased exponentially with a $T_{1/2}$ of about 10 months. In comparison the maximum growth of the PAFP per spleen starting at one month of age was only 10-fold, but then it also decreased exponentially with a $T_{1/2}$ of about 10 months. On the other hand, the spleen cell mass, indicative of the total number of cells per spleen, grew only 4-fold without any evidence of decrease.

Discussion. – The lack of correlation between change with age in the SAFP per spleen and the spleen cell mass is consistent with the notion that immunologically competent cells constitute a minority among the lymphoid cells of the spleen. These results also indicate that once a young mouse is adequately primed, its SAFP per spleen will always be higher than the PAFP per spleen. The significance of the apparently exponential nature of decrease with age in the PAFP and SAFP per spleen and the SAFP per mouse to the

life expectancy of individuals must await further studies. However, it is suggestive that immunologic rejuvenation of the aged is feasible.

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7.2 HOMEOSTATIC REGULATION AND THE DEPLETION OF IMMUNOLOGICALLY COMPETENT CELLS BY EXPOSURE TO EXCESS ANTIGEN

J. F. Albright T. W. Evans
Julia W. Deitchman

Introduction. – In previous studies[1] it has been observed that the reservoir of immunologically competent (progenitor) cells can be temporarily depleted by exposure of an unprimed animal to a large dose of antigen. This was assessed by testing the residual response to a second, non-cross-reacting antigen. It was found that the fund of progenitors could be reduced but not completely exhausted. These results raised the possibility that either: (1) there were two types of progenitors, those that were pluripotential with respect to antigens and those that were committed, or (2) homeostatic factors prevented stimulation of all of the progenitor cells owing to the impossibility of introducing sufficient antigen within a short period of time. There is evidence that there are more progenitor cells in a normal mouse than react to a given antigen exposure. Thus, if the second alternative were correct, nondiscriminatory reduction in the number of progenitors by irradiation should permit temporary exhaustion, more or less, of the remaining progenitor cells by a supraoptimum antigen exposure. This would be revealed by temporary failure of a lightly irradiated animal to respond to a test antigen (Ag_2) after a massive injection of non-cross-reacting antigens (Ag_1).

Methods and Results. – Normal, young-adult $C3lF_1$ mice were treated as four separate groups. One group was irradiated with 200 r of x rays and injected with a test dose of rat erythrocytes (Rrbc); antibody response of these mice was followed with time. A second group was similarly irradiated, then injected with sheep erythrocytes (Srbc) followed 24 hr later by the test dose of Rrbc; their response was also followed with time. A third group of mice was irradiated, then injected with chicken erythrocytes; 24 hr later these mice received Srbc, and after an additional 24-hr period they were administered the test dose of Rrbc and their response to Rrbc followed. A final group of mice were unirradiated. They received Srbc, and after 24 hr they received the test dose of Rrbc. Antibody production against Rrbc over a ten-day period by all four groups of mice is illustrated in Fig. 7.2.1.

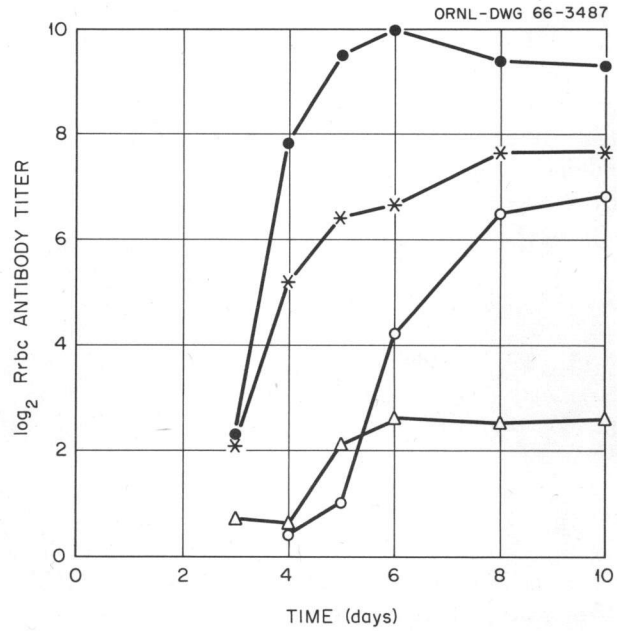

Fig. 7.2.1. Antibody Response Profiles to Rrbc Antigen Administered to Mice After the Following Pretreatments: (•) 200 r, 4×10^9 Rrbc; (*) 2×10^9 Srbc $\xrightarrow{24\ hr}$ 4×10^9 Rrbc; (o) 200 r, 4×10^9 Srbc $\xrightarrow{24\ hr}$ 4×10^9 Rrbc; (Δ) 200 r, 10^9 Crbc $\xrightarrow{24\ hr}$ 3×10^9 Srbc $\xrightarrow{24\ hr}$ 2×10^9 Rrbc.

Discussion. – It is clear from the results that prior exposure of immunologically competent cells to non-cross-reacting antigens severely impairs the response to a given test antigen administered a short time later. That this effect is only slightly evinced in the normal, intact animal is the result

of the reserve progenitor cells which are not stimulated by the initial antigens even when given in the highest possible dosages. The operation of this homeostatic mechanism is not yet fully understood. It seems clear, however, that immunologically competent cells are pluripotential toward antigenic determinants.

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7.3 THE POSSIBILITY OF DETERMINING THE ABSOLUTE NUMBER OF HEMAGGLUTINATING ANTIBODY MOLECULES IN ANTISERA

W. R. Somers R. A. Brown
Takashi Makinodan

Introduction. – If an expedient method for translating hemagglutinating activity into an absolute number of active molecules per milliliter could be devised and made routine, antibody assays would be placed on a much firmer basis. Levine, Puck, and Sagik[1] have demonstrated that the absolute number of virus hemagglutinins can be evaluated by observing the sedimentation rates of red blood cells in the presence of virus. Subsequently Kabat and Mayer[2] have suggested that their technique may be applicable to hemagglutination by antibody.

The analysis of Levine *et al.*[1] presumes that one antibody is sufficient to produce a red blood cell dimer and that dimer concentration is proportional to antibody concentration. This assumption has been examined experimentally.

Results. – Counts of the various red blood cell polymers in the presence of different concentrations of antibody were made in a hemacytometer after incubation with agitation for 45 min at 37°C. Statistical analysis indicated that the data are best represented by the equation: frequency of dimers = 2.24 (antibody concentration)$^{1/3}$. At the lower antibody concentrations there is an excellent fit of the data to this equation.

Discussion. – Since there is no range of antibody concentration for which the frequency of dimers is a linear function of antibody concentration, the fundamental assumption on which the technique of Levine *et al.*[1] is based is not valid. It is not possible to make an estimate of the absolute number of agglutinating particles in antisera by their technique.

The complex dependence of dimer formation upon antibody concentration probably reflects the necessity of more than one antibody molecule to overcome the electrostatic forces between red blood cells[3] and the saturation of a complex mosaic of antigenic sites by a highly heterogeneous antiserum.

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7.4 FUNCTIONAL RIBOSOMES IN ANTIBODY-FORMING CELLS

Toshiteru Morita R. A. Brown

Introduction. – It is known that the synthesis of protein takes place on larger ribosomal units (polyribosomes) bound together by messenger RNA in the mammalian systems. In the immune system, investigators have been unable to obtain these larger ribosomal units, which would correspond to the L and H chains of γ-globulin. These difficulties have arisen because the lymphatic tissues consist of heterogeneous cells and comparatively high RNase activities. The present study was carried out to obtain the functional ribosomal units in antibody-forming cells under the condition where the activity of RNase is minimized.

Method and Results. – Primed $C31F_1$ mice were restimulated with antigen (1 ml of 1% sheep RBC), and at varying intervals after restimulation their spleens were removed. The teased cell suspensions were cultured in Eagle's basal culture medium supplemented with 10% newborn calf serum for 30 min at 37°C and then labeled with

^{14}C-amino acids for 5 to 10 min. The washed cells were resuspended for 10 min in cold hypotonic buffer (10^{-2} *M* Tris-HCl, pH 7.4; 10^{-2} *M* KCl; 10^{-2} *M* $MgSO_4$) containing 0.5% polyoxyethylene cetyl ether, nonionic detergent, which enhanced cell breakage, and 5 mg of bentonite per milliliter, and then the cells were homogenized. In some experiments, the supernatant from mouse regenerating liver extracts obtained by centrifugation at 105,000*g* from 90 min was added prior to homogenization. Either the postnuclear fraction or the postmitochondrial fraction was treated with 0.5% sodium deoxycholate and 2×10^{-4} *M* spermine, layered upon a 10 to 30% sucrose density gradient, and centrifuged at 24,500 rpm for 150 min.

Under these conditions seven or eight peaks of optical density at 260 mμ were observed, corresponding to polyribosomes with up to seven or eight ribosomal units in "immunized" spleen cells. (TMV was used as a sedimentation marker.) The distribution of radioactivity from "immunized" spleen cells was found in the region of two to three and five to ten ribosomal units. Cells treated with puromycin and "nonimmunized" cells contained little radioactivity in these regions of the gradient. The addition of supernatant from mouse regenerating liver shifted polyribosome distribution to the larger polyribosomes, and this would suggest that this supernatant contains an inhibitor of RNase.

Discussion. – Polyribosomes containing five to ten ribosomal units would correspond to the peptides 20,000 to 30,000 in molecular weight and may be of sufficient size for the synthesis of γ-globulin peptides. The results presented above would suggest that the breakdown of polyribosomes by RNase was an important factor in the earlier observation of ^{14}C-amino acid incorporation predominantly into monomer and dimer ribosomal units.

7.5 EFFECT OF PRIOR STIMULATION OF DONORS ON THE IN VITRO ENGULFING CAPACITY OF PERITONEAL PHAGOCYTES

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Introduction. – Previous studies have shown that peritoneal phagocytes destroy sheep red blood cell (RBC) antigens and that the minimum antibody response was obtained when opsonized sheep RBC were injected intraperitoneally into previously stimulated mice.[2] The development of a simple quantitative method[3] has permitted us to measure the in vitro engulfing capacity of peritoneal exudate (PE) cells from stimulated or nonstimulated mice.

Results. – Peritoneal exudate cells harvested from mice which had been stimulated with 2 ml of 4% gelatin 48 hr earlier had a markedly enhanced engulfing capacity. This engulfing capacity was associated with greater numbers of histiomonocytic cells in the exudates of stimulated mice. Sheep RBC number was therefore adjusted relative to the number of histiomonocytic cells in the exudate, to give a constant RBC:histiomonocytic cell ratio. This enabled us to demonstrate that the enhanced phagocytic capacity was not due solely to the increase in the number of histiomonocytic cells, but that it was also related to an increased engulfing efficiency of the stimulated histiomonocytic cell population. Both particulate and soluble material (sheep and rat RBC, lipopolysaccharide, gelatin, sterile pyrogen-free saline) were injected into mice to determine what types of stimuli might result in a highly efficient histiomonocytic cell population. Differential counts demonstrated that while different stimuli resulted in different numbers of histiomonocytic cells, the relative engulfing efficiency per cell was comparable, and in every instance markedly superior, to nonstimulated exudate cells.

Discussion. – The results clearly show both quantitative and qualitative changes in the phagocytic elements of the peritoneal cavity as a result of intraperitoneal injection of various stimulants. Whether this enhanced capacity can be correlated to the maturation of preexisting cellular elements within the cavity, or whether it represents the mobilization of fully functional elements into the cavity as a result of stimulation, or a joint contribution of both, is being investigated.

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Mammalian Genetics

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Effects of Radiation on Mammalian Gametogenesis (8.3)

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Overall Measures of Genetic Damage (8.4)

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Cytology and Tissue Culture in Genetic and Developmental Studies (8.5)

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8.1 MUTATION FREQUENCY IN MOUSE SPERMATOGONIA AT HIGH RADIATION DOSE RATE

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The first dose rates compared in the original discovery of a dose-rate effect of radiation on mutation in mice[1] were approximately 90 and 0.009 r/min. The effects of lower and intermediate dose rates have been tested since then, but no work had been done at higher dose rates. In the experiment reported here, a substantially higher dose rate has now been used.

A dose of 300 r of x irradiation was given at the rate of approximately 1000 r/min to young adult male mice. Mutation induction in spermatogonia is being scored by our standard specific-locus method. To date, a total of 15 mutations at the 7 specific loci have been observed in 24,911 offspring.

Earlier experiments in which the same dose of 300 r of x irradiation was given at approximately

90 r/min yielded a total of 40 mutations in 65,548 offspring. On the basis of that frequency, and assuming no effect of increased dose rate, the expected number of mutations in the 24,911 offspring scored in the current experiment is 15.2. The observed value of 15 indicates that there is no change in mutation frequency in spermatogonia when the dose rate is increased from 90 r/min to 1000 r/min.

One interpretation of the lower mutation frequency at low dose rates is that repair of mutational or premutational damage is occurring. At the high dose rates it is assumed that the repair process is either saturated or damaged. On this interpretation, the present data indicate that when a dose of 300 r is delivered at 90 r/min, the repair process in spermatogonia is completely saturated or damaged. If it were not, then an increase in dose rate to 1000 r/min would be expected to have an additional effect on the repair process and result in more mutation.

Putting the present results together with those from earlier experiments[2] it would appear that, for doses in the range of 300 r and above, dose rates of from 0.001 to 0.8 r/min have little or no effect on the repair process in spermatogonia, a dose rate of 9 r/min has some effect, and dose rates of 90 r/min and above have reached the level that produces the maximum possible effect on the repair process.

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8.2 RADIATION-INDUCED SEX-CHROMOSOME ABNORMALITIES IN FEMALE GERM-CELL STAGES OF THE MOUSE

Liane B. Russell Clyde S. Montgomery

The two sexes of mammals offer different opportunities for comparing the genetic effects of radiation in various germ-cell stages. The female has no separate haploid gametic stage, and gonial stages are relatively inaccessible. On the other hand, it is possible to compare different meiotic stages with each other.

Radiation induction of sex-chromosome abnormalities in female mice was studied by observing the offspring of crosses in which the parents carried different sex-linked markers. This makes it possible not only to distinguish paternal and maternal sex-chromosome losses, but to detect maternal nondisjunction (production of X^MX^MY or $X^MX^MX^P$), should it be induced. Presumed exceptional offspring were tested genetically and/or cytologically.

In one series, marked females were irradiated with 150 to 250 r (mostly 250 r) at one of eight stages of development, namely, days $13\frac{1}{2}$ to $20\frac{1}{2}$ postconception (daily intervals), the last two (days $19\frac{1}{2}$ and $20\frac{1}{2}$) being postnatal. Oocyte development at the time of irradiation ranges from the last DNA synthesis (preleptotene) to first appearance of the dictyate stage (postdiplotene). The irradiated embryos and newborns were permitted to grow up and then were mated to appropriately marked males. Sex-chromosome abnormalities were scored in 2402 offspring produced throughout life. There were 785 control offspring. In another series, females received 400 or 100 r as adults. The 331 offspring observed were conceived 1 to 32 days postirradiation (irradiated dictyate).

Spontaneous maternal sex-chromosome abnormaltities are known to be exceedingly rare, and none was observed in controls. Induced trisomy of sex chromosomes (i.e., positive evidence for nondisjunction of maternal X's) was not observed. Induced maternal-X loss occurred with frequencies of 2.3 and 3.1%, respectively, for the two doses, after irradiation of dictyate in adults. For embryonic and neonatal stages, on the other hand, the overall frequency of X^M loss was only 0.3%, with the highest incidences in the $13\frac{1}{2}$-, $18\frac{1}{2}$-, and $17\frac{1}{2}$-day groups (differences between groups, however, were not significant). Although this frequency is very much lower than for dictyate, it is considerably above the spontaneous rate. As expected, paternal sex-chromosome losses in both series were no more frequent than in controls.

Effects on fertility of the females irradiated in utero or as newborns in general confirm those found earlier. Females irradiated days $13\frac{1}{2}$ or $20\frac{1}{2}$ postconception are exceedingly sensitive, while those irradiated at intermediate stages are less sensitive than are adults.

8.3 RADIATION RESPONSE OF SPERMATOGONIA IN MAN

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Volunteers were given local x-ray exposures of 20, 25, 50, 100, 200, 300, 400, and 600 r to the testes, and biopsies were taken at many intervals between 4 hr and 487 days after irradiation. Tissues were fixed in Cleland's solution, embedded in paraffin, sectioned at 5 μ, and stained with Heidenhain's iron hematoxylin and eosin.

Cell types were classified according to the descriptions given by Clermont.[2] Stages of the cycle of the seminiferous epithelium of man occupy such small areas of the tubule that an average of three stages normally is seen in a single tubule cross section. This makes quantitation of cell types extremely tedious, and selection of identifiable stages of the cycle for scoring as is done in the mouse or rat is not practical. Instead, we have scored any sections of tubule cut at right angles to the basement membrane (i.e., cell layers are not cut tangentially), and have identified each cell type present. Sixty such areas, both longitudinal and cross sections, were scored for each biopsy, and all counts were expressed in relation to the number of Sertoli cells.

Frequency of degenerating spermatogonia was significantly elevated 4 hr after irradiation. Maturation of spermatocytes and spermatids led to progressive loss of these stages owing to failure of replacement from spermatogonia. Eventually, repopulation of the seminiferous from surviving type A spermatogonia occurred. Thus the basic radiation response in man is the same as in experimental animals.

The time sequence of events in man, however, is different from the mouse, and more than just the difference in duration of spermatogenesis is involved. For example, duration of spermatogenesis in mouse and man is about 35 and 72 days respectively. Yet minimum spermatogonial numbers occur at 5 to 7 days after irradiation in the mouse; and though at present our time sequence is not extensive, the time of occurrence of maximal depletion is increased to 80 to 100 days in man, and frequency of spermatogonial degeneration remains high for a much longer time. Differences in cell dynamics, and especially the control of spermatogonial differentiation, must be involved.

Spermatogonial survival in man was much lower than would be predicted on the basis of data for experimental animals, and at 400 and 600 r, it was only one-tenth that observed in the mouse. The ratio of doses required to give this effect suggests a factor of about 2 or 3 in comparative sensitivity. However, both the more protracted decline in spermatogonial numbers and the slower recovery in man make exact quantitative comparisons difficult, and emphasize the care which must be taken in estimating relative radiation sensitivity in mouse and man.

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8.4 GENETIC CONTROL OF RADIATION STERILIZATION OF FEMALE MICE

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Large strain differences in the sterilizing effects of radiation on female mice have been reported.[1] In order to determine the cause of the intraspecific variation, crosses and backcrosses were made between the more sensitive strain (SEC) and the more resistant strain (DBA). The experimental procedures and preliminary results have been described in an earlier report.[2]

The total numbers of offspring and litters produced in the whole life span of females irradiated with 50 r of x rays are summarized in Table 8.4.1. The reproductive performances of irradiated inbred SEC and DBA females are similar to the results of earlier experiments.[1,3] The differences in the reproductive capacities between inbred and hybrid females indicate that the radiation response is controlled by a chromosomal factor. The differences between the reciprocal crosses of hybrid females are attributable to a maternal factor. The results of the backcross females support the conclusion that both chromosomal and maternal factors are responsible for the strain differences in the reproductive capacity of irradiated female mice.

Table 8.4.1. Reproductive Capacity of Female Mice After 50 r of X Rays Mated to C_3H Males

Strain of Irradiated Female	No. of Females	No. of Offspring	Offspring per Female	No. of Litters	Litters per Female
SEC	25	267	10.7 ± 0.8	40	1.6 ± 0.1
DBA	24	535	22.3 ± 1.5	123	5.1 ± 0.3
(SEC × DBA)F_1	26	399	15.3 ± 0.7	74	2.8 ± 0.2
(DBA × SEC)F_1	26	546	21.0 ± 1.3	99	3.8 ± 0.2
[SEC × (SEC × DBA)F_1]BC	51	594	11.6 ± 0.7	115	2.3 ± 0.1
[DBA × (DBA × SEC)F_1]BC	51	1316	25.8 ± 1.8	281	5.5 ± 0.3

On the basis of a chromosomal control of the radiation response, one would expect the number of offspring per backcross female to be intermediate between the productivity of irradiated inbred and hybrid females. But owing to the maternal factor the reproductive performance of the irradiated backcross females is closer to the number of offspring produced by the maternal inbred female. In the case of the [DBA × (DBA × SEC)F_1] backcross females, the resistance is more pronounced than in the inbred DBA females. This difference is very likely due to the fact that inbred DBA females have an unfavorable uterine environment for the embryonal development.[4]

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8.5 PRELIMINARY TEST FOR POSSIBLE MUTAGENIC EFFECTS OF DNA IN MICE

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There has been much recent interest in possible mutagenic or transforming properties of DNA in higher organisms. Several papers have claimed induction of mutations in *Drosophila* by the inclusion of calf thymus DNA into the medium (see, for example, Gershenson[1] and Mathew[2]). Fox and Yoon[3] have produced mosaics for specific loci in *Drosophila* at frequencies significantly higher than control levels by treating permeable eggs with DNA extracted from flies mutant at these specific loci. The present partially completed experiment is designed to look for any striking mutagenic effect of DNA administered directly to the gonads of mice.

Method. – DNA was extracted[4] from the testes of males of two stocks of mice. One of these is the (101 × C_3H)F_1 hybrid, and the DNA is referred to as H-DNA. The other is the multiple recessive test stock or T-stock used by W. L. Russell and his co-workers to study mammalian mutation phenomena. This stock is homozygous for seven recessive visible mutations, and the DNA from this source is referred to as T-DNA. DNA derived from these two sources was dissolved in isotonic saline solution at a concentration of 1 mg of DNA per ml of solution. The yield of the extraction process was approximately 1 mg of DNA per gram of tissue.

Thirty-eight male (101 × C_3H)F_1 hybrids, 14 weeks old at the start of treatment, were checked for fertility and divided into three groups.

Group I (12 animals) received intratesticular injections of 0.05 ml of isotonic saline solution per testis two times a day for ten consecutive days.

Group II (14 animals) received intratesticular injections of 0.05 ml of T-DNA solution per testis two times a day for ten consecutive days.

Group III (12 animals) received intratesticular injections of 0.05 ml of H-DNA solution per testis two times a day for ten consecutive days.

At the end of the treatment each mouse of groups II and III had received 1 mg of DNA per testis administered in 20 equal doses. Ten days following treatment all animals were mated to $(101 \times C_3H)F_1$ ♀♀ to check for fertility.

The experimental matings were begun 25 days after the end of DNA or saline treatment. The timing of these matings was designed so that the first litters would test sperm which had been exposed to DNA as primary spermatocytes, and later litters would test sperm which had been exposed to DNA as spermatogonia. The matings were planned to score approximately 1000 offspring of each group at weaning. Each of the treated animals was placed with six females of the multiple-recessive test stock, and a daily check was made of these females for vaginal plugs. Plugged females were removed and replaced. Litters are checked and sexed on the day of birth, are rechecked at five days of age, and are weaned at approximately 21 days of age.

All offspring of these experimental litters are examined for mutations and abnormalities. However, only litters for which there is a complete count on the day of birth are considered in litter-size determinations. Only litters which, in addition, had all offspring (live and stillborn) clearly sexable were used to compute sex ratios at birth.

Results and Conclusions. – At present, 465 litters have been obtained for the three groups with 3007 offspring; about half of these offspring have been weaned. Several abnormalities have shown up in the offspring. However, their distribution and frequency do not support the conclusion that they are related to the DNA treatment.

Two probable mosaics for one of the seven loci have appeared. One of these was in group I (saline control), and the other was in group II (T-DNA treated). No whole-body mutations have shown up for any of the seven loci involved in the test. The background mutation rate for the seven loci in males is one mutation for about 20,000 offspring.[5] We have weaned to date only about 500 offspring per group.

There is no significant difference in litter size for the three groups. Mean litter sizes for the three groups computed from present data are as follows:

Group I (133 litters)	6.77
Group II (181 litters)	6.47
Group III (145 litters)	6.44

The sex ratio at birth of offspring for the DNA-treated groups does not differ significantly from that of the saline control group. Data collected to date are as follows:

Saline control (885 offspring)	441 ♂♂	49.8%
	444 ♀♀	50.2%
DNA treated (2064 offspring)	1091 ♂♂	52.9%
	973 ♀♀	47.1%

It is concluded from the data available at present that no genetic effect of DNA administered by intratesticular injection has been demonstrated by this experiment. This does not exclude the possibility that a small effect of DNA at this dose level may exist nor the possibility that an effect may be demonstrated at higher dose levels. The results to date suggest several possibilities for further experimentation.

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Human Cytogenetics

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9.1 INDUCTION OF CHROMATID ABERRATIONS BY TRITIATED THYMIDINE

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Introduction. – In a previous report[1] the problems of using chromatid aberrations as a measure of a cell's radiosensitivity were discussed. Preliminary experiments were described in which mammalian cells were exposed to chronic beta irradiation from incorporated tritiated thymidine. The dose-response curves were found to be contrary to what would be expected on the basis of our knowledge of radiation-induced chromosomal aberrations. These experiments have been completed, and the present report summarizes them.

Dewey *et al.*[2] recently reported that when Chinese hamster cells were irradiated with chronic gamma or beta radiation the dose-response curves of all aberrations (exchanges and simple breaks) fit a two-component equation, that is, one and two hit. This is in contrast to the generally accepted notion that chronic irradiation leads to a reduction of the two-hit component and also that simple breaks increase linearly with dose for all types of radiation. The present experiments were performed to test the hypothesis that the dose-response curves obtained by Dewey *et al.* were merely artifacts of stage sensitivity and mitotic delay.

Materials and Methods. – A Chinese hamster cell line was partially synchronized with 5-aminouracil (5AU). Upon removal of 5AU, all the cells that eventually divided were in the DNA-synthetic stage (S) of the cell cycle. At this time the cells were pulse labeled with varying concentrations of tritiated thymidine. The tritiated thymidine served as a source of radiation. Three measurements were made, and all experiments were done in replicate. The various classes of chromatid aberrations were scored in the first division following the addition of the thymidine. Cultures were scored for the length of time it took for the occurrence of the mitotic peak to occur after labeling with the different concentrations of thymidine and for the length of time it took cells to leave S, as measured by ^{14}C-thymidine incorporation into the acid-insoluble nucleic acid.

Table 9.1.1. Number of Aberrations and Silver Grains Scored at the Different Tritiated Thymidine Treatments

Tritiated Thymidine Treatment (2 hr)		Number of Cells Scored for Silver Grains	Mean Number of Silver Grains per Metaphase	Number of Silver Grains per Hour	Number of Cells Scored for Aberrations	Number of Aberrations		
curies/millimole	μc/ml					Chromatid Deletions	Isochromatid Deletions	Chromatid Exchanges
				Part 1				
0[a]	0[a]	100	1.11 ± 0.1[b]	0.28 ± 0.025	200	0	28	12
0.75	0.625	100	6.74 ± 0.2	1.69 ± 0.05	200	22	41	18
1.5	1.25	100	13.36 ± 0.7	3.34 ± 0.02	200	16	50	18
3.0	2.5	100	38.95 ± 1.3	9.74 ± 0.43	200	73	63	44
4.5	3.75	100	51.53 ± 1.8	12.88 ± 0.45	200	103	84	59
6.0	5.0	100	65.72 ± 2.7	16.43 ± 0.55	150	129	70	68
				Part 2				
0[a]	0[a]	50	1.1 ± 0.15[c]	0.55 ± 0.075	200	2	46	16
0.75	0.625	50	4.2 ± 0.4	2.10 ± 0.20	200	20	47	29
1.5	1.25	50	7.2 ± 0.9	3.60 ± 0.45	200	36	65	26
3.0	2.5	50	11.6 ± 1.1	5.8 ± 0.55	200	70	80	60
4.5	3.75	50	21.5 ± 1.7	10.75 ± 0.85	200	183	108	87
6.0	5.0	50	29.5 ± 2.2	14.75 ± 1.1	200	274	131	133

[a]Control.
[b]4-hr film exposure.
[c]2-hr film exposure.

Table 9.1.2. Theoretical Effective Doses Calculated According to Mitotic Delay and Relative Stage Sensitivity and Corrected for Background

Tritiated Thymidine Treatment		Number of Silver Grains per Hour of Film Exposure	Time in		Effective Dose (D_E)		
curies/millimole	μc/ml		S (T_S)	G_2 (T_{G_2})	Terminal Deletions	Isochromatid Deletions	Chromatid Exchanges
				Part 1			
					$\times 10^{-1}$	$\times 10^{-1}$	$\times 10^{-1}$
0[a]	0[a]	0	7.0	3.0	0.0	0.0	0.0
0.75	0.625	1.4 ± 0.1	7.0	3.0	3.1	1.4	2.2
1.5	1.25	3.1 ± 0.2	6.5	3.5	7.4	3.1	5.3
3.0	2.5	9.5 ± 0.4	5.5	4.5	26.6	9.5	18.1
4.5	3.75	12.6 ± 0.5	5.0	5.0	37.8	12.6	25.2
6.0	5.0	16.2 ± 0.6	5.0	6.0	56.7	17.8	37.3
				Part 2			
0[a]	0[a]	0	7.0	3.0	0.0	0.0	0.0
0.75	0.625	1.6 ± 0.2	7.0	3.0	3.4	1.6	2.5
1.5	1.25	3.1 ± 0.5	6.5	3.5	7.4	3.1	5.3
3.0	2.5	5.3 ± 0.6	6.0	4.0	14.7	5.3	9.6
4.5	3.75	10.2 ± 0.9	5.0	5.0	30.8	10.2	20.5
6.0	5.0	14.0 ± 1.1	5.0	6.0	49.0	15.4	32.2

[a]Control.

Results. – The results of the latter two experiments showed that increasing amounts of tritiated thymidine resulted in a delay in the onset of mitosis, whereas there was no delay in the time cells left S. This means the mitotic delay was incurred almost solely in the post-DNA-synthetic (G_2) phase of the cell cycle. Table 9.1.1 summarizes the data on the yield of chromatid aberrations observed at the varying doses, as measured by silver grain counts over the chromosomes. When the data are plotted against dose, terminal deletions and chromatid exchanges give a best fit to the quadratic equation $Y = a + bD + cD^2$, while isochromatid deletions give a best fit to the linear equation $Y = a + bD$.

Discussion. – That the yield of terminal deletions best fits a quadratic equation is strange since it is generally believed that the deletions result from single tracks of ionization. It is also unusual that chromatid exchanges have a large two-hit component in their dose curve with chronic radiation. These contradictions can be explained if stage sensitivity and mitotic delay are accounted for. G_2 is five times as sensitive as S for the production of terminal deletions and three times as sensitive as S for the production of chromatid exchanges, while the two have equal sensitivities for the production of isochromatid deletions.[3,4] The experiments on mitotic delay and when the cells left S show that the cells that received the higher doses of radiation spent up to 3 hr longer in G_2 than those receiving very little radiation. If these differences in sensitivity and time spent in the respective phases of the cell cycle are accounted for, different relative doses can be calculated (see Table 9.1.2). When the yields of aberrations are plotted against these estimated doses, all classes of aberrations best fit a linear equation. It appears, therefore, that stage sensitivity and mitotic delay can explain otherwise peculiar results.

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9.2 EVIDENCE FOR THE ORIGIN OF SISTER CHROMATID EXCHANGES

Gregorio Olivieri J. G. Brewen

Introduction. – Whether the sister chromatid exchanges first described by Taylor are spontaneous or radiation induced is still an unanswered but important question. At this time it is impossible to answer it conclusively because tritiated thymidine must be used to see the sister chromatid exchanges.

If it is assumed that all radiation-induced chromatin breaks rejoin in a random manner and that sister chromatid exchanges are radiation induced, it is expected that for every isochromatid deletion seen at the first division after labeling there would be 1.2 to 2 sister chromatid exchanges seen at the second division after labeling. These ratios are not seen, and from this it can be concluded that either one or both of the above assumptions are not correct. An experiment was performed in an effort to determine what assumption is incorrect.

Materials and Methods. – Two cytological systems were employed, a Chinese hamster cell line and human peripheral leukocytes. Endoreduplicated cells were induced in the Chinese hamster cells with 5-aminouracil and in the human cells by 250 r of x rays. Endoreduplicated cells were used because they offer the unique opportunity of studying exchanges between paired chromatids without employing tritiated thymidine (see Fig. 9.2.1). Therefore, it is possible to study the occurrence of exchanges in cells not treated, as well as treated, with tritiated thymidine. Due to the similarity of the spatial relations of the daughter chromatids of diplochromosomes and those of sister chromatids of normal chromosomes, one can study the relative yields of symmetrical to asymmetrical exchanges and compare these with the relative yields of sister chromatid exchanges to isochromatid deletions.

Immediately after the 5AU or x-ray treatment, some of the cells were labeled with tritiated

thymidine. Both first- and second-division metaphases were collected with colchicine. The first-division diploid cells were scored for isochromatid deletions, and the first-division endoreduplicated cells were scored for both symmetrical and asymmetrical exchanges within diplochromosomes. Second-division diploid cells were scored for sister chromatid exchanges.

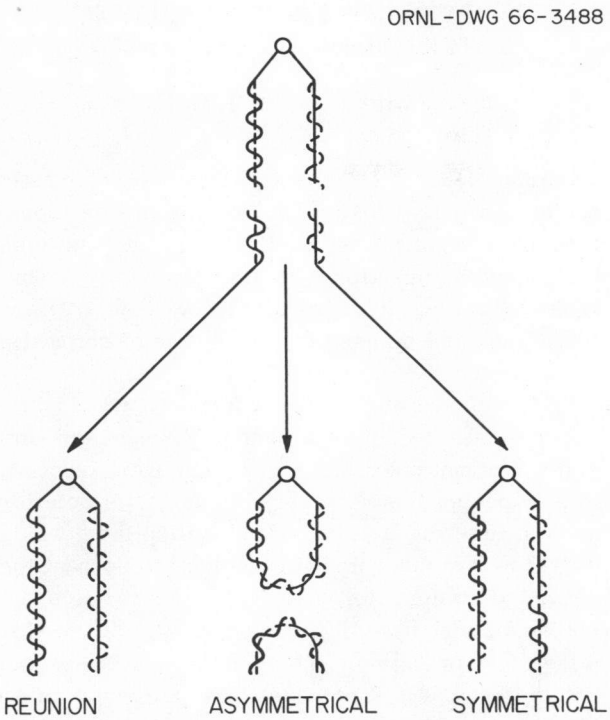

Fig. 9.2.1. Possible Types of Reunion in an Endoreduplicated Chromosome Having Two Isolocus Breaks.

Results and Discussion. – Tables 9.2.1–9.2.3 summarize the results of the scoring. There are several obvious results: (1) the exchanges in the endoreduplicated cells are induced; (2) tritiated thymidine can induce these interchanges (see human data); (3) reunion to form symmetrical or asymmetrical interchanges is not random; and (4) the ratio of sister chromatid exchanges to isochromatid deletions does not differ from the ratio of symmetrical to asymmetrical exchanges.

The authors believe these data can be interpreted to mean that sister chromatid exchanges are radiation induced. This belief derives from, first, the fact that, in a system (diplochromosomes) analogous to a normal chromosome where equivalent exchanges can be seen without the addition of tritiated thymidine, no exchanges are seen unless the cells are treated with known chromosome-breaking agents, tritiated thymidine being one of them. Second, the type of reunion of induced breaks that would give rise to either sister chromatid exchanges or isochromatid deletions is not random in a comparable system, and the ratio of the types of reunion in these diplochromosomes is the same as seen in the case of sister chromatid exchanges to isochromatid deletions.

Although these data do not represent absolute proof that sister chromatid exchanges are radiation induced, they certainly suggest such an interpretation.

Table 9.2.1. Frequencies of Isochromatid Deletions and Sister-Strand Exchanges Observed at First or Second Postlabeling Metaphase

Treatment	^{3}H-Thymidine		Number of First-Division Cells Scored	Number of Isochromatid Deletions per Cell	Number of Interchanges	Number of Second-Division Cells Scored	Number of Second-Division Chromosomes Scored	Number of Sister-Strand Exchanges	Corrected Number of Sister-Strand Exchanges per Cell	Ratio of Sister-Strand Exchanges to Isochromatid Deletions
	Specific Activity (curies/millimole)	Concentration (μc/ml)								
5AU only			200	0.14	10	*a*				
5AU + ^{3}H-thymidine	0.75	0.625	200	0.21	16	20	173	109	5.9	28:1
5AU + ^{3}H-thymidine	1.5	1.25	200	0.25	16	26	176	109	4.5	18:1
Total[b]			400	0.23	32	46	349	218	5.1	22:1

[a]No sister-strand exchanges could be scored because no ^{3}H-thymidine was added.

[b]Only cells receiving ^{3}H-thymidine are pooled.

Table 9.2.2. Frequency of Symmetrical and Asymmetrical Interchanges Within Diplochromosomes of Endoreduplicated Chinese Hamster Cells

Treatment	^{3}H-Thymidine		Number of Cells Scored	Number of Symmetrical Interchanges	Number of Asymmetrical Interchanges	Ratio of Symmetrical Interchanges to Asymmetrical Interchanges
	Specific Activity (curies/millimole)	Concentration (μc/ml)				
Control			26	0	0	
5AU only			22	101	3	34:1
5AU + ^{3}H-thymidine	0.75	0.625	13	83	5	17:1
5AU + ^{3}H-thymidine	1.5	1.25	16	107	7	15:1
5AU + ^{3}H-thymidine	3.0	2.5	15	105	2	53:1
Total[a]			66	396	17	23:1

[a]Only cells treated with ^{3}H-thymidine and/or 5AU are pooled.

Table 9.2.3. Frequency of Symmetrical and Asymmetrical Interchanges Within Diplochromosomes of Endoreduplicated Human Cells

Treatment	Number of Cells Scored	Number of Symmetrical Interchanges	Number of Asymmetrical Interchanges	Ratio of Symmetrical Interchanges to Asymmetrical Interchanges
1. Control	28	0	0	
2. X ray alone	43	14	1	14:1
3. X ray + ^{3}H-thymidine,[a] unlabeled cells	23	7	0	
4. X ray + ^{3}H-thymidine, 0.1 μc/culture	39	47	1	47:1
5. X ray + ^{3}H-thymidine, 0.5 μc/culture	30	31	0	
6. X ray + ^{3}H-thymidine, 2.0 μc/culture	36	43	5	8.6:1
7. Total x ray, no ^{3}H-thymidine (2 + 3)	66	21	1	21:1
8. Total x ray plus ^{3}H-thymidine (4 + 5 + 6)	105	121	6	20:1

[a]Unlabeled cells at all ^{3}H-thymidine concentrations pooled.

Radiation Microbiology

Alexander Hollaender[a]	
Mutagen Mechanisms – Bacterial Metabolism (10.1) G. E. Stapleton[a] Annie S. Angel V. L. Orce[b]	**Bacterial Genetics (10.2–10.9)** H. I. Adler L. J. Charamella Roy Curtiss III Alice A. Hardigree C. E. Terry[c] D. E. Axelrod[d] Claire M. Berg[e] Janet Renshaw

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10.1 RADIATION RESISTANCE AND MACROMOLECULAR SYNTHESIS

G. E. Stapleton W. D. Fisher[1]
Annie S. Angel

There seems to be a correlation between the development of radioresistance in many strains of *Escherichia coli* during the stationary phase and the ability to initiate rapid protein synthesis upon regrowth of cells in fresh medium.[2,3] Moreover, the ability to initiate rapid protein synthesis seems correlated with the size of pools of 30- and 50-S ribosomes which exist in cells. A sensitive strain of *E. coli*, B_{s-1}, does not develop radioresistance under those growth conditions which have been shown to increase radioresistance in other strains.

Ribosome profiles of *E. coli* B_{s-1}, grown in glucose-broth and broth, are similar to those obtained for other strains. Protein synthesis, however, in this sensitive strain, upon regrowth, is independent of the previous growth conditions. Net synthesis of protein is measurable only after a lag of 45 min or longer, as it is for broth-grown (sensitive) cells of other strains.

Thus for *E. coli* B_{s-1}, regardless of the size of pools of small ribosomes, initiation of new protein synthesis is delayed. The correlation of types of ribosomes and protein synthesis as found for other strains is not apparent. However, the correlation between ability to initiate protein synthesis and radioresistance is still obtained. It will be of interest to examine the RNA and protein moieties of the 30- and 50-S ribosomes in this very sensitive strain.

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[1]Subcellular and Viral Physiology section.

[2]G. E. Stapleton and W. D. Fisher, *Radiation Res.* (submitted for publication).

[3]G. E. Stapleton, W. D. Fisher, and Annie S. Angel, *Biol. Div. Semiann. Progr. Rept. July 31, 1965,* ORNL-3855, pp. 88–89.

10.2 ROLE OF DONOR PILI IN SPECIFIC PAIR FORMATION AND CHROMOSOME TRANSFER IN *ESCHERICHIA COLI* K-12

Roy Curtiss III L. G. Caro[1]

Donor strains of *Escherichia coli* K-12 possess F-pili which adsorb the donor specific RNA phages. Starvation of log-phase Hfr cells in buffer, or starvation for an amino acid in synthetic medium, causes an exponential loss in time in the number of F-pili as scored by electron microscopy. This is paralleled by an exponential decline in the ability to adsorb RNA phage and ability to form specific pairs with F^- strains. Loss of pili, giving rise to "F^- phenocopies," is dependent on metabolic processes since the rates of loss at 2°C and with nitrogen bubbling are negligible. Readdition of amino acid to the pili-less Hfr auxotroph allows the reappearance of pili after 5 min, with complete restitution occurring by 20 min. Hfr mutants incapable of adsorbing RNA phages and of forming specific pairs with F^- bacteria have been used to assess the role of pili for chromosome transfer. They were mated with F^+ donors which have F-pili and, therefore, can form specific pairs with the pili-less Hfr mutants. No chromosome transfer from the Hfr mutants into the F^+ "recipients" was observed. We, therefore, tentatively conclude that a donor strain must possess its own pili for both pair formation and chromosome transfer.

References

[1]Biophysics section.

10.3 MECHANISMS OF CHROMOSOME TRANSFER BY F^+ DONORS OF *ESCHERICHIA COLI*

Janet Renshaw Roy Curtiss III

There are two types of F^+ strains in *Escherichia coli* K-12: those which yield stable Hfr mutants (I) and those which seldom if ever do (II). These strain differences were demonstrated by fluctuation tests[1] in which individual F^+ cultures, descended from small inocula, were mated with F^- cells. The difference between type I and type II strains is not due to differences in F, the presence of λ, any known genetic marker, or to environment. Types I and II F^+ donors give equal recombinant frequencies (*ca.* 10^{-4} to 10^{-5}). Kinetic experiments indicated that both donor types transfer F and chromosome as independent elements. Ultraviolet stimulated chromosome transfer equally in both types (50- to 100-fold), with maximum recombinant yield being obtained in matings initiated 90 min after uv. Thereafter, recombinant frequency declined, indicating the absence of stable Hfr mutants. The use of recombination deficient F^+ donors proved that F need not integrate to effect chromosome transfer. Thymine deprivation before and during mating with F^+ *thy*$^-$ donors caused a twofold increase in chromosome transfer. This result suggests that chromosome replication need not accompany chromosome transfer by F^+ donors. Time of entry experiments for *leu*$^+$, *ade*$_3^+$, *try*$^+$, and all pairwise combinations of these proved that type I and type II strains transfer their chromosomes in a linear oriented manner and not as loops. The results indicate that, in addition to the infrequent integration of F to yield Hfr mutants, another important mechanism for chromosome transfer by F^+ cells must exist. We propose that replication of the bacterial chromosome, which results in two interlocking circular structures, is resolved by breakage to yield two linear chromosomes. Should this happen in a conjugating F^+ cell, there would be a low probability (*ca.* 10^{-4}) that a linear chromosome could transfer through the conjugation tube. A necessary condition of this model is that the breaks in the circular chromosomes must be randomly distributed to allow early transfer of any F^+ marker.

References

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10.4 ROLE OF THE F^- PARENT DURING BACTERIAL CONJUGATION IN *ESCHERICHIA COLI*

Roy Curtiss III L. J. Charamella

By use of *Escherichia coli* K-12 Hfr and F^- strains unable to utilize certain energy sources, it has been possible to prove that both parents

require energy for maximum frequencies of chromosome transfer during bacterial conjugation. Chromosome transfer and resultant recombinant formation are decreased 80% when the Hfr is deprived of an energy source, 65% when the F^- is deprived of an energy source, and 99% when both parents are deprived of an energy source during mating. The F^- parent is therefore not a passive partner as was previously recognized as a fact of life.

Time-of-entry experiments with various Hfr donor strains have shown that there must be homology between the proximal portion of the Hfr chromosome and the comparable region of the F^- chromosome in order to allow initiation of chromosome transfer and to obtain "normal" recombination frequencies. Thus, a deletion of 2 to 3% of the F^- chromosome, corresponding to the region first transferred by an Hfr, results in a delay of 25 to 30 min in the appearance of the lead Hfr marker in recombinants. The frequency of these recombinants is also reduced by a factor of 10 to 50 as compared with the frequency in a mating with an F^- strain having a point mutation instead of a deletion.

We interpret these results by postulating the following role for the F^- parent: Immediately after transfer of the lead region of the Hfr chromosome, pairing between this segment and the homologous region of the F^- chromosome would be required before further transfer could take place. Once pairing was established, the F^- would "wind in" the Hfr chromosome; thus, pairing of homologous chromosomes would be ensured. This would allow efficient integration of the transferred Hfr chromosome into recombinants. Such conjugal activity of the F^- parent would be energy requiring.

10.5 THE *pro-lac* REGION OF THE *ESCHERICHIA COLI* K-12 GENOME

L. J. Charamella Roy Curtiss III

The gene order and distances in minutes in the *leu* to ade_3 segment were determined to be: *leu* – 6.2 – pro_1 – 2.5 – pro_2 – 0.8 – lac_{ac} lac_y lac_z lac_i – 0.2 – pro_3 – 2.0 – *T6* – 2.0 – ade_3. Mapping was accomplished with Hfr strains OR 6 (O-pro_1 ... pro_2 F); OR 11 (O-pro_2 ... lac_{ac} F); OR 1 (O-lac_z ... lac_i F); OR 21 (O-pro_3 ... lac_i F); OR 7 (O-*T6* ... ade_3 F); and OR 5 (O-ade_3 ... *gal* F) and with F′ strains obtained from them. Deletion mutations for pro_1 to pro_2, pro_2 to *lac*, and for various portions of the *lac* operon have also been used. P1*kc* cotransduction between pro_2 and *lac* is 1 to 5% and between *lac* and pro_3 is 25 to 30%. All pro_3 mutants crossfeed all pro_1^- and pro_2^- mutants.

It has been observed that different substrains of *Escherichia coli* K-12, that have not shared a common ancestor for 15 to 20 years, give rise to distinct classes of pro^- mutants. Thus the number of $pro_1^-:pro_2^-:pro_3^-$ mutants isolated from derivatives of W1485 was 311:5:2, of W945 was 244:5:50, of C600 was 0:39:6, and of 58-161 was 0:25:3. We postulate that the absence or near absence of a given class of pro^- mutants is due either to duplication of that *pro* cistron or to some controlling element closely linked to the pro_2 cistron. In support of these postulates a derivative of W945 with the pro_2-*lac*-pro_3 segment from W1485 only gave rise to pro_1^- mutants, and a derivative of C600 with the pro_2-*lac* segment from W945 has only given pro_1^- mutants. Further tests to differentiate between these are in progress.

10.6 GENETICALLY MARKED *ESCHERICHIA COLI* STRAINS FOR STUDIES ON INTESTINAL FLORA IN MICE

Roy Curtiss III R. C. Allen[1]
R. T. Jones[1] Paula E. Harris[1]

To determine the feasibility of using genetically marked bacteria capable of being selected for or against in gnotobiotic or conventional mice, four *Escherichia coli* strains of mouse origin, each differing in at least four genetic traits, were used. Tests on genetic stability, pathogenicity, and ability to establish in presence of other microorganisms were done in germfree mice. Strain DXM-2 caused infantile diarrhea and became genetically unstable in vivo. High mutation frequencies in sugar fermentation and phage, drug, and antibiotic resistance were noted. All 250 DXM-2 variants studied reverted to wild type after reisolation. DXM-2 was genetically stable during all in vitro experiments. The temperate phage in lysogenic strain M-12 lysogenized other *E. coli* strains in vivo.

With all *E. coli* strains tested singly in germfree mice, *lac*$^-$ mutants at final frequencies of 0.5 to 50% were detected approximately six weeks after infection. The suckling mice derived from the mono-contaminated parents contained no *lac*$^-$ variants until approximately six weeks after weaning. The genetically stable, nonpathogenic strain M-13 was chosen for further work. Mutations to streptomycin, azide, and cycloserine resistance were obtained as aids in isolating this strain from a mixed flora. Mutations for requirement of proline and tyrosine were induced to allow selection against this strain. The derived strain M-13-24 has been stable in quantitative titer and for all of its genetic traits for over two years in a variety of intestinal environments. The use of genetically marked *E. coli* strains for in vivo physiological and genetic interactions between members of different or of the same genera is feasible.

References

[1]Pathology and Physiology section.

10.7 INVERTED AND NONINVERTED TRANSPOSITIONS IN THE GENOME OF *ESCHERICHIA COLI* K-12

Claire M. Berg

Selection from Hfr OR11 (O pro_2 pro_1 *leu---try* ade_3 pro_3 *lac* F), of derivatives capable of early transfer of the pro_3 marker, yielded two classes of aberrant strains. Both types arose by a reciprocal exchange between nonhomologous sites bracketing the integrated F factor. In one type the F-*lac* region was inverted, producing Hfr strains with an inverted order of transfer (O pro_3 ade_3---pro_1 pro_2 *lac* F). In the second type the single haploid chromosome was split into two structures; the one containing F is termed an F′ factor. Two inversion Hfr and eleven F′ strains were thus obtained. It is proposed that the orientation of chromosome segments during preexchange pairing determines whether the progeny contain a region inverted or a genome split into two chromosomes. In the F′-containing strains the two chromosomes did not possess homologous segments, but could still rejoin by reciprocal exchange between nonhomologous sites. Joining yielded Hfr strains with inverted, as well as noninverted, transpositions. In none of the 200 derivatives tested did transposition result in mutation to auxotrophy or the inability to ferment lactose, showing that the exchanges had occurred in regions of the genome which do *not* control these conditionally lethal functions and that the genome comprises many units of transcription (each operon is probably an independent unit).

10.8 THE MECHANISM OF PHOTOREACTIVATION IN *NEUROSPORA CRASSA*

C. E. Terry

Lethal effects produced by irradiation of *Neurospora crassa* conidia with far-ultraviolet light (2537 A) can be partially reversed by light of longer wavelengths (3100 to 4500 A) administered after uv.

Irradiated conidia lose photoreactivability when held in aqueous suspension in the dark after irradiation. The rate at which this loss occurs is temperature dependent, with the ability to respond to photoreactivating light completely lost after 120 min at 37° but completely retained after 20 hr at 3°.

Action spectra indicate that the most effective wavelengths for photoreactivation of *N. crassa* are close to 4000 A, in a region relatively intermediate between the optima for *E. coli*[1] and *Streptomyces griseus*.[2]

The rate of photoreactivation in *N. crassa* is dependent upon the temperature during illumination, with an activation energy of approximately 16 kcal for a 10° change between 25 and 35°.

Extracts of *N. crassa* can carry out in vitro photoreactivation of uv-irradiated *Haemophilus influenzae* transforming DNA. The activity of these extracts is roughly comparable to that of crude yeast extracts (on a milligrams of protein per milliliter basis).[3]

The action spectrum, temperature dependence, and ability of extracts to reverse uv damage in vitro suggest that photoreactivation of lethal damage in *N. crassa* is of the direct type and involves light-dependent repair enzymes.

References

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[2]A. Kelner, *J. Gen. Physiol.* **34**, 835–52 (1951).

[3]J. K. Setlow and M. E. Boling, *Photochem. Photobiol.* **2**, 471–77 (1963).

10.9 RADIATION-RESISTANT MUTANTS OF *ESCHERICHIA COLI* K-12

D. E. Axelrod H. I. Adler

We have reported the isolation of ultraviolet (2537-A) and x-ray resistant mutants derived from *Escherichia coli* K-12 (HfrH).[1] Attempts are now being made to understand the genetic and physiological basis of radiation resistance in one of these mutants (R-5).

The R-5 mutant and its parental culture HfrH both act as high-frequency donors of chromosomal material in matings with a variety of recipient strains. The order of transfer of the genetic determinants of biochemical properties is the same in R-5 as it is in HfrH. To date, however, we have never detected recombinants that have inherited the enhanced radiation resistance characteristic of R-5. This is true even in cases where recombinants are known to have received all or almost all of the R-5 chromosome during mating.

In the course of these genetic tests we have detected several additional phenotypic differences between R-5 and HfrH. R-5 has a greater plating efficiency when grown on media containing the radiomimetic agent Mitomycin C, or when exposed to a 60°C heat shock. R-5 has a lower plating efficiency than HfrH when plated in the presence of certain amino acids, notably leucine. R-5 lacks a flagellar antigen characteristic of HfrH and, in contrast to HfrH, is nonmotile. The nonmotility characteristic of R-5 is transferred normally in crosses with motile recipient strains and is not linked to radiation resistance.

Since the radiation-resistance characteristic does not seem to behave as a normal genetic determinant in a variety of experiments, we are currently constructing new strains containing the radiation-resistance determinant in order to test various ideas that may explain the nature of the genetic control of radiation resistance.

References

[1]H. I. Adler and A. A. Hardigree, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1965,* ORNL-3768, p. 86.

Mammalian Recovery

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Secondary Disease (11.1, 11.2)

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11.1 MODIFICATION OF SECONDARY DISEASE MORTALITY

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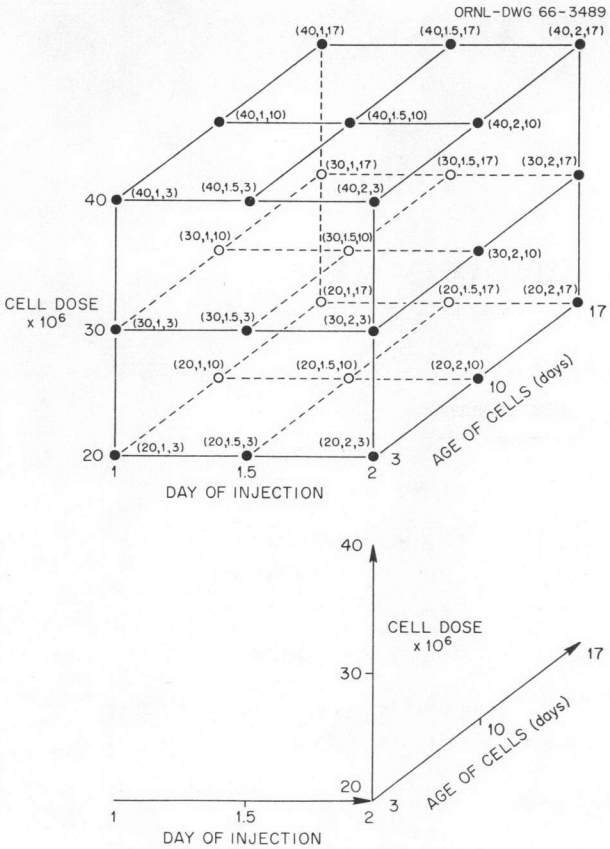
Introduction. – A program to determine the effects of bone marrow cell dose, age of donor cells, day of cell injection, and sex on 90-day cumulative mortality from secondary disease in foreign-bone-marrow radiation chimeras was undertaken, using a series of experimental designs.[2] The designs were arranged to show effects of different levels of the single factors and their interactions. In the initial designs, the major effort was aimed at finding a combination of variables that would result in the least 90-day mortality.

In the first three designs, newborn donor cells significantly reduced 90-day deaths, but the least mortality was unexpectedly noted in mice that received 30×10^6 cells from 44- to 61-day-old donors at $1\frac{1}{2}$ days after irradiation, these levels



being center points in the designs. Variation in mortality from cage to cage was greater than would be expected from a binomial distribution of deaths, but this variation tended to be less in the "center-point" groups.

A fourth design (Fig. 11.1.1) was carried out to see the effect of combining levels of all variables except donor cell age, in the range of the original "center-point" groups, with newborn donor cells of three different ages. Thus, donor cells from newborn $B6D2F_1$ mice at 3, 10, and 17 days of age were given in doses of 20, 30, and 40 million cells on days 1, $1\frac{1}{2}$, and 2 after 950 r of whole-body irradiation to $1C3F_1$ mice. Both sexes were used to make 54 treatment groups of five mice each. A second replicate was also carried out.

Results. – In the first three experimental designs, 90-day mortality ranged from about 34 to 62% in a total of 1429 mice (Text-Fig. 5, ref. 2). The fourth design showed a 90-day mortality varying between 12 and 25% in 548 mice (Fig. 11.1.2).

Fig. 11.1.1. Geometric Illustration of the Levels of Variables and Treatment Groups Observed in the Fourth Factorial Design.

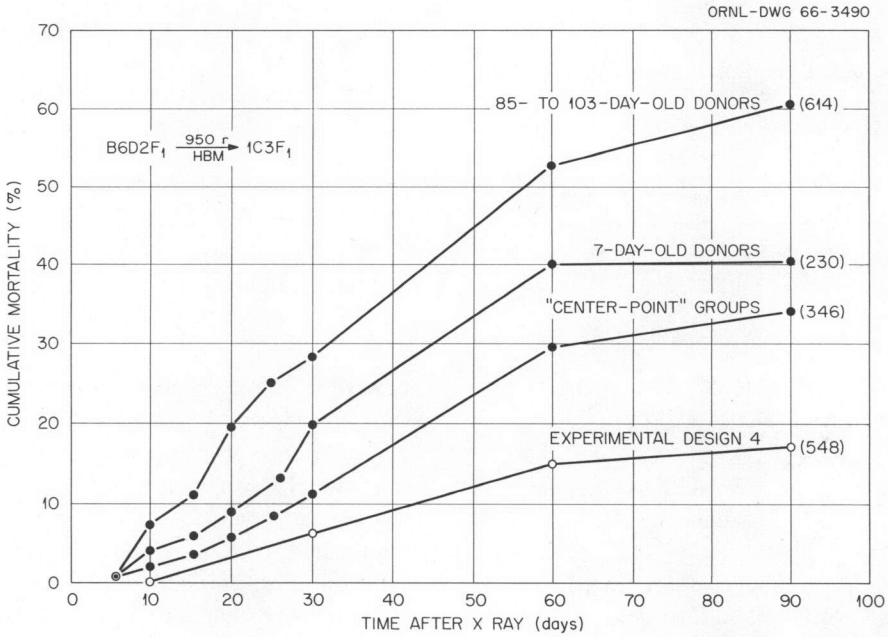


Fig. 11.1.2. Low 90-day Cumulative Mortality in Design 4 Compared with Young and Older Donors, and "Center-Point" Group from First Three Designs.

With 3-day-old donor marrow, the 90-day mortality was only 12%, a value quite comparable to that achieved with isologous bone marrow. These percentages apply to the main effects. In certain subgroups the values were as low as 6% and as high as 36%. It was amazing in this design to see no 10-day mortality in the 548 mice, whereas the other three designs combined had about 4% 10-day deaths. Variance in 90-day deaths among cages in the fourth design was reduced essentially to the theoretical value.

References

[1]Biometrics section, Mathematics Division.

[2]C. C Congdon, M. A. Kastenbaum, and D. A. Gardiner, *J. Natl. Cancer Inst.* **35**, 227 (1965).

11.2 SECONDARY MORTALITY AND BODY WEIGHT LOSS IN LETHALLY IRRADIATED MICE GIVEN SMALL DOSES OF ISOLOGOUS BONE MARROW CELLS

A. L. Kretchmar[1] W. R. Conover[1]

Introduction. – Barnes *et al.*[2] have described "secondary disease" in lethally irradiated mice given isologous bone marrow cells and believe that the syndrome is related to lymphoid aplasia. We have been interested in their work because of the possible connection between their observations and the disease of radiation chimeras believed to be related to a graft-host immunological incompatibility.

Methods. – Mice were given 950 r of total-body irradiation and then given 1×10^5, 1.5×10^5, or 1×10^6 isologous bone marrow cells intravenously. The animals were weighed periodically, and a record was made of the date of death if mortality occurred.

Results. – The results are summarized in Table 11.2.1. Secondary mortality (30 to 100 days after treatment) was usually below 13% in our experiments. In only two was the mortality comparable to that reported by Barnes *et al.*: 33% compared to 43% observed by the English group. In four of our experiments there was no mortality at all after 30 days. Pathological data are being collected to ascertain the cause of death in these animals.

In other experiments we have observed that, at 70 to 96 days after treatment, lethally irradiated mice given 1.5×10^5 isologous cells show anemia, subnormal erythropoietic capacity, and a reduction to 8.5 from a normal 15.3 spleen-colony-forming units per 10^5 marrow cells. It is, therefore, apparent that hemopoietic recovery in these animals is poor.

Conclusions. – We conclude, on the basis of work up to the present, that "secondary disease" in lethally irradiated mice given small numbers

Table 11.2.1. Mortality in Lethally Irradiated Mice Given Isologous Bone Marrow Cells

Group	Number of Mice	Percent Mortality After						
		X Ray Only	X Ray and Administration of Isologous Bone Marrow Cells					
			1×10^5 Cells		1.5×10^5 Cells		1×10^6 Cells	
K-20, 15	55	100[a]	27[a]	13[b]	7[a]	13[b]	0[a]	0[b]
K-21, 16	53	100	23	7	21	7	0	10
K-22, 17	55	100	33	0	7	13	20	0
K-23, 13	55	100	20	0	13	0	0	0
K-24, 14	55	100	73	0	13	7	0	0
K-25	34	100	40	33			0	0
K-26	35	100	33	33			0	0
Average		100	35.6	12.3	12.2	8.0	3.0	1.5

[a]Numbers in these columns refer to deaths occurring 0 to 30 days after treatment.

[b]Numbers in these columns refer to deaths occurring 31 to 100 days after treatment.

of isologous bone marrow cells is not prominent and that hemopoietic recovery requires more than 1×10^6 isologous marrow cells in mice given 950 r of total-body irradiation.

References

[1]Medical Division, Oak Ridge Associated Universities; work carried out in collaboration with the Mammalian Recovery section.

[2]D. W. H. Barnes, J. F. Loutit, and H. S. Micklem, *Ann. N.Y. Acad. Sci.* **99**, 374–85 (1962).

11.3 SINGLE vs SPLIT X-RAY EXPOSURE TO MOUSE BONE MARROW CELLS

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

Introduction. – A dose of ionizing radiation given in two parts separated by a short interval is less lethal to mammalian tissue culture cells than the same dose given at one time.[1] A similar effect was observed for mouse bone marrow cells exposed in vivo.[2] Using an in vitro bone marrow system,[3] we compared single vs split exposure followed by incubation at 25°C or below.

Methods. – Marrow preparation, in vitro irradiation, incubation, and subsequent testing have been described.[3] For the single-dose series, cells were exposed to 100 r at time 0 and tested at several intervals thereafter. For the split-dose series, cells were exposed to 50 r at time 0, followed by another 50 r at one of several intervals up to 24 hr.

Results and Conclusion. – Activity was reduced to 50 and 80% of the control value by 100 and 50 r respectively. At 25°, progressive recovery was observed during the first 6 hr as the interval between exposures increased (Fig. 11.3.1). A maximum recovery ratio of ~2.2 (ratio of split-exposure activity to single-exposure activity) was obtained at 6 hr, followed by a decline to a ratio of 1 at 12 hr and subsequent increase to ~1.5 at 18 and 24 hr. Interpretation of the decline and, in particular, the second increase in recovery is complicated by a small but steady reduction in activity of unirradiated cells maintained at 25°. The recovery effect was tested at the 6-hr interval when cells were incubated at 2, 6, or 15°. The

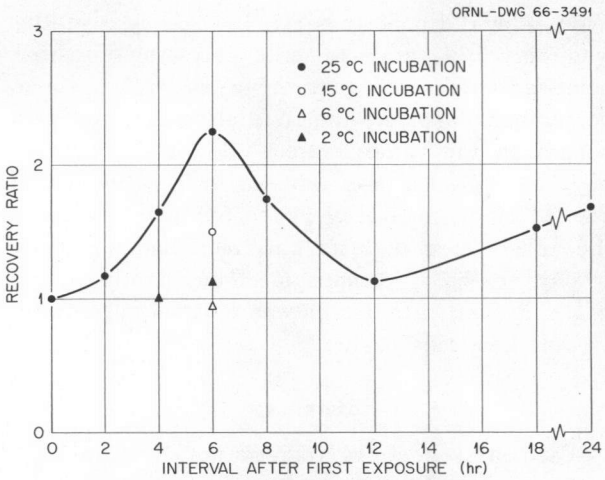


Fig. 11.3.1. Recovery Ratio as a Function of Time After First Exposure. Recovery ratio equals split-exposure value (50 r + 50 r) divided by single-exposure value (100 r). Exposure values are percent ^{59}Fe incorporation by spleen.

ratios for these temperatures were 1, 1, and 1.5 respectively. These results show that recovery can be demonstrated for normal mammalian cells in an in vitro system and that this recovery is temperature dependent. It is important to emphasize that these are purely in vitro effects to which cells are exposed and that, regardless of the treatment to cells, they are returned to in vivo conditions to assess their activity.

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[2]J. E. Till and E. A. McCulloch, *Radiation Res.* **18**, 96 (1963).

[3]L. H. Smith and T. W. McKinley, Jr., *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1963,* ORNL-3498, p. 102.

11.4 SOME UNUSUAL EFFECTS OF IMMUNIZATION WITH LIVING PARENTAL CELLS

Joan Wright Goodman Gayle C. Bosma

Introduction. – Recent experiments in our laboratory have dealt with a fruitless search for parental

(P) antigens not expressed in F_1 hybrid mice. Several approaches have been used: (1) Tolerance has been induced in newborn P1 mice by the injection of F_1 spleen cells. As adults these P1 animals were tested for tolerance to P2 spleen and skin antigens. (2) Adoptive immunization was tried by transfer of isologous F_1 lymph node cells to F_1 mice that had been irradiated and were then given P bone marrow. (3) Active immunization was attempted by injection of living P cells at a time before irradiation and transplantation treatment of F_1 hybrid hosts. (4) Systematic histologic study is being made of tissues from $P \rightarrow F_1$ chimeras killed at intervals after radiation transplantation. From these studies have come no convincing data to indicate the presence of parent-specific antigens. Yet, the well-established observation of poor growth of certain parental-strain cells in F_1 hybrids remains to be understood. We have continued looking for parental antigens as a way of seeking an explanation of poor growth and an understanding of factors relating to good growth.

Methods. – The protocol used in these experiments was similar to that of Celada and Welshons in which 30 million living (dye-excluding) P spleen cells were injected intraperitoneally into F_1 recipients ten days before the recipients were to be irradiated. After x irradiation the "preimmunized" F_1's were given bone marrow cells, and the success of these transplants was assessed a week later by measuring 24-hr incorporation of ^{59}Fe into spleens and erythrocytes.

Results and Conclusions. – The first unusual effect was that P1 spleen cells did not decrease the performance of P1 but did of P2 bone marrow (Table 11.4.1, experiments A and B). Although the result is unusual from the point of view of the initial objective of the experiment – specific preimmunization – it is not so unpredictable. Immunocompetent parental cells should be expected to take part in a graft-vs-host (GVH) reaction in the F_1 hybrid and even after 900 r to be able to reject cells of the second parental strain. This fact alone renders the concept of immunization of F_1 hybrids against living immunocompetent parental cells difficult at best.

The second unusual finding was that the preimmunization with spleen cells of one particular parental type (B6) consistently gave rise to increased ^{59}Fe uptake in chimeric spleens but not in erythrocytes. This is illustrated by experiment B of Table 11.4.1. The increased uptake was proportional to spleen size and would seem to be related to the preceding GVH reaction. When "preimmunizing" cells were heavily irradiated, the effect was lost (Table 11.4.1, experiment C).

Table 11.4.1. Erythropoiesis in Irradiated $B6D2F_1$ Mice[a]

Preimmunized to	Numbers of Mice	24-hr ^{59}Fe Uptake (% of Injected Dose)[b] by RBC	Spleen
A. Donor Marrow: 3×10^6 D2 cells			
B6	22	12.46 ± 1.17	9.35 ± 0.82
D2	19	30.96 ± 4.68	11.05 ± 1.33
$B6D2F_1$	21	30.93 ± 2.42	11.95 ± 0.70
B. Donor Marrow: 10^7 B6 cells			
B6	40	30.7 ± 1.5	11.8 ± 0.52
D2	40	22.3 ± 1.9	6.2 ± 0.47
$B6D2F_1$	39	34.6 ± 2.4	7.0 ± 0.42
C. Donor Marrow: 10^7 B6 cells			
$B6D2F_1$	23	25.48 ± 2.51	8.36 ± 0.62
D2	24	5.78 ± 1.29	5.44 ± 0.83
D2[c]	24	14.64 ± 1.45	6.40 ± 0.65
B6	25	21.77 ± 1.87	20.37 ± 0.64
B6[c]	28	17.11 ± 1.44	6.30 ± 0.45

[a]Preimmunized to 3×10^7 dye-excluding cells in A and B; 3×10^8 in C.

[b]0.5 μc of ^{59}Fe as citrate given six days after marrow.

[c]Cells given 10,000 r in vitro.

11.5 POLYCYTHEMIC MICE PRODUCED BY HYPOXIA IN SILICONE RUBBER MEMBRANE ENCLOSURES

Robert D. Lange[1] M. L. Simmons
Norman R. Dibelius[2]

Introduction. – This report presents results which show that specially built cages covered with dimethyl silicone rubber can be used to produce polycythemia in laboratory mice.

Methods. – The dimethyl silicone rubber was hand fabricated in the laboratories of the General Electric Company. Silicone rubber film 0.001 in. thick was bonded on both sides with 0.005-in.-thick Dacron mat in order to improve the membrane's durability, protect it from abrasion, and to support a pressure differential. Cages were fabricated by modifying two standard Lexan animal cage bottoms. One cage bottom, which was subsequently inverted to become the top of the enclosure, was modified by cutting openings (amounting to 143 in.^{2}) in the sides and top. The openings were covered with the Dacron-backed silicone rubber membrane. Two tube fittings were installed, one in each end of the top of the enclosure, for the purpose of flushing the cage with gas mixtures. An additional hole was cut to allow the positioning of an oxygen sensor. A standard plastic animal cage, which was easily removable for cleaning, was placed in the lower enclosure.

The cages were opened three times a week for cleaning. Routinely, after being resealed they were flushed for approximately 2 min with a mixture of 10% oxygen and 90% nitrogen. In one series of experiments, the cages were allowed to come to equilibrium without flushing with the gas mixture, and the time to reach equilibrium was noted.

Female A/Jax mice six to eight weeks old were used. For most experiments, 11 mice weighing approximately 16 g each were placed in the enclosures for periods up to three weeks. In other experiments, different numbers of mice were placed in the enclosure in order to measure the effect on the oxygen concentration of the air in the enclosure.

Results. – In a series of six experiments, different numbers of animals were allowed to reach oxygen equilibrium without flushing the cages with 10% oxygen. An average of 470 min (range 442 to 520 min) was required for initial equilibrium to be reached. The subsequent oxygen concentration was found to be dependent on the number of animals in the enclosure.

When 11 animals were placed in an enclosure for a period of three weeks, there was a progressive rise in hemoglobin and hematocrit values (Table 11.5.1). The hematocrits rose from 47.7 to 73.5%. Eight days after the animals were removed from the enclosure the mean hematocrit was 56.9%. The response of each animal to erythropoietin was measured at this time by determining the ^{59}Fe in circulating erythrocytes,[3] and the following uptake values (percent of ^{59}Fe injected) were obtained: normal mice, 47%; polycythemic mice + saline, 0.67%; polycythemic mice + erythropoietin, 20%.

Conclusion. – Hypoxia and consequent polycythemia in mice can be produced by the use of cages covered with a selectively permeable dimethyl silicone rubber membrane. The oxygen concentration within the enclosure is directly related to the number of mice. The method produces sensitive erythropoietic-assay animals and is adaptable to other physiological experiments.

Table 11.5.1. Effect on Hemoglobin, Hematocrit, and Reticulocyte Count After Enclosure of Mice in Cages Covered with Permselective Membrane

Results are recorded ± the standard error of the mean. Numbers in parentheses refer to the number of observations.

	Hematocrit (%)	Hemoglobin (g %)	Reticulocytes (%)
Normal (room air)	47.65 ± 0.64 (18)	18.05 ± 0.22 (18)	3.29 ± 0.28 (18)
In enclosure:			
One week	63.90 ± 1.22 (5)	15.76 ± 0.42 (5)	5.20 ± 0.54 (5)
Two weeks	68.60 ± 1.22 (5)	24.35 ± 0.42 (5)	5.30 ± 0.54 (5)
Three weeks	73.50 ± 0.96 (8)	25.28 ± 0.33 (8)	4.75 ± 0.42 (8)
8 days after enclosure:			
Saline	55.89 ± 0.91 (9)	19.03 ± 0.54 (3)	0.10 ± 0.40 (9)
0.5 unit erythropoietin	57.69 ± 0.91 (9)	20.65 ± 0.47 (4)	0.94 ± 0.40 (9)

References

[1]University of Tennessee Memorial Research Center, Knoxville.

[2]Research and Development Center, General Electric Co., Schenectady, N.Y.

[3]V. Pavlovic-Kentera *et al.*, *J. Lab. Clin. Med.* **65**, 577 (1965).

Subcellular and Viral Physiology

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Molecular Physiology (12.1, 12.2)

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AEC-NIH Zonal Ultracentrifuge Development (23.22–23.25)

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12.1 ATTEMPTS TO ISOLATE SUBSTANCES WHICH PROMOTE DIVISION IN FILAMENT-FORMING STRAINS OF *ESCHERICHIA COLI*

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Alice A. Hardigree[1]

We reported previously[2,3] that intact cells of certain strains of *Escherichia coli* can serve as donors of a diffusible substance which promotes division of x-irradiated *E. coli* K-12 (1899 NM). We have found that extracts of the same donor cells can likewise stimulate cell division in the test strain. This report describes attempts to characterize the division-promoting substances.

Materials and Methods. – *Preparation of Extracts.* – Suspensions (about 30 mg/ml net weight) of washed stationary-phase cells of *E. coli* B/r (ORNL) from shaken glucose-broth cultures were broken in an Aminco pressure cell (12,000 psi). The material was then centrifuged at 15,000 rpm for 30 min. The pellet P_1 and supernatant S_1 were thus obtained. P_1, composed of unbroken cells and large cell debris, was discarded. S_1 was centrifuged at 50,000 rpm from 300 min (2°C) and gave a pellet P_2 and a supernatant S_2. P_2 was resuspended in phosphate buffer (0.067 *M*, pH 6.8).

Biological Assay. – Routine assays measured survival of stationary-phase cells of *E. coli* K-12 (1899 NM) after x irradiation (40 kilorads). The pour plate method described by Adler *et al.*[2] was used. Division-promoting activity of various fractions was determined by addition of these materials, along with irradiated test cells, to the plating medium. In this assay, increase in numbers of colony-forming cells is equated to division-promoting activity. An arbitrary unit of activity, the division index, as used, is the ratio of colony-forming cells in the presence of added substances to the number in their absence.

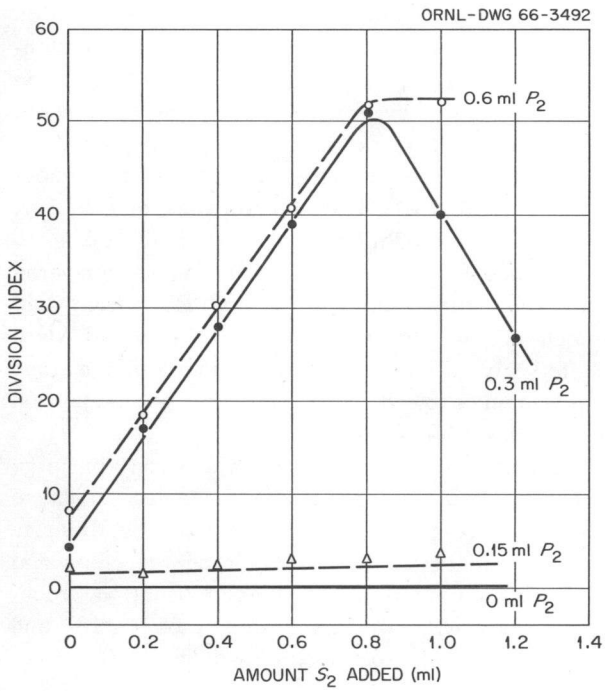

Fig. 12.1.1. Division-Promoting Activity of Fractions from *E. coli* B/r (ORNL) on X-Irradiated *E. coli* K-12 1899 NM. P_2 is 3× concentrated as compared with S_1; S_2 is 8× concentrated as compared with S_1.

Results and Discussion. – Fraction S_1 (1 ml/plate) increases survival of the routine test organism by a factor of 6 to 10. Survival is increased further by addition of larger quantities of this fraction. No change in survival is detected by addition of S_1 to irradiated cells of other strains of this species that are not filament formers.

High-speed centrifugation (50,000 rpm for 300 min) of S_1 yielded a pellet P_2 of low activity as compared with S_1, and a supernatant S_2 with no activity. Recombination of P_2 and S_2, however, gave an activity similar to that of S_1. P_2 is heat labile, while S_2 is dialyzable and heat stable (2 min at 100°C). The activity of P_2 is recovered from sucrose density gradients associated with particles with an estimated sedimentation coefficient of 100 S. Gel filtration of S_2 suggests active components(s) with molecular weight(s) in the range of 500 to 1000.

Data for the activity of recombined P_2 and S_2 are shown in Fig. 12.1.1. For such experiments P_2 is concentrated threefold and S_2 concentrated eightfold as compared with S_1. The following main points are demonstrated:

1. S_2 is inactive without added P_2.
2. P_2 has a low activity without added S_2, but its activity is markedly increased by increasing concentrations of S_2.
3. A marked threshold requirement of P_2 is indicated by the lack of response of 0.15 ml of P_2 to increasing the concentration of S_2.

The 50-fold increase in survival obtained at high concentrations of S_2 plus P_2 is the maximum expected for the dose of x rays used, if one assumes that the increase represents exclusively the prevention of filament formation. This conclusion is based on the fact that the maximum survival is that obtained at this x-ray dose for the parent strain of K-12 (1899), which is not a filament former.

Two pieces of evidence suggest that S_2 contains inhibitory as well as stimulatory activity: (1) the existence of a maximum in the curve relating activity to S_2 concentration with suboptimal concentrations of P_2, and (2) preliminary evidence for two such components of S_2 based on ion exchange chromatography.

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[1]Radiation Microbiology section.

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12.2 STUDIES ON THE GENESIS OF RIBOSOMES IN *TETRAHYMENA PYRIFORMIS*

A. W. Prestayko W. D. Fisher

Introduction. – Investigations on protein synthesis in bacterial systems have shown that abnormal ribosomes are generated during conditions of unbalanced growth. Such ribosomes, when formed under conditions of stress, particularly where protein synthesis has been inhibited by antagonists, become incorporated into normal ribosomes when these inhibitors are removed.[1,2]

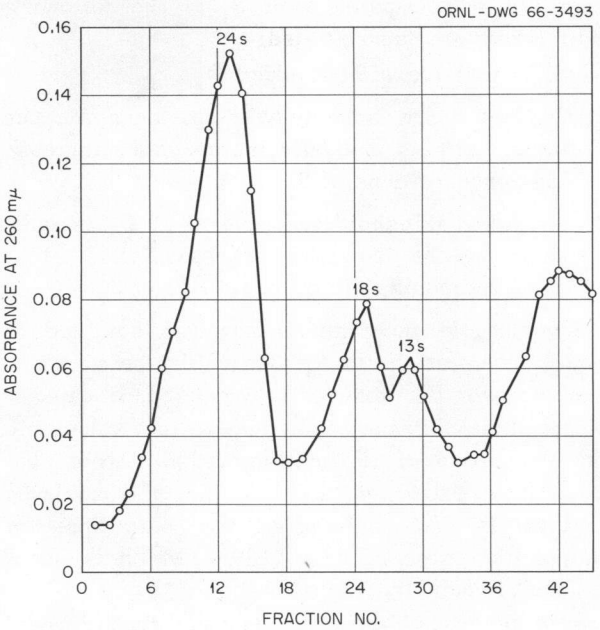

Fig. 12.2.1. Centrifugation Pattern in Sucrose Density Gradient (4 to 18% Sucrose in 0.1 *M* Tris, 0.14 *M* NaCl, pH 7.0) of *Tetrahymena pyriformis* Ribosomal RNA Prepared by Phenol Extraction from Isolated Ribosomes. Centrifugation was at 38,000 rpm for $5\frac{1}{4}$ hr in the Spinco model L centrifuge (SW39 rotor).

Experiments are now being performed with *Tetrahymena pyriformis* GL with the purpose of demonstrating the mechanisms and pathways of ribosome synthesis in these cells.

Results and Discussion. – Preliminary studies were carried out on the effects of different concentrations of chloramphenicol on cell division in *Tetrahymena pyriformis* GL. The cells were grown into log phase in a proteose-peptone medium, and chloramphenicol was added to aliquots of the culture to final concentrations of 0.5, 1.0, and 2.0 mg/ml. Cell division was inhibited 40 to 50, 85, and 99 to 100%, respectively, as compared with the untreated control culture, which had tripled in cell number in 8 hr. Cells which were treated with 2 mg/ml of chloramphenicol remained viable and showed good recovery on incubation into fresh media.

Log-phase cells were shown to contain large amounts of 81- and 125-S ribosomes. These ribosomes were isolated in a sucrose gradient in the B-IV zonal ultracentrifuge, and the ribosomal RNA was obtained by a phenol extraction procedure. This RNA was separated into 24-, 18-, and 13-S components on a sucrose gradient in an SW39 rotor (see Fig. 12.2.1).

Studies are now in progress pertaining to the isolation and characterization of unstable normal ribosomes generated during unbalanced growth.

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Cell Physiology

Peter Mazur
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Janice J. Schmidt

13.1 CALORIMETRIC DETERMINATIONS OF "BOUND" WATER IN BIOLOGICAL SYSTEMS

Peter Mazur

Introduction. – There is increasing evidence that water serves not just as an inert solvent in biological systems but as an important factor in determining the conformation and, therefore, the function of macromolecules. The structure of water, for example, is involved in hydrophobic bonding.

It has also been known for some time that macromolecules are surrounded by a hydration layer composed of water molecules that exhibit reduced mobility on the basis of dielectric and nuclear magnetic resonance measurements, or water molecules that appear to move or be associated with the macromolecule in hydrodynamic measurements such as ultracentrifugation.

Finally, there is a third group of experimental data indicating that a fraction of the water in biological systems does not exhibit certain properties of normal water. This fraction either does not dissolve solutes, or it has a reduced vapor pressure, or it does not freeze. Water with one or more of these properties is what has been referred to as "bound."

Although there is probably some relation between the water involved in macromolecular structure, hydration water, and "bound" water, the three have not been shown to be equivalent.

The long-range purpose of the current studies is to investigate these relations by determining the function of water in cells and solutions of macromolecules that can freeze. When water freezes it is converted from a quasi-crystalline, partly hydrogen-bonded substance to a completely hydrogen-bonded, crystalline one. The heat of fusion released in the process depends largely on the degree of structural change involved. Thus, if liquid water were more structured than normal, it ought to release less heat upon conversion to ice than normal water. Accordingly, measurements of the heat of fusion may provide information about the structure of the liquid water in a given biological system under a given set of conditions.

Method and Preliminary Results. – The experiments involve the following procedure. A 1- to 2-g sample of accurately known mass (±0.0001 g) is frozen to a given temperature (–5 to –70°C) and equilibrated at a closely controlled temperature (±0.01°C). It is then dropped into a Dewar flask containing about 300 g of water, the mass of which is known to ±0.01 g and the temperature to ±0.01°C. The drop in temperature of the water in the Dewar produced by the melting of the sample is about 0.6 to 0.8°C, and it is measured to ±0.0001°C.

Knowing the initial and final temperatures of the sample, the temperature drop in the calorimeter, and the masses and specific heats of the sample water, the sample solids, the calorimeter water, and the sample container, one can calculate the mass of ice originally present in the sample.

Independent measurements will be made of the total water in the sample. This is to be done with three gravimetric methods and possibly an electrolytic technique. The figure of interest will be the ratio of ice to total water.

Samples consisting of suspensions of cells or subcellular particulates will also require determining the fraction of the total water that is

intracellular or intraparticulate. This will be done by the so-called "space" technique, which involves adding a known quantity of a solute that cannot penetrate the cell or particulate to a packed suspension and measuring the dilution of the added solution produced by the movement of the solute into the intercellular or interparticulate space.

Although the method is simple in principle, attaining the required precision has necessitated the solution to a number of problems, including the control and measurement of the sample temperature, the avoidance of condensation on the sample container, the minimizing of temperature changes and condensation during transfer of the sample to the calorimeter, the control and measurement of the temperature of the water in the calorimeter, the minimization of evaporation in the calorimeter, and the measurement of water lost from the calorimeter from run to run without disassembling the instrument.

The construction of the apparatus and the development of procedures are nearly complete. Three preliminary runs using distilled water frozen at −20°C yielded temperature drops in the calorimeter of 0.7659, 0.7689, and 0.7700°C. The range of 0.0041°C indicates that the precision of the replicate runs will be in the order of ±0.3%. Among the procedural matters still remaining are the determination of the heat capacity of the calorimeter and the specific heats of the sample containers.

13.2 DENSITY OF BACTERIOPHAGES T4B AND $T4BO_1$ AS A FUNCTION OF DILUTION TREATMENT

S. P. Leibo Peter Mazur

A continuing series of experiments on the survival and buoyant densities of bacteriophages T4B and $T4BO_1$ after osmotic shock and various dilution treatments have demonstrated the following significant points:

1. Phage T4B was sensitive to osmotic shock and to low monovalent-salt concentrations. $T4BO_1$ was resistant to osmotic shock but sensitive to low salt concentrations.
2. Sensitivity of T4B to dilution from a concentrated salt solution depended on dilution rate. Sensitivity of $T4BO_1$, however was independent of rate.
3. Sensitivity of T4B to osmotic shock depended critically on the initial salt concentration in which the phage was suspended.
4. Sensitivity of both T4B and $T4BO_1$ to low salt concentrations depended only partially on initial salt concentrations. However, initial exposure to at least 0.5 *m* salt for T4B and to 0.5 *m* salt for $T4BO_1$ was necessary to cause inactivation from exposure to low salt concentrations.
5. Phage T4D was intermediate to T4B and $T4BO_1$ in all these respects.
6. Since no more than 60% of osmotically shocked T4B lost DNA when subjected to a treatment that caused 99% loss of infectivity, loss of DNA from the phage head could not be the primary cause of inactivation from osmotic shock.
7. No DNA was lost from $T4BO_1$ exposed to low salt concentrations which produced a 90% loss of infectivity. This and other facts strongly suggest that the effects of osmotic pressure changes and low salt concentrations on T4B and $T4BO_1$ were distinct.

Preliminary density analyses previously described[1] have been extended. The results shown in Table 13.2.1 substantiate the preliminary conclusions regarding density changes of T4B and $T4BO_1$ as a function of the dilution treatment to which the phages were subjected. The density (g/ml) of 1.502 for T4B diluted from 0.1 *M* phosphate buffer into 0.1 *m* NaCl agrees with the value of 1.505 for T2L reported by Cummings (1963).[2] The reality of all these density differences was confirmed by centrifuging mixtures of variously treated phages in CsCl gradients.

We propose that these data can be explained in terms of the different relative permeabilities of the phages and the polyelectrolyte behavior of DNA in the phage heads. Phage T4 normally contains inorganic (Mg^{2+}, Na^+, K^+) and organic cations (polyamines, internal protein) which serve to neutralize the PO_4^- groups of DNA.[3,4] These counterions have different relative binding affinities for DNA.[5] Assuming that cesium may replace any or all of these counterions to an extent determined by phage permeability, cation binding affinity for DNA, and dilution treatment,

Table 13.2.1. Density of Phages T4B and $T4BO_1$ as Function of Dilution Treatment

Phage	Suspending Medium		Dilution Rate	Survival (%)	Density (g/ml)
	Initial	Final			
T4B	Phosphate buffer	0.1 *m* NaCl	Fast	100	1.502 ± 0.003
$T4BO_1$	Phosphate buffer	0.1 *m* NaCl	Fast	100	1.536 ± 0.005
T4B	3 *m* NaCl	0.003 *m* NaCl	Slow	100	1.525 ± 0.003
	3 *m* NaCl	0.003 *m* NaCl	Fast	0.5	1.561 ± 0.006
$T4BO_1$	3 *m* NaCl	0.3 *m* NaCl	Fast	82	1.558 ± 0.003
	3 *m* NaCl	0.03 *m* NaCl	Fast	80	1.554 ± 0.003
	3 *m* NaCl	0.003 *m* NaCl	Fast	65	1.557 ± 0.003
	3 *m* NaCl	0.0003 *m* NaCl	Fast	10	1.559 ± 0.002

Table 13.2.2. Calculated Density of Phages T4B and $T4BO_1$ as Function of Dilution Treatment

Phage	Treatment	Observed Density	Assumed Cations Replaced[a]	Calculated Density Assumptions[b]		
				1	2	3
T4B	PO_4 buffer → 0.1 *m* NaCl; fast dilution	1.502	Na, K	1.491	1.489	1.496
$T4BO_1$	PO_4 buffer → 0.1 *m* NaCl; fast dilution	1.536	Na, K, Mg	1.515	1.532	1.526
T4B	3 *m* NaCl → 0.003 *m* NaCl; slow dilution	1.525	Na, K, Mg	1.515	1.532	1.526
T4B	3 *m* NaCl → 0.003 *m* NaCl; fast dilution	1.561	Na, K, Mg, polyamines, internal protein	1.558	1.605	1.555
$T4BO_1$	3 *m* NaCl → 0.3 to 0.0003 *m* NaCl; fast dilution	1.557	Na, K, Mg, polyamines, internal protein	1.558	1.605	1.555

[a]Normal cations assumed to be replaced by Cs^+.

[b]Calculated density assumptions:

1. Assume that all cations bind to one PO_4^- group; phage density is due to difference between weight of normal counterions and that of cesium; phage head volume does not change; water enters or leaves to account for volume differences between counterions and cesium.
2. Assume that Mg^{2+} binds to two PO_4^- groups and all other cations bind to one PO_4^- group; phage density change is due to difference between weight of normal counterions and that of cesium; phage head volume changes to account for volume difference between normal counterions and cesium.
3. Assume polyamines bind according to charge and all other cations bind to one PO_4^- group; phage density change is due only to difference between weight of normal counterions and weight of cesium.

we have calculated several possible, resultant densities. For this purpose we have also made assumptions regarding cation equivalence and physical characteristics of these phages. These calculated densities and some possible assumptions are shown in Table 13.2.2.

Because of the necessary assumptions and uncertainties regarding some phage characteristics, we emphasize that these calculated densities must be viewed with extreme caution. Nevertheless, since the calculated densities are of the same order of magnitude as the observed, we suggest that the observed density changes of T4B and $T4BO_1$ are due to the replacement of various endogenous cations by cesium.

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Nucleic Acid Chemistry

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14.1 NUCLEOTIDE SEQUENCES IN RNA

Mayo Uziel Waldo E. Cohn
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Various fractions and one highly purified product ($tRNA^{Phe}$) obtained by the chromatographic separation of crude tRNA (*E. coli*)[1] have been analyzed for their base ratios and end groups, using the rapid micro techniques described earlier[2] (gel filtration for desalting, modified Anderson nucleotide analyzer for base ratios and end groups). No correlation was found between base ratios (ignoring methylated nucleosides) and chromatographic position of the various tRNA species.

However, small amounts of RNA of higher molecular weight, subsequently discovered as contaminants, may have obscured such differences, if they exist. The purified preparation of $tRNA^{Phe}$ had a composition of $1 \pm 0.4\ \psi$, 4.4 ± 0.7 U, 28.1 ± 0.4 G, 12.8 ± 0.2 A, and 18.8 ± 0.2 C, for a total of 65 ± 2 residues (calculated molecular weight = 27,700), plus 0.8 ($\pm$ 0.1) thiouridine per adenosine end group. One nanomole of adenosine is found in 0.76 ± 0.03 absorbancy unit at 260 mμ in H_2O. Adenosine was found to be the only nucleoside end group, although some less purified fractions had small amounts of cytidine as a terminal group. The recovery in the base analysis, in terms of absorbancy, was 86%. The phosphorus content of this preparation is 85 moles per mole of end group (adenosine), and the phosphate released by phosphatase amounts to 4–6 moles per adenosine. Thus we suspect the presence of a small amount of nonnucleotide, phosphorus-containing material in this purified $tRNA^{Phe}$ preparation.

The recent discovery of thiouridine as a constituent of some tRNA's[3] motivated an investigation into its stability toward alkaline hydrolysis, as well as a consideration of the possibilities of chain-chain bonding by oxidation to an –S–S– form. Reduced thiouridine survives the conditions of alkaline hydrolysis and is easily distinguished in our chromatographic analysis.[1] Oxidized thiouridine (a "dimer"), however, forms about 0.9 mole of thiouridine and an equivalent amount of an unstable intermediate (presumably the sulfenic acid) and about 0.2 mole of uridine. Thus the appearance of 4-thiouridine in an alkaline hydrolyzate of tRNA may not be taken as evidence of its occurrence as such in the parent substance. Nevertheless, thiouridine is one more minor nucleoside useful as a marker in the elucidation of nucleotide sequences.

The need by others for specific trinucleotide sequences has prompted us to separate, from a crude tRNA mixture, a suspected "terminating" codon UAG. The final separation of this from its isomer (AUG), both obtained from a T_1 RNase digest, was accomplished by Dowex 1 chromatography.[4] The product will be made available to those investigating the significance of specific trinucleotides in mRNA-ribosome binding.[5]

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Pathology and Physiology

Section Chief – A. C. Upton[a]

Pathological Effects of Neutrons, High-Energy Radiation, and Chemicals (15.1)

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15.1 LATE HEMATOLOGIC EFFECTS OF 14-Mev NEUTRONS IN MICE

G. E. Cosgrove E. B. Darden, Jr.
M. L. Davis

Introduction. – In mice exposed to 14-Mev neutrons, hematologic changes were observed in the early postirradiation period.[1] Similar studies have been completed throughout the life of the mice in the experiment and are summarized here.

Results and Discussion. – Female RF/Un mice were exposed to 50, 100, 200, or 400 rads of 14-Mev whole-body neutron radiation at ten weeks of age. Selected groups averaging 21 mice from sham-irradiated controls and from each radiation level were examined for hematologic changes at 100-day intervals throughout life. After the first 50 days following irradiation, the total leukocyte counts (Fig. 15.1.1) in the irradiated groups did not vary significantly from those in controls, decreasing slightly with time in all groups (detailed data on the counts during the first 50 days after exposure have been published).[1] The lymphocyte counts decreased correspondingly with time (Fig. 15.1.2), whereas the neutrophil counts increased slowly with time in all groups of mice up to 600 to 700 days of age (Fig. 15.1.3). Those changes occurring later than 50 days after exposure were not significantly correlated with radiation dose. The packed red-cell volume in all groups decreased slowly with time. After 200 days, packed red-cell volumes in the irradiated groups were often significantly lower than those in controls (Fig. 15.1.4). The blood findings in individual aging mice were dependent on the health of the animals in question, being significantly affected in the presence of diseases such as leukemia. Individual variations of this type, however, are not thought to have accounted for the age-dependent trends observed. The absence of long-term dose-dependent changes in peripheral blood contrasts with the presence of early hematologic changes and the induction of leukemia into the irradiated populations.

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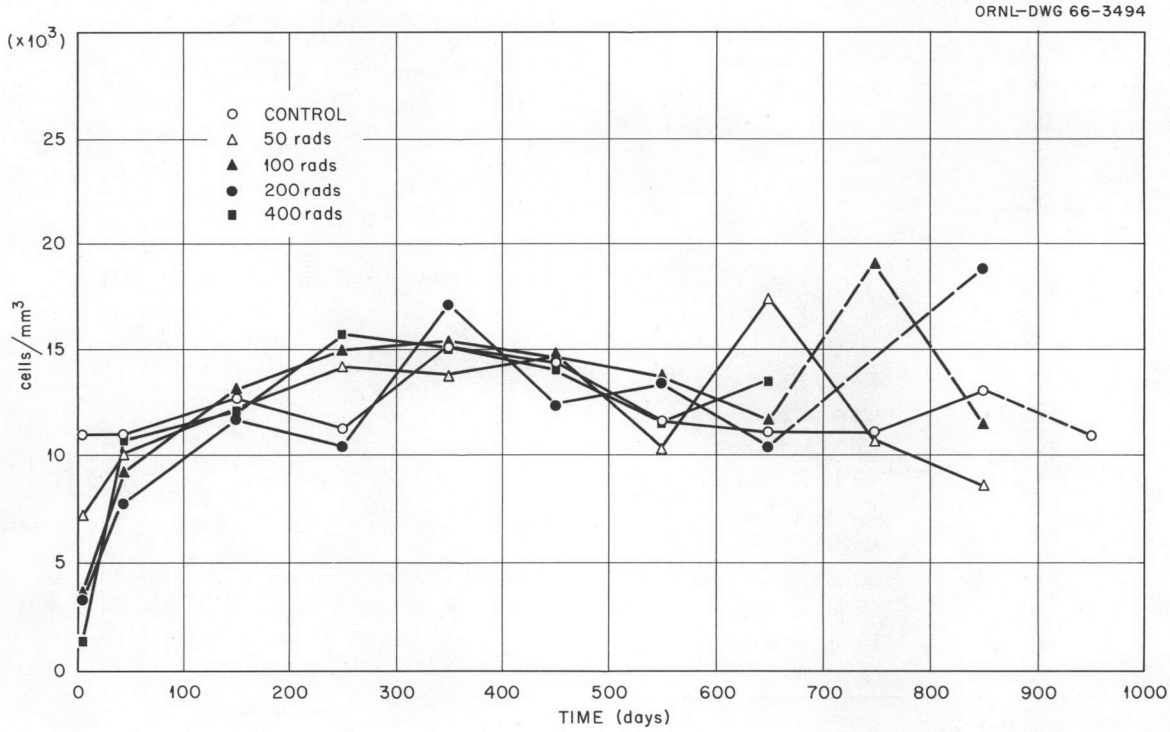

Fig. 15.1.1. Leukocyte Count in Relation to Dose and Time After Irradiation.

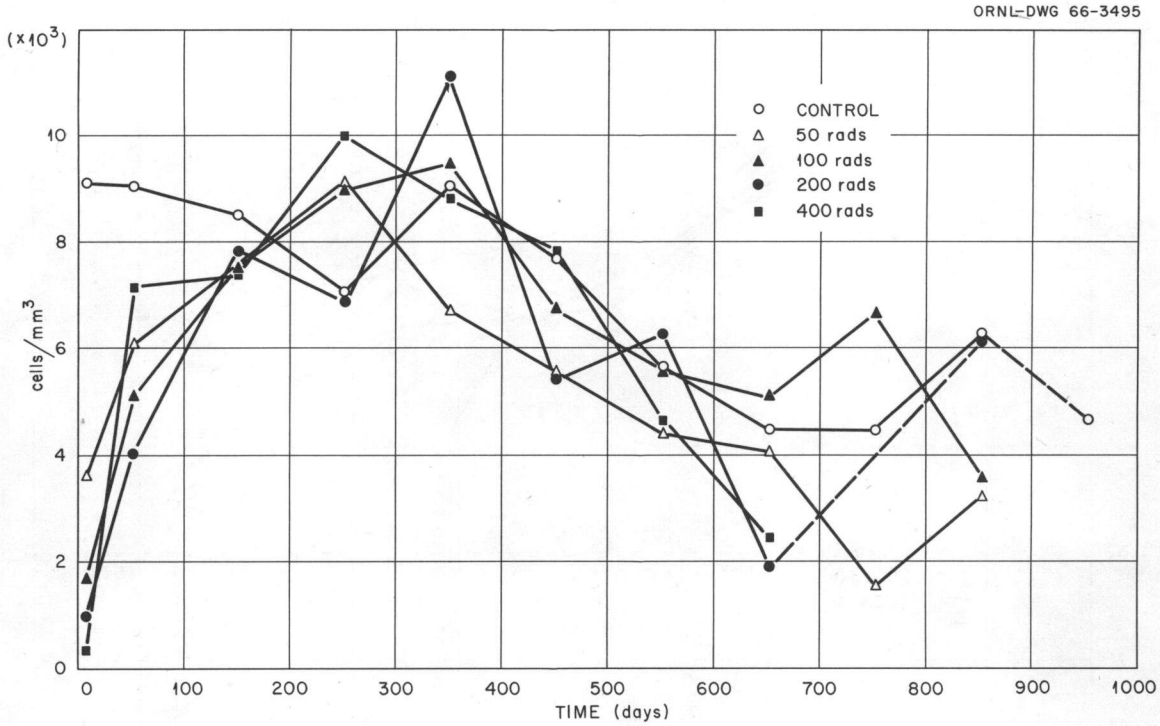

Fig. 15.1.2. Lymphocyte Count in Relation to Dose and Time After Irradiation.

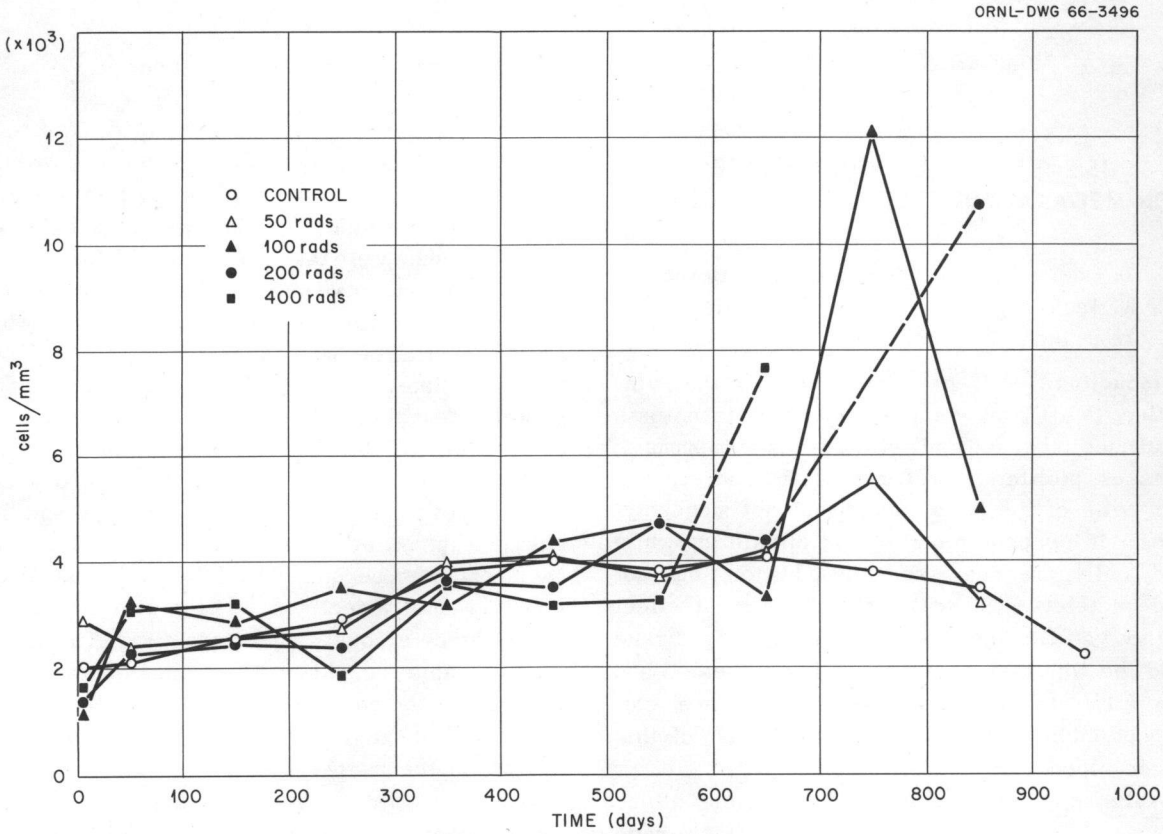

Fig. 15.1.3. Neutrophil Count in Relation to Dose and Time After Irradiation.

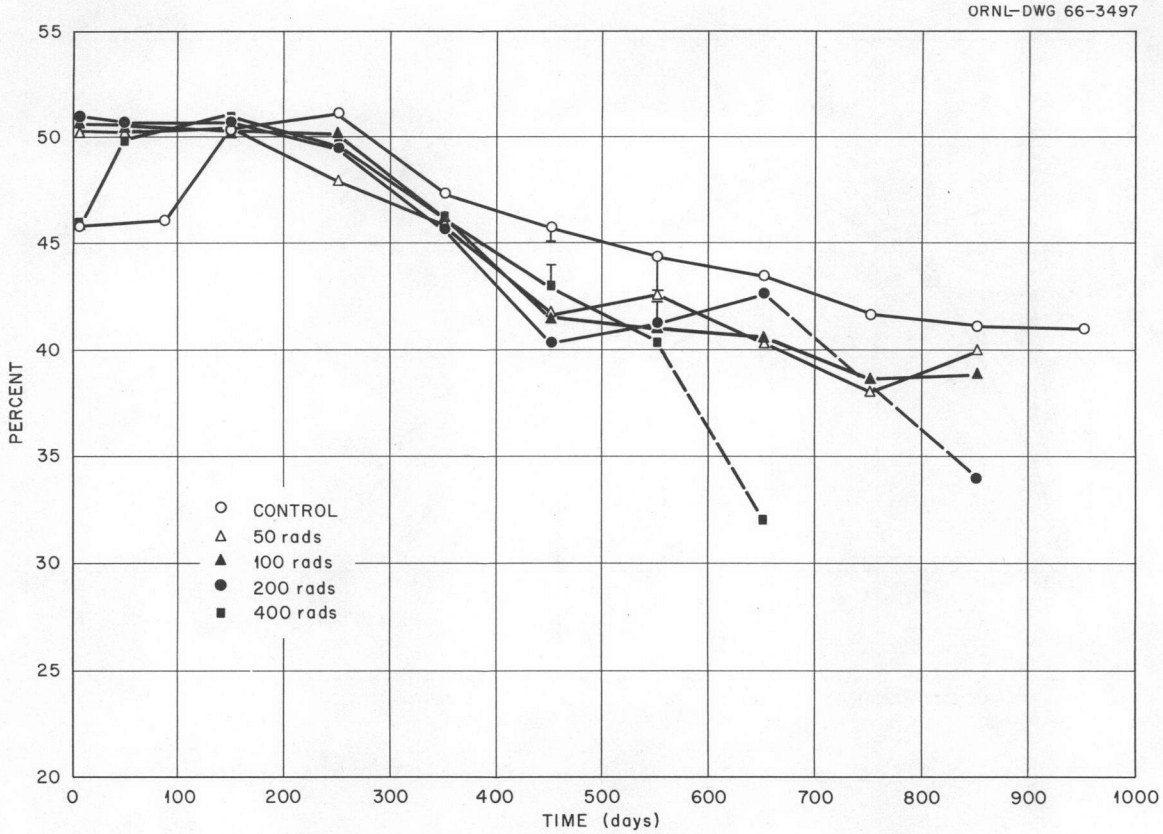

Fig. 15.1.4. Packed Red-Cell Volume in Relation to Dose and Time After Irradiation.

15.2 EFFECT OF VINBLASTINE ON MEGAKARYOCYTES AND PLATELETS

T. T. Odell, Jr. Marianne L. Jefferson
C. W. Jackson Rebecca S. Reiter

Introduction. – It has been reported that vinblastine (VLB), an alkaloid that inhibits mitosis and causes other radiomimetic effects in mammals, depresses proliferation of myelocytic and erythrocytic cells of the bone marrow but not megakaryocytes. It has been reported that circulating white blood cells are depressed, in addition, but not blood platelets. Since such a differential effect on the various elements of hemopoietic tissue would be valuable in studying hemopoiesis, and especially megakaryocytopoiesis, we have carried out additional studies of the action of this drug on blood-forming tissue.

Results and Discussion. – Eight male albino rats weighing approximately 300 g each were injected intravenously each day for two to five days with 0.5 mg of vinblastine sulfate per kilogram of body weight. Erythrocyte, leukocyte, and platelet counts were taken daily. Two VLB-injected and two control rats were killed at two, three, four, and five days after beginning injections, at which time bone marrow was taken for smears and sections. Platelet counts of treated rats dropped sharply, reaching less than 10% of a normal count by five days (Table 15.2.1). The fall of the peripheral count was even more rapid than that observed after exposure to an $LD_{50/30}$ of x rays and suggested that no new platelets were produced after treatment was begun. The total white blood cell count gradually declined. There were no changes in erythrocyte counts or hematocrits. The marrow became aplastic, having an appearance similar to that observed after exposure to large doses of whole-body radiation. The number of megakaryocytes in the marrow declined sharply during the five days of treatment. The average number of megakaryocytes per high-power field of eight

Table 15.2.1. Effect of VLB on Marrow and Blood Cells

Time After VLB (days)	Marrow Count	Blood Count		
	Megs/HPF[a]	Platelets/mm^3	WBC/mm^3	RBC/mm^3
		$\times 10^6$	$\times 10^3$	$\times 10^6$
0	14.1	1.019	16.2	8.7
1		0.579	13.3	7.9
2	11.1	0.383	12.0	8.4
3	6.3	0.317	10.9	8.7
4	5.5	0.215	8.0	8.6
5	3.3	0.078	9.4	8.6

[a]Number of megakaryocytes per high-power field.

control rats was 14.1, whereas that of VLB-treated rats had dropped to 3.3 by the fifth day. The percentage of naked and degenerating megakaryocyte nuclei was markedly increased, and notably fewer of the more mature megakaryocytes were present in VLB-injected rats than in controls. In addition, many of the large megakaryocyte nuclei were spherical in shape rather than having the usual highly lobulated form. Microspectrophotometric measurements of the DNA of nuclei of individual megakaryocytes revealed a shift in distribution of ploidy values from that of controls, the percentage of megakaryocytes in the 16*N* class being much reduced, while that of the 8*N* class was increased. Since the megakaryocytes decreased in number along with other marrow cells, it appears that they are not differentially resistant to VLB, as had previously been suggested, at least not in the rat. The results demonstrate that vinblastine has a marked depressive effect on megakaryocytes and platelets in this species.

15.3 INDUCTION OF RESISTANCE TO A TRANSPLANTABLE HOMOLOGOUS LYMPHOID LEUKEMIA

D. M. Morris

Introduction. – The tumor-host relationship is being studied in the Fischer, Wistar, and Wistar/Furth strains of rats bearing the IRC-741 transplantable lymphoid leukemia. This neoplasm arose spontaneously in a Fischer 344 rat bearing a transplanted acetylaminofluorene-induced adenocarcinoma, and on transplantation into rats of the Fischer strain it causes 100% mortality after an average survival time of 11 to 20 days.[1]

Results and Discussion. – In the present study it has been observed that subcutaneous injection of a cell suspension containing 10^4 to 10^7 IRC-741 leukemia cells causes about 85% mortality in adult Wistar rats and 20% mortality in adult Wistar/Furth rats after 60 days. Five different responses can be seen, however, based on rate of tumor growth and mortality. Thus, the recipient rat may die with a large tumor (greater than 20 cm^3) at the site of inoculation within about 50 days; it may die with a relatively small tumor (less than 20 cm^3) after about 60 days; it may continue to live beyond 80 days with a small or large tumor; it may develop a detectable tumor which regresses after 30 or 40 days; or it may never show signs of tumor development at all. To explore the possibility of an immunological basis for these differences, 17 Wistar and 7 Wistar/Furth rats which had rejected a growing tumor (6W, 4W/F), had shown no signs of tumor growth after inoculation (10W, 2W/F), or had developed only a small scab-like nongrowing tumor mass (1W, 1W/F) received a second subcutaneous inoculation of leukemia (5.5×10^4 cells). At the same time, 8 previously untreated Wistar and 9 Wistar/Furth rats were similarly injected. After 20 days, none of the previously injected rats showed any evidence of tumor development, whereas 4 of the 8 control Wistar rats and all the control Wistar/Furth rats showed a palpable tumor.

A second series of experiments was designed to determine if the lymphoid cells of the "resistant" rats were immunologically reactive against the neoplasm. The axillary and inguinal lymph nodes, spleen, and thymus of a "resistant" Wistar rat (one which had previously rejected a primary implant and was negative for neoplasm after a second tumor implant) were minced with scissors and suspended in Tyrode's solution. A similar preparation was made from the same lymphoid tissues of a previously untreated Wistar rat. These preparations were diluted to concentrations of 15.8×10^6 and 15.9×10^6 cells per 0.1 ml respectively. An aliquot of each of these suspensions was added to an equal volume of Tyrode's solution containing 12.5×10^3 leukemia cells per 0.1 ml. Eight Wistar rats were injected subcutaneously with 0.2 ml of the first preparation, and 6 Wistar rats received similar injections of the second preparation. After 30 days, 7 of the 8 rats receiving the suspension containing "rejector" lymphoid cells remained negative, while 4 of the 6 rats receiving the suspension containing "normal" lymphoid cells had developed large tumor masses.

These data strongly suggest an immunological basis for the resistance to tumor development shown by some Wistar and Wistar/Furth rats. It is not clear, however, whether the response is to a specific leukemia antigen or to a nonspecific histocompatibility antigen normally present in the Fischer rat. This question is being explored.

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15.4 EFFECT OF THE MICROBIAL FLORA ON AGING IN ICR MICE

H. E. Walburg, Jr. G. E. Cosgrove

Introduction. – The life span and incidence of disease have been studied in ICR mice reared in different microbial environments. Conventional mice were reared with "conventional" techniques such that the microbial environment was essentially uncontrolled. Barrier mice were raised and maintained in an isolation facility[1] designed to prevent uncontrolled variations in the microbial flora and fauna. Germfree mice were raised in rigid isolation inside plastic-film isolators free of detectable microorganisms.

Results and Discussion. – The mortality rate was approximately the same in all groups over the entire life span. In 65% of the germfree mice dying in the first 500 days of life, however, the cause of death was cecal volvulus, a condition seen almost exclusively in germfree mice and associated with the large cecum. If animals with this disease are removed from the sample, there is a lower mortality rate in the germfree mice than in mice of the other two groups over the first two-thirds of the life span, but this difference tends to diminish and may disappear in the last third of life (Fig. 15.4.1).

Approximately 35% of the mice in all groups died with leukemia, either lymphoma (mean age of death 300 to 400 days) or reticulum-cell sarcoma (mean age of death 575 to 675 days), no difference in the occurrence of these diseases being apparent among the three groups (Table 15.4.1). The numbers of mice with solid tumors at various sites did not differ greatly among the three groups, but the incidence of mammary tumors was almost three times as great in the barrier mice as it was in

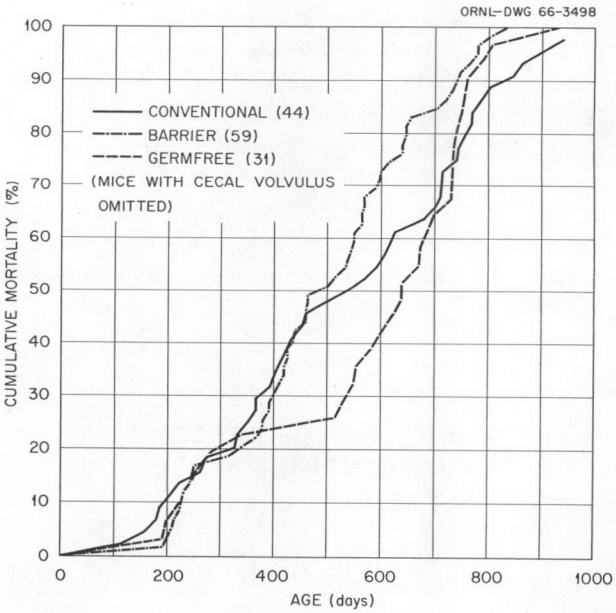

Fig. 15.4.1. Cumulative Mortality in Relation to Age in ICR Mice Reared Under Various Conditions of Microbial Contamination (Each Group Contains Approximately Equal Numbers of Males and Females).

Table 15.4.1. Incidence of Various Diseases in Aging ICR Mice Reared Under Various Conditions of Microbial Contamination

Disease	Experimental Group								
	Conventional Mice			Barrier Mice			Germfree Mice		
	Number with Disease	Number at Risk[a]	Percent Incidence	Number with Disease	Number at Risk	Percent Incidence	Number with Disease	Number at Risk	Percent Incidence
All leukemias	13	35	37.1	17	53	32.1	13	38	34.2
Lymphoma	3	35	8.6	8	53	15.1	5	38	13.2
Reticulum-cell sarcoma	10	26	38.5	9	40	22.5	8	30	25.7
Mice with solid tumors	23	36	63.9	36	53	67.9	25	38	79.0
Mammary tumors	3	25	12.0	12	40	30.0	3	30	10.0
Glomerulosclerosis	10	19	52.7	6	12	50.0	7	27	25.9
Auricular thrombosis	3	11	27.3	3	11	27.3	0	19	0.0
Amyloidosis	0	12	0.0	0	8	0.0	5	15	33.3
Intestinal volvulus	0	36	0.0	1	53	1.9	10	38	26.3
Urinary bladder dysfunction	5	16	31.3	7	23	30.4	7	17	41.2

[a]The number of animals at risk are those which lived at least as long as the first animal dying with the disease and which were examined sufficiently to detect the characteristic pathology.

the germfree and conventional mice (see Table 15.4.1).

All groups of mice showed glomerulosclerosis, although the incidence and severity of the disease appear to be lower in the germfree mice than in the barrier or conventional mice (see Table 15.4.1). Auricular thrombosis was observed in several mice in the two microbially contaminated groups but was not seen in the germfree mice. Amyloidosis was seen only in the germfree mice. Lesions associated with infectious microorganisms (such as otitis media, abscesses, and ulcerative dermatitis) were seen only in the conventional and barrier mice. Late in life (mean age at death 640 to 740 days), males reared in all three microbial environments showed a disease characterized by a gradually enlarging urinary bladder and leakage of urine into the abdominal cavity, resulting in a pelvic phlegmon and often hydronephrosis.

The similarity of widely diverse pathological lesions in germfree, barrier, and conventional mice suggests that the detectable microbial flora and fauna generally alter the aging processes relatively little; however, the possible exceptions to this generalization (e.g., amyloidosis, cecal volvulus, glomerulosclerosis, and auricular thrombosis) call for further study.

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15.5 GROWTH IN SEMISOLID AGAR OF CELLS INFECTED WITH THE RAUSCHER LEUKEMIA VIRUS

Ernestine Teeter R. L. Tyndall
N. D. Bowles

Introduction. – MacPherson and Montagnier have recently reported that virus-transformed cells have the ability to form colonies when planted as monodispersed cell suspensions in semisolid agar, whereas uninfected control cells are unable to form colonies under identical conditions.[1] We have recently observed morphological transformation of JLS V6 mouse cell cultures infected with the Rauscher leukemia virus when such cells were subcultured from NCTC-109 to Eagle's basal medium (BME).[2] The present study was undertaken to determine the effects of Rauscher virus infection

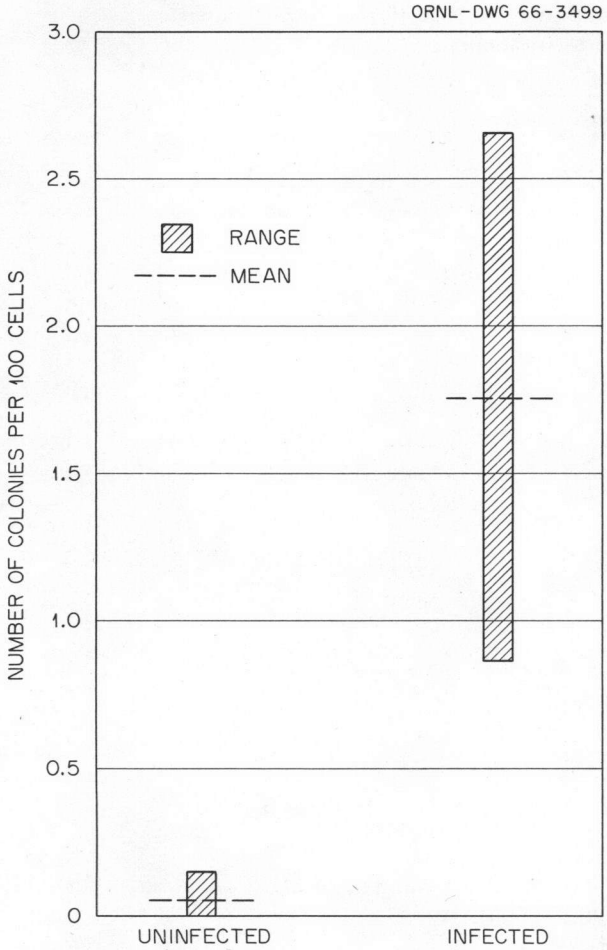

Fig. 15.5.1. Effects of Rauscher Virus on the Ability of JLS V6 Cells to Form Colonies in Semisolid Agar.

on the ability of JLS V6 cultures to form colonies in semisolid agar.

Results and Discussion. – Equal numbers (7×10^4) of cells of uninfected or of Rauscher virus–infected JLS V6 cultures were planted in duplicate bottles containing a layer of semisolid agar and NCTC-109 medium. In four experiments, cultures infected with the virus exhibited a tenfold higher yield of colonies (Fig. 15.5.1) than did the uninfected control cultures (i.e., 1.8 vs 0.18 colonies per 100 cells). Infected cells harvested from such colonies and replanted on glass in BME did not appear morphologically transformed; conversely, they showed an epithelial pattern of growth which contrasted sharply with the fibroblastic crisscross pattern seen when infected cells were subcultured from NCTC-109 to BME without intermediate propagation in agar. Growth of Rauscher virus–infected cells in semisolid agar thus appeared to select for colony formation by morphologically "reverted" cells rather than by morphologically transformed cells. The virus content and tumorigenicity of cell lines developed from clones of morphologically "reverted" cells are now being investigated.

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15.6 TRANSPLANTABILITY AND LEUKEMOGENIC ACTIVITY OF MYXOFIBROSARCOMAS INDUCED BY THE RAUSCHER VIRUS

R. L. Tyndall Ernestine Teeter
J. A. Otten

Introduction. – Previous studies have shown that an established culture of BALB/c spleen and thymus cells (JLS V6) can be morphologically transformed on infection with the Rauscher leukemia virus.[1] Such transformed cells are highly tumorigenic when injected subcutaneously into newborn BALB/c mice, causing myxofibrosarcomas at the site of inoculation. The majority of mice developing primary myxofibrosarcomas from injection of the infected, transformed cultures also develop a concomitant leukemia.[2] The control, uninfected cell cultures are much less tumorigenic and show no leukemogenic activity. When tumors do arise from injection of control cells, they are spindle-cell sarcomas.[2] It was of interest to determine if the myxofibrosarcomas associated with Rauscher virus infection could be serially transplanted and, if so, if they would retain their myxomatous character and leukemia virus on serial passage.

Results and Discussion. – A primary myxofibrosarcoma was excised, finely minced in Tyrode's solution, and injected subcutaneously into newborn BALB/c mice (2×10^5 cells/mouse). Resultant tumors were passaged in a similar manner. The incidence of tumors and leukemias in successive

Table 15.6.1. Characteristics of Transplantable Myxofibrosarcomas

Passage No.	Tumor Incidence	Average Tumor Latency (days)	Histological Type of Tumor	Concomitant Leukemia[a]
1	2/6	23	Myxofibrosarcoma	4/4
2	4/7	27	Myxofibrosarcoma	3/4
3	13/15	21	Myxofibrosarcoma	0/7
4	15/19	22	Myxofibrosarcoma	0/2
5	10/14	20	Myxofibrosarcoma	0/4
6	13/13	18	Myxofibrosarcoma	0/4

[a]Number of mice with histologically confirmed leukemia/number of mice per group examined.

passages is summarized in Table 15.6.1. Although the myxofibrosarcomas were transplantable and retained their myxomatous character, their leukemogenic potency decreased with serial passage. Whether virus antigens, but not whole virus, persist in these tumors remains to be determined.

References

[1]R. L. Tyndall *et al.*, *Proc. Soc. Exptl. Biol. Med.* **119**, 186 (1965).

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15.7 VIRAL TRANSMISSION OF RADIATION-INDUCED MYELOID LEUKEMIA: DONOR VARIATION IN VIRUS

V. K. Jenkins R. L. Tyndall

Introduction. – Although the myeloid leukemias induced by irradiation in RF mice have been found to be serially transmissible by passage of leukemia cells or cell-free extracts,[1] the leukemogenic potency of cell-free passage materials has varied greatly from donor to donor. Efforts to determine the basis for this variation have disclosed that the infectivity of the tissues from a given leukemic mouse depends on the type of cell-free passage material which caused leukemia in that mouse.

Results and Discussion. – The leukemogenic potency of ultracentrifugates of leukemic spleen tissue prepared from various donors was observed to be greater if those donors had been rendered leukemic by inoculation of the resuspended pellet than by inoculation of the ultracentrifugate (Table 15.7.1 and Fig. 15.7.1), although the clinical and morphological features of the disease were the same in either case. The observed variation in

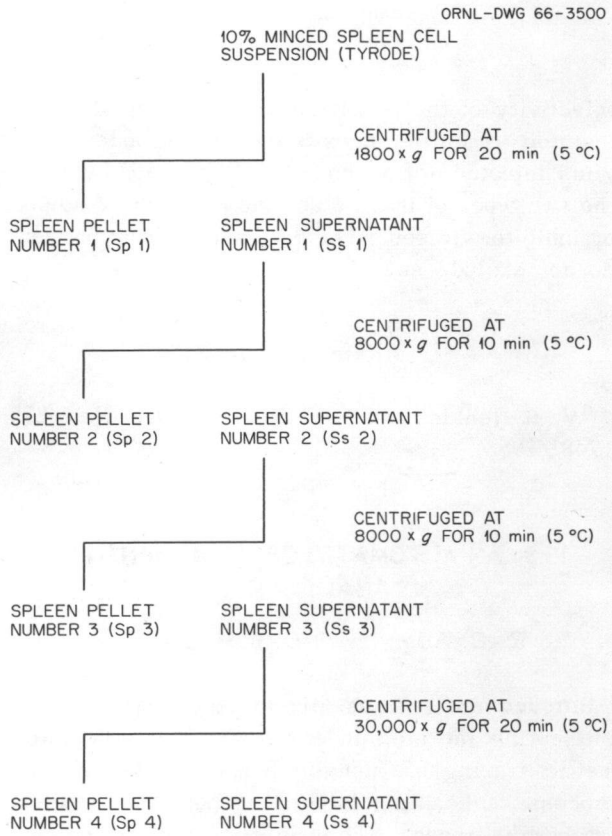

Fig. 15.7.1. Schematic Diagram of Procedure for Preparing the Ultracentrifugates from Spleens of Mice with Myeloid Leukemia.

Table 15.7.1. Leukemogenic Potency of Donor Spleen Ultracentrifugates in Relation to Type of Passage Material Responsible for Leukemia in Donor

Passage Material Which Caused Leukemia in Donor[a]	Potency of Donor Spleen Ultracentrifugates[a] as Measured by Number of Recipients That Develop Leukemia[b]					
	Sp 3[c]		Sp 4[d]		Ss 4[e]	
	Fraction of Animals	Percent of Animals	Fraction of Animals	Percent of Animals	Fraction of Animals	Percent of Animals
Sp 3	1/8	12	74/200	37	6/40	15
Sp 4	5/72	7	80/326	24	44/213	21
Ss 4	1/75	1	55/314	18	1/235	0.4

[a]For definition of terms and schematic description of procedure for preparing passage materials, see Fig. 15.7.1.

[b]The results tabulated are based on the combined data from several dozen separate passages. The mice used were young adult RF males and females injected with ultracentrifugates and scored within 90 days thereafter, according to the techniques described elsewhere [V. K. Jenkins and A. C. Upton, *Cancer Res.* **23**, 1748 (1963)].

[c]Second 8000 × *g* pellet.

[d]30,000 × *g* pellet.

[e]30,000 × *g* centrifugate.

infectivity of the leukemias may conceivably have resulted from differences in the amount of leukemia virus injected or from possible differences between the two types of inocula and the presence of helper or inhibitor viruses. The data available at present do not exclude any of these possibilities.

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15.8 AN AUTOMATIC OPTICAL-DENSITY ANALYZER

R. C. Allen G. R. Jamieson[1]

Introduction. – Presently available methods for integrating the area under the curve of a densitometric tracing are usually based on the tedious process of counting integrating pen pips or disk integrator traces. This is time consuming and inaccurate in areas with minute or closely associated bands, and subject to considerable variation in interpretation on the part of the operator. Thus the following instrument was developed in conjunction with the vertical, discontinuous flat-bed electrophoretic system.

Results and Discussion. – The analyzer consists of a Leitz Ortholux microscope equipped as a scanning microspectrophotometer. The signal from the photomultiplier tube is passed through a Photovolt 520M photometer to an Infotronics CRA 10A integrator and from there to a Minneapolis Honeywell Electronic 19 recorder modified so that the tracing pen also acts as an event marker. The topical density of each band is converted to the inverse log, and the concentration expressed numerically on a tape. Each area integrated is delineated on the densitometric trace by vertical slash marks. With previous instruments, a total count of 300 over the entire tracing was the upper limit; however, with this system, total counts around 1 million are routinely possible. Thus, bands ranging in density from <50 up to 10^6 counts may be recorded on the same trace. This instrument electronically senses slope changes; thus the actual boundaries of each peak are defined by the instrument, and the area between zero slope changes is integrated and counted. This eliminates any operator bias on location of the curve boundaries. In regions of shoulders which may not pass through a zero slope

change, areas may be integrated manually by throwing a trip switch. Instrument reproducibility on the same sample is within ±1% or less on automatic operation and is within ±2% on manual operation.

References

[1]ORGDP Instrument Development Division.

15.9 DISCONTINUOUS, VERTICAL FLAT-BED ELECTROPHORESIS AS A PREPARATIVE SYSTEM

R. C. Allen Dorothy J. Moore

Introduction. – The high degree of electrophoretic separation of proteins obtained with discontinuous, vertical flat-bed electrophoresis prompted efforts to employ the technique as a preparative system.

Results and Discussion. – Gels prepared from recrystallized acrylamide monomer were cast in 11-ml rectangular quartz cells as described previously[1] or in intact quartz Tiselius-type cells. The cells were inverted, the former type run in a specially designed tank, and the latter in a Beckman model E free-electrophoresis apparatus. Location and removal of bands separated by either method were accomplished in either of two ways: (1) A 6-mm slice from one edge of the gel was removed and stained for protein. Then, R_F values, from the dye front to the boundaries of the zones, were calculated, and the zones removed from the gel with a cutting device designed specifically for this purpose. (2) A Polaroid picture of the schlieren pattern was obtained, the gel was laid directly over the photo, and the bands were located and cut out with the cutting device. The latter method is rapid and probably more precise than the staining technique. A third method has been employed with mouse plasma, using the nonspecific esterases as a means of locating the bands.

The isolated bands from gels were pooled, frozen at −85°C to dehydrate them, finely minced, and then suspended in 50% sucrose buffered to a pH of 6.9 with Tris-phosphate buffer. The suspension was placed in a single quartz cell[1] and overlaid with 5% spacer gel. The cell was then encased in a dialysis bag to the bottom of the cell plate. The total volume of buffer within the bag and the proportion of the gel were adjusted by increasing or decreasing the sample and spacer-gel depth. The protein was then eluted electrophoretically into the buffer in the dialysis tubing. Ten milligrams or more was successfully isolated per cell in this manner. The eluted proteins, on re-electrophoresis, have indicated only single bands and no contamination. Thus, proteins may be separated in this manner in a state of high purity, suitable for amino acid analysis.

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15.10 SEPARATION AND PURIFICATION OF ERYTHROPOIETIN

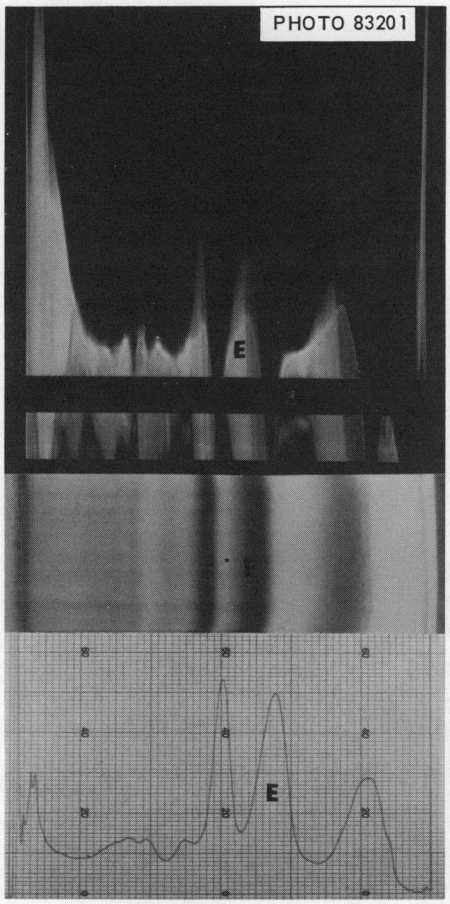
R. C. Allen Dorothy J. Moore
J. W. Fisher[1]

Introduction. – Use has been made of vertical, discontinuous flat-bed electrophoresis in acrylamide gel to attempt the purification of sheep erythropoietin.

Results and Discussion. – Sheep erythropoietin purified to stage 3 with the Goldwasser technique was found to contain some 13 electrophoretically distinct components, including up to 50% albumin. Individual bands separated electrophoretically within the gel were assayed in mice to determine their erythropoietic activity. The activity was found in the α-1-globulin region, which was isolated from the gel by elution. A single separation and elution, utilizing the staining technique for band location, are shown in Fig. 15.10.1. One separation, followed by elution, resulted in a purification of from 29 to 93%, as determined by optical-density analysis of the stained protein bands. Sufficient differences in electrophoretic mobility were present to allow the components to be separated easily with the cutting device.

References

[1]Department of Pharmacology, University of Tennessee School of Medicine, Memphis.



A
SCHLIEREN
OPTICS

B
GEL SLAB
AMIDO SCHWARTZ

C
DENSITOMETRIC
TRACE

Fig. 15.10.1. Protein Zone Location of Erythropoietin for Stage 3 Purified Sheep Plasma. (*A*) Schlieren optics of unstained gel slab; (*B*) same slab stained for protein with Amido Schwartz dye; (*C*) densitometric trace of gel *B*. Erythropoietic activity was found only in zone marked "E" corresponding to α-1-globulin.

15.11 AUTORADIOGRAPHIC STUDY OF VAGINAL EPITHELIA OF MICE BY SERIAL BIOPSY

Iracema M. Baccarini T. T. Odell, Jr.
W. D. Gude

Introduction. – Cell renewal in the vaginal epithelium has been studied previously by sacrificing animals at intervals after injection of tritiated thymidine (^{3}HTdr). It was the aim of this experiment, however, to study sequential changes in the vaginal epithelium of the same individual during a single estrous cycle. For this purpose we examined autoradiograms of sections of vaginal epithelia obtained by serial biopsy in (101 $\times$ C3H)F_1 mice injected with ^{3}HTdr at a known stage of the estrous cycle.

Results and Discussion. – A series of vaginal smears were taken to determine the timing of the estrous cycle. Mice were selected in proestrus, estrus, and metestrus, and were injected intraperitoneally with 1 μc of ^{3}HTdr per gram of body weight. Biopsies of the vaginal epithelium were taken at 1, 24, and 48 hr from the posterior, right lateral, and left lateral walls of the vagina, respectively, and the mice were killed and the remaining vaginal tissue taken at 60 hr.

The major synthesis of DNA among cells of the vaginal epithelium occurred in the basal cells, these cells being well labeled 1 hr after injection of ^{3}HTdr, whereas very few Malpighian or mucous cells were labeled at that time. DNA synthesis was most prominent and the mitotic index highest in basal cells during the early estrus stage of the estrous cycle. The number of Malpighian cells

labeled 1 hr after injection of ^{3}HTdr was low but increased markedly in most mice within 24 hr, presumably indicating an entry of already-labeled cells into this compartment, namely, the labeled basal cells that were differentiating into Malpighian cells. The grain count of labeled basal cells declined with time after injection, indicating that these cells continued to divide throughout the greater part of the estrous cycle. The average grain count of Malpighian cells at 24 hr was about half that of basal cells at 1 hr, indicating that the labeled basal cells divided once during this 24-hr period before maturing to Malpighian cells.

By taking serial biopsies of the vaginal epithelia of mice injected with ^{3}HTdr, it has been possible to trace DNA synthesis, cell division, and maturation of basal and Malpighian cells of the vaginal epithelium during the course of the estrous cycle.

Nucleic Acid Enzymology

Elliot Volkin

Microbial Systems (16.1–16.6)

Elliot Volkin
W. E. Barnett
R. K. Fujimura
J. X. Khym
C. G. Mead
J. L. Epler[a]
M. Helen Jones
Dawn R. Zimmerman

Developmental Biochemistry (16.7–16.9)

F. J. Finamore
Sumiko Iyeiri[b]
A. H. Warner[c]
Rose P. Feldman
Carolyn G. Mohler[d]

Media Kitchen – 3rd Floor, BT

Elliot Volkin
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[d]Student trainee.

16.1 ATTEMPTS AT ISOLATION OF BACTERIOPHAGE MESSENGER RNA

Elliot Volkin M. Helen Jones

Since the discovery in our laboratory of T_2 messenger RNA, it has been determined that this material undergoes continual turnover in vivo and probably represents about 1% of the total cellular RNA. We have now developed a method for accumulating relatively large amounts of messenger RNA in the T_2-infected cell. The RNA appears to be particularly potent in activating the incorporation of amino acids into protein in an in vitro incorporating system.

Experiments in progress attempt to purify the messenger RNA on a relatively large scale and further characterize its properties in directing the synthesis of protein.

16.2 OLIGODEOXYRIBONUCLEOTIDE TRANSFERASE SYSTEM OF T2-INFECTED *E. COLI*

C. G. Mead

The product of the reaction catalyzed by the oligodeoxyribonucleotide transferase system has previously been examined by chromatography on methylated albumin kieselguhr columns and by sucrose density gradient centrifugation. The results of these experiments were consistent with the proposed mechanism, that is, the condensation of an oligodeoxyribonucleotide with a polydeoxyribonucleotide. This report is concerned with experiments designed to determine whether the oligodeoxyribonucleotide is attached to the 5′ end, the 3′ end, or both ends of the acceptor polyribonucleotide. Reactions were carried out using ^{32}P-labeled oligodeoxyribonucleotides and un-

Table 16.2.1. Release of Acid-Soluble Products by Snake Venom Phosphodiesterase

Time (min)	Optical Density at 260 mμ	Percent of Total Optical Density	Radioactivity (counts/min)	Percent of Total Counts per min	Specific Activity[a] (counts/min per optical density unit)
0	0.001		122	2.4	
5	0.022	3.6	1093	21.5	49,600
30	0.069	11.3	2808	55.0	40,800
60	0.201	32.8	3953	77.5	19,700

[a]Specific activity of product = 7300 counts/min per optical density unit.

labeled acceptor polydeoxyribonucleotides. At various times of incubation the reactions were stopped, and the product was isolated by column chromatography on hydroxylapatite. The product was subjected to sequential hydrolysis from the 3′ end using the 5′-exonucleases isolated from snake venom and *E. coli* (exonuclease I of Lehman). In Table 16.2.1 are data obtained from such a sequential hydrolysis of a product isolated after 60 min of incubation and treated with snake venom phosphodiesterase (free of endonuclease and nucleotidase). It is clear that the radioactivity is released as acid-soluble products initially at a higher rate than the ultraviolet-absorbing material, indicating that the labeled oligodeoxyribonucleotide was attached to the 3′ end of the acceptor polydeoxyribonucleotide. Similar results were obtained when products were examined in reaction mixtures with up to 240 min of incubation time. Experiments designed to examine this question by sequential hydrolysis from the 5′ end of the product have so far been unsuccessful due to the inability of the spleen phosphodiesterase preparation used to attack DNA.

16.3 INHIBITORY ACTION OF COLICINE E2 ON BACTERIOPHAGE R17–INFECTED CELLS

R. K. Fujimura

Colicine E2 is a bacteriocidal agent which is known to inhibit DNA, RNA, and protein syntheses and which causes degradation of DNA in sensitive bacteria but does not inhibit the multiplication of T-even bacteriophages. It was found, however, that infection of E2-treated *E. coli* by the RNA-containing bacteriophage, R17, is inhibited. Furthermore, if R17-infected cells are treated subsequently with E2, viable phage formation and viral RNA synthesis are inhibited. It takes longer for the inhibition to take effect on such a system if the time of addition of E2 is delayed. However, the concomitant addition of chloramphenicol at a late stage of infection causes a more rapid inhibition of viral RNA synthesis, although chloramphenicol alone has no noticeable effect. The inhibitory action on viral RNA synthesis appears to be on the formation of new replicative forms, but it is still not clear whether it is due to the damage on viral RNA, on viral enzymes, or on the host system.

16.4 PARTITION STUDIES OF TRANSFER RIBONUCLEIC ACIDS

J. X. Khym

Introduction. – A variety of conditions have been explored for the partition of amino acid acceptor RNA's between an organic phase of 5 w/v % tricaprylyl methylammonium chloride dissolved in trichlorotrifluoroethane and aqueous salt solutions. The extraction coefficients for the individual transfer RNA's (tRNA) are changed markedly by slight changes in the concentration of salt in the aqueous phase and are also influenced by the pH of this phase.

Experimental. – The source of tRNA was a commercial preparation of soluble RNA from *Escherichia coli* B. This RNA material, dissolved in 0.2 *M* NaCl, KCl, LiCl, or CsCl, was extracted into an equal volume of organic phase

Table 16.4.1. Extraction Coefficients[a] for Transfer Ribonucleic Acids

tRNA Assayed	0.30 *M* NaCl,[b] pH 7.5	0.32 *M* NaCl,[b] pH 7.5	0.35 *M* NaCl,[b] pH 7.5	0.35 *M* NaCl,[c] pH 3.5
Glutamyl-	0.7	0.1	0.02	0.1
Lysyl-	0.5	0.1	0.01	0.03
Isoleucyl-		0.1	0.02	1
Methionyl-		0.2	0.02	0.1
Valyl-	2	0.2	0.02	4
Alanyl-	4	0.4	0.02	1
Aspartyl-		0.6	0.03	0.4
Asparagyl-		0.6	0.03	0.5
Glycyl-		1	0.03	0.2
Histidyl-	10	1	0.05	0.3
Threonyl-	15	1	0.05	2
Arginyl-	5	2	0.05	0.1
Leucyl-	51	3	0.3	0.7
Seryl-	36	6	0.4	2
Prolyl-	220	14	0.2	1
Tryptophanyl-		10	5	4
Tyrosyl-	75	30	6	1
Phenylalanyl-	120	60	6	21

[a]Extraction coefficient $(E_a^o) = \frac{\text{concentration of acyl-tRNA in organic phase}}{\text{concentration of acyl-tRNA in aqueous phase}}$.

[b]0.01 *M* Tris chloride buffer, pH 7.5, also present.

[c]0.1 *M* sodium acetate buffer, pH 3.5, also present.

by gently shaking by hand for about 10 min. Aliquots of this organic phase were then equilibrated with an equal volume of salts of a given concentration and known pH for over 16 hr on a Dubnoff metabolic shaker. This machine was set to give about 130 "back-and-forth horizontal motions" per minute. Separation of phases and the assay method for the determination of incorporation of ^{14}C-amino acids were carried out as previously described.[1]

Results and Discussion. – The extraction coefficients for individual tRNA's are not changed significantly relative to one another when either KCl or CsCl is replaced for NaCl in the aqueous phase, although more concentrated solutions of the former two salts are necessary to achieve the equivalent partitions that are obtained with the use of NaCl solution. A slight increase in the concentration of NaCl in the aqueous phase produces marked decreases for the extraction coefficients of tRNA's, as is shown in Table 16.4.1. Also, as shown in Table 16.4.1, all the transfer RNA's (tRNA) studied showed an increase in their distribution coefficients in the presence of acetate buffer of pH 3.5, with the exception of tyrosyl- and tryptophanyl-tRNA. The distribution coefficient of the former tRNA decreased by a factor of 6, while for the latter compound only a slight decrease was noted. The effect of the concentration of quaternary amine in the organic phase upon the extraction coefficients of the tRNA's is currently being investigated.

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16.5 MULTIPLE AMINOACYL-RNA SYNTHETASE SYSTEMS

W. E. Barnett J. L. Epler
Dawn R. Zimmerman

Fractionation of cell-free extracts of *Neurospora* has revealed the presence of two chromatographically separable aminoacyl-RNA synthetases for

both L-phenylalanine and L-aspartic acid. Two *Neurospora* phenylalanine tRNA's have been resolved by countercurrent distribution, and each is specifically aminoacylated by only one of the phe-RNA synthetases. Studies with the two aspartyl-RNA synthetases and countercurrently distributed aspartic tRNA's show specificity in aminoacylation also, although poor separation does not permit identification of the tRNA's involved.

We have examined the synthetase activities for 17 other amino acids in *Neurospora* preparations, and those for phenylalanine, aspartic acid, and leucine in *E. coli*, for multiplicity of enzyme forms by DEAE-cellulose chromatography. In none of these cases has more than one activity been found. This, of course, does not prove that there is only a single enzyme for these amino acids, and we are currently developing more sensitive assays for multiple systems.

16.6 sRNA'S OF *NEUROSPORA* – FRACTIONATION, CODING PROPERTIES, AND INTERSPECIES REACTIONS

J. L. Epler W. E. Barnett

The amino-acid-specific RNA's of *Neurospora* have been isolated and characterized.

Fractionation. – Countercurrent distribution (Holley's system) and hydroxylapatite chromatography have been used to separate the individual species of acceptor RNA's. Multiple forms were found for the aspartic acid-, glutamic acid-, glutamine-, glycine-, leucine-, lysine-, methionine-, serine-, and phenylalanine-specific RNA's.

Coding Properties. – RNA code words of *Neurospora* sRNA's were established using synthetic polyribonucleotides in the ribosomal binding system of Nirenberg. The responses were similar to those reported for *E. coli* sRNA's. When possible, the coding properties of the individual sRNA's were determined. For example, subspecies of leucine-specific RNA's responding, respectively, to poly U, UC, or UG were observed.

Interspecies Reactions. – The ability of enzymes isolated from *E. coli*, yeast, and various fungi to charge *Neurospora* sRNA's is being investigated. Information reflecting on the nature of enzyme recognition sites on sRNA's and their evolution will be accumulated.

16.7 AN AUTORADIOGRAPHIC AND BIOCHEMICAL STUDY OF AMPHIBIAN LETHAL HYBRID DEVELOPMENT

S. K. Brahma[1] F. J. Finamore
Rose P. Feldman

Introduction. – In the cross between *Rana pipiens* ♀ and *Rana catesbiena* ♂, development proceeds normally until the early gastrula stage and then stops abruptly. A variety of embryological and biochemical studies may be made on these embryos because they remain viable for several days after the onset of the developmental block.

We have endeavored to compare normal embryonic development with lethal hybrid development in an attempt to determine the consequences of the developmental block on nucleic acid and oligonucleotide synthesis. In addition, we attempted to correlate biochemical data obtained from whole embryos with microscopic information gathered by autoradiography.

Materials and Methods. – Each of 15 female *R. pipiens* received an intraperitoneal injection of 250 μc of uridine-^{3}H for four consecutive days. On the fifth day each was administered whole frog pituitary glands to stimulate ovulation, and on the following day labeled eggs were obtained. The unfertilized eggs from each female were randomly distributed among several dishes; half the eggs were fertilized with homologous spermatozoa and half with spermatozoa from *R. catesbiena*. At stages corresponding in normal embryos to cleavage, gastrulation, neurulation, and hatching, both normal and hybrid embryos were denuded chemically, or manually for autoradiography, and the concentrations and specific activities were determined for the acid-insoluble nucleotides, oligonucleotides, RNA, and DNA. Furthermore, random samples of embryos were fixed in Smith's fixative, and paraplast sections 5 μ thick were made. The sections were treated with cold trichloroacetic acid, and alternate slides were further treated either with RNase, in phosphate buffer, pH 7.1, or with phosphate buffer alone, followed by TCA. The slides were covered with Kodak NTB-3 liquid emulsion and exposed for nearly four weeks.

Results. – *Comparison of Biochemical and Autoradiographic Studies.* – In both the normal

Table 16.7.1. Grain Counts per Unit Area of Normal and Hybrid Embryos[a]

Stage		Oligonucleotides		Polyribonucleotides		DNA	
Normal	Hybrid	Normal	Hybrid	Normal	Hybrid	Normal	Hybrid
8	8	7.76	5.08	4.15	6.72	12.88	2.88
10	9	2.93	3.06	4.59	5.17	3.57	1.59
13	10	4.89	1.40	7.24	6.44	4.12	3.04
18	10+	3.81	1.68	3.51	5.79	3.46	1.00
20	10+	4.65	1.38	6.12	7.81	0.51	0.34

[a]Only ectoderm in hybrids and presumptive neural area or neural area in normal embryos used for counting.

and hybrid embryos, at all stages of development, the grain counts were localized primarily over the nuclei and, in particular, over the nucleoli when present. Of considerable interest was the observation that a large amount of the radioactivity could be removed from the sections merely by suspending them in phosphate buffer, pH 7.1, followed by a thorough washing with TCA. When whole egg homogenates were carried through the procedure ordinarily used for fixing, mounting, and treating tissue for microscopic examination, it was observed that suspending the homogenate in phosphate buffer released, into the medium, compounds similar to oligonucleotides. Thus we have assumed that radioactivity lost from tissue sections upon buffer treatment is due to removal of oligonucleotides.

An autoradiographic comparison of normal and hybrid development is presented in Table 16.7.1. A large percentage of the total radioactivity of the tissue in both normal embryos and hybrid embryos is released upon washing the sections with buffer followed by TCA. This observation correlates well with the biochemical data, showing that at early stages of development most of the ^{32}P counts in the RNA fraction are due to the presence of oligonucleotides.[2] Furthermore, the autoradiographic data show, at least in the ectodermal regions of the embryos, a relatively constant amount of oligonucleotide material in normal embryos, whereas this fraction decreases in the hybrid after developmental arrest. Biochemical information at these stages shows the oligonucleotide fraction decreasing in *both* the normal and the gastrula-arrested embryos. The reason for this discrepancy is not clear, although it may be related to the fact that only special regions of the normal embryo were used for grain counts.

Table 16.7.1 shows that there is little, if any, difference in the labeling of RNA in normal and hybrid embryos. This observation has been corroborated biochemically since it was found that hybrid embryos under developmental arrest reveal patterns of RNA synthesis *exactly* like those of normal embryos.[3] This table indicates also that labeling of DNA in the hybrid is less than that found in normal embryos. This observation has been confirmed in the biochemical studies.

Discussion. – The major discrepancy between the autoradiographic data and the biochemical information is found in the oligonucleotide labeling pattern. Whereas the hybrid grain counts follow the biochemical data, those found in sections of normal embryos do not. All other microscopic observations in both normal and hybrid embryos correlate well with the data obtained from whole embryos. Perhaps the fact that cellular differentiation is occurring in the neural tissue of the normal embryo and not in the presumptive ectodermal region of the hybrid is reflected in the general oligonucleotide levels of these regions.

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16.8 AN UNUSUAL URACIL-CONTAINING COMPOUND IN AMPHIBIAN EGGS

F. J. Finamore A. H. Warner
Rose P. Feldman

Introduction. – Observations by us[1,2] and other laboratories[3] have indicated that there may be a uracil-containing compound of unusual nature in amphibian eggs. This compound of unidentified composition has appeared both in RNA and oligonucleotide preparations obtained from unfertilized and/or developing amphibian eggs. Most recently, we have observed this compound to be present in the acid-soluble fraction and to be related to, but distinct from, uridine 5′-diphosphate.

Materials and Methods. – Each of 15 female *R. pipiens* received an intraperitoneal injection of 250 μc of ^{32}P as orthophosphate and 250 μc of uridine-^{3}H for four consecutive days. On the fifth day each was administered whole frog pituitary glands to stimulate ovulation, and on the following day labeled eggs were obtained and fertilized. At cleavage, gastrulation, neurulation, and hatching, the embryos were denuded chemically, and the acid-soluble fraction was prepared and subjected to anion exchange chromatography, as described previously.[4] The UMP, UDP, and UTP fractions were rechromatographed, desalted, concentrated, and analyzed for radioactivity. Furthermore, the UDP fraction was subjected to spectrophotometric and phosphorus analyses as well as periodate oxidation.

Results. – Table 16.8.1 shows the specific activities of the various uracil-containing compounds present in the acid-soluble fraction at various stages of development. It can be seen that there is a distinct difference in the specific activities, with respect to ^{3}H, of UMP, UDP, and UTP. Whereas the specific activities at all stages of development of UMP and UTP are essentially equal, those of UDP are approximately twice the values obtained for UMP and UTP. Starting with uridine-^{3}H and a normal operational kinase system in the embryos, after this period of time, the specific activities of EMP, UDP, and UTP should be equal. Thus these data suggest that what ostensibly appears to be UDP is *not* UDP at all.

The UDP fraction was then subjected to periodate oxidation and was found to consume 4.0 moles of IO_4 per mole of uracil, whereas commercial UDP consumed the expected 1.0 mole of IO_4 per mole of uracil. Furthermore, periodate oxidation under neutral conditions and moderate temperatures cleaved the molecule in such a way that inorganic pyrophosphate was liberated in a ratio of micromoles of uracil/micromoles of PP_i = 1.0. No such liberation of PP_i was seen when commercial UDP was used. The remaining fragment obtained from periodate oxidation differed spectrophotometrically and chromatographically from either free uracil or uridine. Only after hydrolysis of this fragment in 1 *N* HCl at 100°C for 2 hr could free uracil be isolated.

Table 16.8.1. Specific Activities of Uracil-Containing Compounds

Stage	Specific Activity (^{3}H counts per minute per micromole)		
	UMP	UDP	UTP
Cleavage	348,000	787,000	337,000
Gastrulation	266,000	552,000	293,000
Neurulation	195,000	520,000	265,000
Hatching	16,300	37,000	

Discussion. – It is clear both from the specific activity data and from the products obtained from periodate oxidation that the compound in the acid-soluble fraction, thought to be uridine 5′-diphosphate, is not UDP. This is, most likely, the unidentified compound found in preparations of RNA and oligonucleotides from amphibian eggs and embryos.

We are currently subjecting the molecule to a variety of chemical and enzymatic hydrolysis procedures in an attempt to elucidate its structure.

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16.9 PRIMING ABILITY OF BRINE SHRIMP DNA ISOLATED FROM DEVELOPING EMBRYOS

Sumiko Iyeiri F. J. Finamore

Introduction. – The development of the brine shrimp, *Artemia salina,* is unique in that neither cell division nor cell multiplication occurs during the course of development subsequent to the blastula stage.[1] This observation suggests that cytoplasmic cleavage as well as nuclear division is not always necessary for differentiation of cells. It is possible, however, that subsequent to blastulation, subtle modifications occur in preexisting DNA that could be reflected in the synthesis of messenger RNA that is necessary for production of specific proteins for the differentiation process. Therefore, we have tested the ability of highly purified brine shrimp DNA to prime the synthesis of RNA in a brine shrimp RNA polymerase system.

Materials and Methods. – DNA was isolated and purified from developing embryos of *A. salina.* The DNA's so prepared were free of RNA and protein and were tested in a highly purified RNA polymerase system prepared from encysted brine shrimp eggs.

Results. – In our previous studies of RNA polymerase from brine shrimp eggs, we found that both native and denatured DNA serve as primer for the synthesis of RNA but that denatured DNA is considerably more efficient in priming ability. Since this difference in priming ability exists, it was necessary to determine by column chromatography on hydroxylapatite the quality of the DNA prepared from the embryos. We observed that the DNA prepared from encysted embryos and embryos that had developed for 2 and 6 hr, respectively, contained insignificant quantities of denatured DNA, whereas the DNA prepared from embryos at the emergence and nauplius stages contained about 30% denatured DNA. The priming abilities of these DNA preparations are reported in Table 16.9.1.

It can be seen that the priming ability apparently decreases with development from encystment through emergence, but the DNA prepared from nauplii reverses the trend and appears to be as effective as DNA from encysted embryos. That this effect is not due to the presence of variable quantities of denatured DNA in the preparations is shown in Table 16.9.2. Each DNA was tested after denaturation by heat in the presence of formaldehyde. A comparison of Tables 16.9.1 and 16.9.2 shows that, indeed, denatured DNA is a more effective primer than native DNA. Furthermore, Table 16.9.2 shows the same general trend, with the denatured DNA's as with the native DNA's, that there is a general decline in priming ability through the emergence period, but DNA from the hatched nauplius appears to reverse the trend.

Table 16.9.1. Priming Ability of DNA's Isolated from Various Stages of Development of *Artemia salina*

Embryonic Source of DNA	Quantity Added[a] (OD_{260} units)	Millimicromoles of ^{3}H-ATP Incorporated in 30 min per Milligram of Protein
Encysted	2.0	4.45
2 hr	2.0	2.74
6 hr	2.0	1.28
Emergence	2.0	1.58
Nauplii	2.0	4.30

[a]OD = optical density at 260 mμ.

Table 16.9.2. Priming Ability of DNA's After Denaturation

Embryonic Source of DNA	Quantity Added[a] (OD_{260} units)	Millimicromoles of ^{3}H-ATP Incorporated in 30 min per Milligram of Protein
Encysted	2.0	6.12
2 hr	2.0	5.28
6 hr	2.0	3.68
Emergence	2.0	3.26
Nauplii	2.0	5.40

[a]OD = optical density at 260 mμ.

Discussion. – Studies of the mitotic indices of brine shrimp embryos from encystment through hatching have indicated that only at the nauplius

stage is there significant nuclear division. Our preliminary data suggest that during the time of development, when differentiation is occurring and there is no synthesis of DNA as evidenced by the lack of nuclear division, there is actually a decrease in the priming ability of the preexisting DNA. At the time when nuclear division resumes, that is, at the nauplius stage, the priming ability of the DNA increases once again.

These experiments were performed using the RNA polymerase system from encysted embryos, but we are now in the process of testing the DNA preparations in RNA polymerase systems isolated from embryos at various stages of development.

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Chemical Protection and Enzyme Catalysis

D. G. Doherty[a] Margaret Turner
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17.1 CHEMICAL PROTECTION

D. G. Doherty Margaret A. Turner

The rationale behind combined treatment for radiation injury lies primarily in the assumption that the overall effect of multiple agents will be additive in providing protection and promoting recovery. If quantitive responses could be obtained, such treatments might also provide clues as to the sensitive systems being protected and aid in the search for more effective compounds. In the previous report[1] we established the quantitative response of BC3F1 mice to various doses of isologous bone marrow cells injected intravenously one day after x radiation. The optimal response was obtained with 5×10^6 nucleated cells. Since the $LD_{50/30}$ response is the most powerful and reliable quantitative measure of effectiveness, it was used to evaluate the effects of single and combined pretreatment with chemicals with posttreatment with isologous bone marrow. The results obtained are presented in Table 17.1.1. It is interesting to note that the $LD_{50/30}$ for the combined treatments of both chemicals and chemicals and bone marrow are close to the values one would expect by adding the increments for each agent alone. The largest deviation was in the combination of AET plus bone marrow, where one would expect a value of over 2000 r. It seems clear from these experiments that neither GSH nor AET is providing maximal protection to the bone marrow system and that, in order to obtain increased chemical protection, agents with increased effectiveness in the marrow must be sought for.

Table 17.1.1. The Effect of Various Treatments on the $LD_{50/30}$ of BC3F1 Mice

Treatment	$LD_{50/30}$ (r)	Observed Increment, $LD_{50/30}$ (r)	Calculated Increment, $LD_{50/30}$ (r)
None	725		
1.6 g/kg GSH	950	225	
5×10^6 cells IBM	1350	625	
360 mg/kg AET	1500	775	
AET-GSH	1700	975	1000
GSH-IBM	1525	800	850
AET-IBM	1825	1100	1400
AET-GSH-IBM	2400	1675	1675

GSH: glutathione.
AET: *S*,2-aminoethylisothiourea diHBr.
IBM: isologous bone marrow.

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Enzymology

G. David Novelli[a]

Protein Synthesis (18.1–18.7)

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Peter Pfuderer
E. F. Phares
Lawrence Rosen[a]
C. J. Wust
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Enzymatic Mechanisms in Protein Synthesis (18.8–18.10)

M. P. Stulberg
L. R. Shugart[b]
K. R. Isham
Jane B. Mills[f]

Mammalian Cell Regulation (18.11–18.14)

K. Bruce Jacobson[a]
Virginia Holten[b]
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Participation in Cooperative Programs:
AEC-NIH Chemical Carcinogenesis (23.2, 23.3, 23.4, 23.5)

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18.1 ROLE OF MEMBRANOUS PARTICLE FRACTIONS IN PROTEIN SYNTHESIS

Lawrence Rosen G. David Novelli

Introduction. – The importance of microsomes in protein synthesis has been established.[1,2] In order to determine the relevance of these findings, mostly based on experiments with rat liver, to trout, a postmitochondrial supernatant (PMS) from trout liver was fractionated by modifying the procedure of Manganiello and Phillips.[3] In this procedure, rough-surfaced microsomes (RSM) are separated from smooth-surfaced microsomes (SSM) and free ribosomes. The latter two are separated from each other by centrifugation in 56% sucrose. Treatment of the RSM with sodium deoxycholate (DOC) yields attached ribosomes (AR) and RSM membranes; treatment of the SSM with DOC yields free ribosomes (FR) and SSM membranes.

Results and Discussion. – Table 18.1.1 shows the yield of the different particulate fractions.

The loss of incorporation which was noted as fractionation proceeded was due in large part to the pelleting and resuspension of the particulate fractions. A loss of 40% in incorporation ability of

Table 18.1.1. Particulate Fraction Yield from Trout Liver

Fraction	Yield[a]	Relative Incorporation[b]
RSM	2	1.0
SSM	4	~0.3
FR	1	1.0
AR	0.5	2.0

[a]In milligrams of protein per gram of liver.

[b]Based on incorporation of L-valine-^{14}C per milligram of protein under standard conditions.

the PMS was noted when the RSM fraction was resuspended and incubated with the supernatant fraction from the RSM to reconstitute the PMS; similarly, a loss of 65% of incorporation ability of the PMS was obtained when all particulate fractions (RSM, SSM, and FR) were incubated in a reconstituted PMS.

Since the AR fraction exhibits greater protein-synthetic ability than the FR fractions, attempts were made to enhance the incorporative capacity of FR by the addition of different membrane fractions. This was not accomplished, but it was found that membranes derived from SSM inhibited incorporation by the FR to the extent of 40%; membranes from RSM had little, if any, effect under similar conditions. Inhibition by a microsomal fraction has been previously reported.[4]

Unfortunately, the procedure of Manganiello and Phillips has led to a lower yield of particulate fractions which are of lower incorporative capacity than ordinarily obtained in this laboratory. Therefore, attempts will be made to combine the two procedures in order to maximize the yield of the distinct particulate fractions.

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18.2 STUDIES OF THE RIBOSOMES OF *CLOSTRIDIUM PASTEURIANUM*

R. D. MacElroy G. David Novelli

Introduction. – In conjunction with a previous report concerning the distribution of ribosomes in cells of *Clostridium pasteurianum*,[1] we have investigated the distribution of newly synthesized RNA in this organism under conditions of exponential growth and during the stationary phase of growth. The RNA of nongrowing cells was labeled with ^{14}C-orotic acid, and that of cells growing exponentially was labeled with ^{32}P. Use of the latter isotope required alteration of the medium to reduce the inorganic phosphate concentration of the medium from 0.072 to 0.001 *M*, an alteration which not only permitted synthesis of RNA with high specific activity but also affected the distribution of the ribosomes between the cytoplasmic and membrane fractions. The methods of extraction and separation of the cytoplasmic and membrane fractions have been described previously.[1]

Results and Discussion. – Newly synthesized RNA of stationary-phase cells sediments with particulate material in sucrose density gradients. When the particles are isolated and separated, it is found that labeled RNA is associated primarily with the ribosomes of the membrane fraction. Extraction and separation of the RNA of these ribosomes suggests that very little ribosomal RNA is synthesized and that most of the labeled material bands as high-specific-activity RNA with sedimentation rates between 8 and 16 S. Material of this nature has been considered to be messenger RNA.[2]

When cells are grown in medium containing low phosphate concentrations, the distribution of ribosomes within the cells is quite different from that observed in cells grown in high concentrations of phosphate. Most of the ribosomes are found to be bound to the cytoplasmic membrane in the form of polysomes, monosomes, and submonosomal particles. The RNA synthesized in these cells after a short pulse of ^{32}P (5-min pulse; generation time 70 min) sediments as a broad peak of activity ranging in sedimentation rate from about 20 to 40 S, with a peak of activity associated with the 30-S submonosomal particle. This material has a base composition which closely reflects that of DNA, and it is estimated that less than 15% is ribosomal

RNA. When the length of the pulse is increased (25 min), the newly synthesized RNA sediments in definite association with the submonosomal particles and the polysomes. Very little activity is found in the region of the monosome. Base-ratio analysis of this material indicates that most of the activity is found in ribosomal RNA, with about 10% as DNA-like RNA.

These results are of interest, particularly because of the association of the newly synthesized material with the naturally occurring submonosomal particles. We are presently studying the relationship between the ribosomes found free in the cytoplasm and those bound to the membrane fraction, as well as the function of the submonosomal particles. It would appear that these particles may be important in the synthesis of polysomes, both as a repository of messenger RNA and in the synthesis of ribosomes.

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18.3 BIOSYNTHESIS OF CYTOCHROME

D. H. Brown
E. F. Phares
D. R. Holladay
C. J. Wust
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G. David Novelli

The site of synthesis of the soluble proteins associated with mitochondria is unknown. If these proteins are synthesized on cytoplasmic polysomes (and then transported to the mitochondria), it should be possible to detect the nascent specific proteins on the polysomes by an immunological titration of fractions from a profile obtained by sucrose-density-gradient centrifugation.[1]

In our investigations we are concerned with the synthesis of cytochrome C in the fungus *Ustilago sphaerogena*. We elected to study the site of cytochrome C biosynthesis because (1) cytochrome C is a well-characterized soluble mitochondrial protein, (2) the quantity of cytochrome C synthesized by the fungus is readily controlled, and (3) *Ustilago* produces large amounts of the protein (up to 1% of its dry weight) under proper cultural conditions.[2]

Methods. – To utilize the immunological procedure described above it was necessary to obtain purified cytochrome C from the fungus. The following is a brief description of the large-scale isolation and purification procedures developed to obtain cytochrome C from *Ustilago sphaerogena*.

Approximately 8 kg of 40-hr-old cells obtained from the 100-gal fermentor were made to 50 liters with a borate–potassium hydroxide buffer (pH 10.0) and extracted at room temperature for 5 hr. After centrifugation the crude supernatant fraction of cytochrome C was desalted by means of an 8-liter Sephadex G-25 column operated automatically at a 2-liters-per-2-hr cycle. The desalted cytochrome C was reduced in volume and chromatographed with a 0.005 *M* borate buffer, pH 9.0, on a 2500-ml DEAE-cellulose column. The partially pure product was fractionated with ammonium sulfate and rechromatographed on the DEAE-cellulose column. The final product was homogeneous with respect to its ultracentrifugation characteristics and gel electrophoresis.

In order to obtain an antiserum to the cytochrome C, rabbits were injected with 15.6-mg samples of cytochrome C at 15 and 30 days. The antiserum was assayed by a difference spectrophotometric method. Antiserum mixed with cytochrome C resulted in a reduction in absorbance at 549 mμ in a Zeiss spectrophotometer. With the known extinction coefficients at these two wavelengths the concentration of the cytochrome C is calculated from the difference spectrum.

Results. – The following information was used as the criteria for purity of the cytochrome C:

1. The sedimentation coefficient, 1.98×10^{-13} sec^{-1} (obtained with a synthetic boundary cell), compared favorably with the value obtained by J. B. Neilands (1.4×10^{-13} sec^{-1}).[3]
2. Electrophoretically the protein moved as a single band even when the current was reversed.[3]
3. The ratio of the extinction at the wavelengths 549 and 271 mμ was 1.095, which compared favorably with the value obtained by Neiland (1.17).[3]

The data indicate that the chromatographic procedure is excellent for separating oxidized cytochrome C from reduced cytochrome C.

Preliminary data indicate that the antiserum obtained from rabbits against the cytochrome C is capable of reducing the concentration of cytochrome C in solution as assayed by a difference spectrophotometric method.

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18.4 CONFIGURATION STUDIES ON *E. COLI* POLYSOMES

Peter Pfuderer

It has previously been demonstrated that some rat liver polysomes are helical. To further investigate polysome configuration, polysomes in whole protoplasts of *E. coli* were examined in the electron microscope. The protoplast was chosen since it could be easily lysed and the contents examined, and also in the hope that the polysomes could be oriented in a favorable plane by centrifuging at low speed before fixation. The *E. coli* were grown on a high-glucose–broth medium to stationary phase, then transferred to broth for a short incubation, and protoplasted. Polysomes and microsome fractions were isolated by differential centrifugation and characterized in the ultracentrifuge. The protoplasts were then harvested, fixed, embedded in Epon, and examined by thin-section electron microscopy. Most of the polysomes appeared to be membrane bound and were evenly distributed throughout the protoplast, while some of the free polysomes have been observed in polysome helices.

18.5 PHYSICAL STUDIES ON *E. COLI* PHENYLALANINE TRANSFER RNA

Peter Pfuderer D. R. Holladay

Introduction. – Physical studies have been started on purified phenylalanine tRNA separated by the Chemical Technology group. The objective of these studies is to try to determine the physical parameters of the "specific interactions" residing in this molecule. These would be interactions with messenger RNA, amino acid, and transfer enzyme.

Results and Discussion. – The first parameters to be investigated were the size, shape, and partial specific volume of the molecule. Purified tRNA sedimented, in the ultraviolet optics of the ultracentrifuge, as a single homogeneous boundary with a sedimentation coefficient of 3.8 S at neutral pH in 0.15 *M* saline. Addition of Mg^{2+} to 0.01 *M* increased this coefficient only slightly, while adding EDTA caused a pronounced lowering of this coefficient, as would be produced by a change in shape.

A molecular-weight value of 27,400 was obtained utilizing the interference optics and concentrations of 0.3 mg/ml and 0.2 mg/ml. This value has not been corrected for charge effects, which would increase it by roughly 20%, although sedimentation titrations with Mg^{2+} would lead one to believe the charge effects were negligible. This value has assumed the $\overline{V}$ = 0.51 reported by Tissieres for pooled *E. coli* tRNA.

A high-precision capillary pycnometer capable of being used with 100 μl or less of material has been designed and calibrated for the partial specific volume determinations. Density measurements of the material have been made but cannot as yet be converted to $\overline{V}$ results since the concentration of the solution is not known on a dry-weight basis.

Preliminary melting curves of the molecule indicate a T_m of about 70°C for the molecule at neutral pH and a T_m of about 80°C in 0.01 *M* Mg^{2+}. There is a slight discontinuity in the melting curve near 30°C and an indication of the biphasic type of curve reported for other pure tRNA's.

18.6 CLEARANCE OF SHEEP AND RAT ERYTHROCYTES IN THE MOUSE: ^{75}Se AS MARKER

C. J. Wust

The clearance of antigen from blood and its deposition in tissue have been examined in mice with erythrocytes (RBC) of a closely related (rat) and distantly related (sheep) species. With the radioisotope ^{75}Se as a marker, the disappearance

of the antigen from the circulation was different for each species.

With sheep RBC, 50 to 60% was cleared within 2 hr after injection. Early hemolysis was noted and attributed to naturally occurring hemolysins. At 4 to 5 hr, the isotope increased in blood and plasma, probably as the result of release of soluble products following phagocytosis. After that time the level gradually decreased. At 24 hr, less than 10% of the label remained in blood, and most of this was in plasma. Both liver and spleen were found to take up 5 to 6% of the label by 2 hr after injection. The spleen on a gram-weight basis was ten times more efficient than liver in taking up the label.

About 20 to 30% of the ^{75}Se from rat RBC was lost from blood within 45 min. After that time, in contrast to the ^{75}Se-labeled sheep RBC, a steady level of isotope was found through 48 hr. From 48 to 72 hr, a rapid disappearance of label indicated the presence and activity of immune opsonins. The isotope was found in plasma but not to the extent as with sheep RBC. In contrast to the tenfold efficiency of spleen over liver in removing sheep RBC on a gram-weight basis, rat RBC were taken up by spleen only twice as efficiently as by liver.

18.7 STUDIES ON ANTIBODY BIOSYNTHESIS

C. J. Wust

Successful attempts to demonstrate the synthesis of hemagglutinin to sheep erythrocytes in a cell-free amino-acid-incorporating system using rat spleen material have been reported.[1] At that time, successful separations of ribosomes by aggregated size had not been fully achieved. During the interim an intensive effort was made to improve ribosomal isolations and to examine the changes in ribosomal profiles occurring during the course of immunization with sheep erythrocytes.[2] After incubation in a suitable mixture for amino acid incorporation followed by sucrose-density-gradient centrifugation of these ribosomes, attempts to demonstrate hemagglutinin synthesis on distinct aggregated classes of the ribosomes were completely negative.

The synthesis of the polypeptide subunits of γ-globulin (IgG) has been shown to be mediated by two genetic loci in humans. The autosomal locus In V determines the synthesis of chain B (light), which appears to be common to all types of human immunoglobulins, whereas the autosomal locus Gm controls the synthesis of chain A (heavy), which is specific for IgG. Both polypeptide chains apparently contribute to antibody specificity. In rabbits, similar findings have been reported. Based on the probability that the synthesis of rat IgG was under the control of at least two genetic loci, a working hypothesis was formulated. The translation of genetic information for each subunit is determined on different classes of ribosomal aggregates. The B chain has a molecular weight of about 20,000, and the A chain, 50,000. Completion of synthesis would result in IgG formation containing two pairs of each chain and having a molecular weight of 160,000.

Fraction C-2 (γ_2-globulin) has been prepared from rat serum by ethanol fractionation. Reduction was accomplished with mercaptoethanol under nitrogen atmosphere, and alkylation of reduced subunits was done with α-iodoacetamide (recrystallized). A and B chains have been separated on Sephadex G-75.

The isolated chains will be used as antigen to prepare antisera to each subunit. These antisera will be fractionated, and the C-2 component labeled. Thus this reagent will be used in future incorporation experiments to test the proposed hypothesis.

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18.8 PHENYLALANYL-RNA SYNTHETASE

M. P. Stulberg K. R. Isham

Introduction. – Phenylalanyl-RNA synthetase from *E. coli* has been purified to a near-homogeneous state. We are now examining the mechanism of interaction between the enzyme and highly

purified $tRNA^{Phe}$.[1] Our immediate goal is to establish the nature of the tRNA recognition site for the enzyme.

Results and Discussion. – Of the numerous properties of the synthetase that have been examined, we have determined a molecular weight of 181,000 and a K_m for $tRNA^{Phe}$ of 2.7×10^{-7}. The purified enzyme, although quite labile in dilute solutions, is stable for at least four months when concentrated and stored at 4°. Preliminary tests[2] have indicated the presence of approximately one thiol group per $tRNA^{Phe}$ molecule. We are presently testing the possible involvement of this thiol during the specific binding of the $tRNA^{Phe}$ to the enzyme. In addition, we have initiated a study of the competitive inhibition of $tRNA^{Phe}$ by degraded fragments of the nucleic acid and by synthetic oligonucleotides. Both of the latter studies should provide information concerning the tRNA recognition site.

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18.9 EFFECT OF METHYLATION ON PHENYLALANINE TRANSFER RNA FROM *ESCHERICHIA COLI*

L. R. Shugart M. P. Stulberg

Introduction. – From recent reports in the literature[1] it is obvious that meaningful information concerning the function of methylated bases in tRNA can be obtained only by using purified methyl-deficient tRNA.

The isolation and purification of tRNA, the first major problems in this study, have been greatly facilitated by the use of a simple method of separation.[2]

Results and Discussion. – *E. coli* $K_{12}W_6$ ($meth^-$, λ^-) was grown under conditions of methionine starvation whereby approximately one-half of its content of tRNA existed in the undermethylated form. The isolated, mixed tRNA's were resolved on a reverse-phase chromatography column,[2] and $tRNA^{Phe}$ was eluted from the column in two completely separated fractions, as determined by phenylalanine acceptance activity. The two $tRNA^{Phe}$ fractions differed in their capacity to be methylated by *E. coli* methylases. Since other undermethylated species of tRNA are probably present in these two fractions of $tRNA^{Phe}$, they are being further purified to determine a valid ratio of methylation to phenylalanyl-tRNA formation. Using a purified phenylalanyl-RNA synthetase,[3] experiments are under way to examine differences between normal and undermethylated $tRNA^{Phe}$. Preliminary experiments with the purified synthetase indicate that the normal $tRNA^{Phe}$ has the capacity to accept 2.5 times as much phenylalanine as that of the undermethylated $tRNA^{Phe}$.

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18.10 RIBOSOME-tRNA SYNTHETASE SEPARATION BY MOLECULAR SIEVING

R. L. Pearson[1] M. P. Stulberg

Introduction. – One of the routine separation tools used in the laboratory to help isolate various particles or molecules is the ultracentrifuge. Unfortunately, the ultracentrifuge is not amenable to throughputs of more than several hundred milliliters per hour. Some other method of separation would be more suitable for large-scale operation. A recent report[2] described the development of porous glass grains which should be able to separate various-sized particles or molecules from each other or from their suspending media by molecular sieving. A separation procedure making use of molecular sieving could be scaled up to large throughputs.

Results and Discussion. – Twenty grams of –50 +100 mesh glass particles permeated with uniform 120-A pores were packed into a column 0.9 × 54 cm. The column volume was determined to be 14.4 ml, exclusive of a pore volume of 10 ml. The initial trial was to isolate soluble proteins from ribosomes in a broken-cell preparation of *E. coli*.

Four hundred grams of frozen *E. coli* cells were thawed and dispersed in buffer. The suspension was passed through a Gaulin press at 10,000 psi, diluted with buffer, and centrifuged at 14,600 × *g* for 90 min. Then 0.5 ml of the supernatant solution was placed on the glass grain column and eluted with buffer; 2.5-ml fractions were collected. These fractions were analyzed for ribosomes by absorption at 420 mμ on the Beckman DU spectrophotometer, and for phenylalanine-tRNA synthetase by radioactive assay. Since ribosomes from *E. coli* have a molecular weight of over 1,000,000 and a size of approximately 170 A and since phenylalanine-RNA synthetase from *E. coli* has a molecular weight of 180,000, it seemed likely that separation could be achieved by this method of molecular sieving. Our first results indicate little separation. The ribosomes were eluted as predicted, their peak appearing 16 ml after application. The phenylalanyl-RNA synthetase peak appeared 18 ml after application of the sample. This lack of separation could be due either to the enzyme having a spatial configuration larger than 120 A or perhaps to the fact that the glass surface is charged and repels the enzyme. Further experiments, such as the use of larger pore sizes and surface treatment of the grains, are under way in order to resolve the problem.

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18.11 ALCOHOL DEHYDROGENASE FROM DROSOPHILA: PURIFICATION AND PROPERTIES

K. Bruce Jacobson E. H. Grell[1]
J. B. Murphy

Introduction. – An isozymic group of alcohol dehydrogenases were discovered in *D. melanogaster* which are under control of a single genetic locus.[2] Also reported was the purification of one of these enzymes to about 50% purity. Certain characteristics of the enzyme have been under study to determine whether it is indeed a dimer.

Results and Discussion. – The final step in the purification procedure has been chromatography of the alcohol dehydrogenase on hydroxylapatite, and two peaks of enzyme have resulted, widely separated. Each of these peaks has all three electrophoretic bands of enzyme. Furthermore, on rechromatography each of these peaks is partially converted to the other. Therefore they do not correspond to the isozymes observed after electrophoresis, but are two interconverting forms.

The molecular weight of the purified enzyme is about 60,000 as determined on Sephadex G-200 and by sucrose-density-gradient centrifugation.

The dimer structure postulated for the alcohol dehydrogenase may not be correct. A reference substance is available to measure the effect of known charge difference on the electrophoretic mobility of a protein with a molecular weight of 66,000, namely, hemoglobin A, S, and C. The comparison of these known charge forms with the positions of the various bands of alcohol dehydrogenase reveals a difference of two charges between adjacent bands. This is inconsistent with a dimer structure but may be indicative of a tetramer form. Other evidence on this point is being sought.

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18.12 USE OF LACTATE DEHYDROGENASE AS A MARKER IN RAT-MOUSE CHIMERA STUDIES

L. V. Kaufman[1] K. Bruce Jacobson

Introduction. – In the rat-mouse chimera it is often of interest to determine the organs in which rat cells are located. In the course of other studies, C. Wust noted that the isozyme patterns of certain enzymes of mice are quite different from those of rats. This study was undertaken to

develop a technique to detect rat lactate dehydrogenase in mouse tissues.

Methods. – Extracts of mouse, rat, and chimera organs were made in 0.05 *M* Tris, pH 8.5. Samples were subjected to electrophoresis in gels of 5% polyacrylamide buffered with 0.05 *M* glycine, pH 10.0. Lactate dehydrogenase was demonstrated in the gel upon adding NAD, lactate, phenazine methosulfate, and nitroblue tetrazolium.

Results. – Of the five basic isozymes of lactate dehydrogenase in the mouse and rat, only band 1 of each animal migrated coincidently. Bands 2 to 5 of mouse migrated at rates different from the corresponding bands of rat. When a mixture of the two was examined, all the bands were observable.

In the chimera animal, the spleen and erythrocytes showed the strongest activity of the rat-type lactate dehydrogenase. Other organs also yielded rat-type isozymes, namely, liver, bone marrow, skin, small intestine, and kidney. On the other hand, the heart of the chimera did not contain rat cells, according to this technique. The pattern characteristic of each organ was not affected appreciably in the chimera, except for the presence of the rat lactate dehydrogenase bands.

Conclusion. – The technique of using the isozyme patterns of lactate dehydrogenase to detect the presence of rat cells in mouse tissues was developed. The detection of rat-type lactate dehydrogenase in the kidney of the chimera was unexpected.

Acknowledgment. – The authors wish to thank Dr. C. C Congdon for helpful discussions and for supplying the chimeras.

References

[1]Student trainee; Mammalian Recovery section.

18.13 LACTATE DEHYDROGENASES IN THE RODENTS

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J. B. Murphy

Introduction. – The multiple subbands of isozymes of lactate dehydrogenase of the laboratory mouse (*Mus musculus*) indicate that this enzyme has many configurational forms, controlled by primary structure and secondary structure as well. To determine how widely these multiple subbands exist in rodents we examined a variety of genera and species in three families of rodents for lactate dehydrogenase isozymes. This study revealed a striking similarity among LDH band 1 of this family and further defined several dissimilarities.

Methods. – Homogenates of organs were made in 0.05 *M* Tris, pH 8.5, centrifuged, and subjected to electrophoresis on 5% polyacrylamide gel buffered with 0.05 *M* Tris, pH 8.5. The enzyme was localized as a purple area after incubation with NAD, lactate, phenazine methosulfate, and nitroblue tetrazolium.

Results. – Band 1 of lactate dehydrogenase migrated identically in the cases of all the rodents except one, *N. floridana* (wood rat). Band 5 appeared to exist in either of two forms, a "mouse" form, which is faster migrating, and a "rat" form, which is slower migrating. The isozyme forms found in erythrocytes were band 5 in all cases except one, *R. humulis* (eastern harvest mouse), which has bands 2 and 3. In the case of the hearts, all the isozyme patterns contained bands 1 and 2, but there was variation in the appearance of bands 3 and 4.

Conclusion. – The original purpose of seeking new subband patterns for lactate dehydrogenase was useful since many of these animals exhibit this characteristic. The utilization of the isozyme pattern by the taxonomist will allow the comparison of closely related animals and perhaps afford a classification technique based on primary gene products, in this case the multiple form of lactate dehydrogenase.

References

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18.14 INHIBITION OF VALYL-tRNA SYNTHETASE BY OLIGONUCLEOTIDES

Virginia Holten K. Bruce Jacobson

At present, little information is available as to the type of interaction between an aminoacyl-tRNA synthetase and the tRNA molecule to which it

transfers the amino acid. To examine the specificity of this reaction in greater detail, we undertook a study of the effects of some oligonucleotides on the incorporation of valine into partially purified tRNA (approximately 30% pure). The valyl synthetase used in these studies was purified from *E. coli* according to the procedure of Bergmann *et al.*[1] Since it has been shown[2] that modified tRNA competitively inhibits the aminoacylation of tRNA, a search for the nucleotide binding site on the synthetase seemed a logical approach. Fifty-three synthetic oligonucleotides of known content but unknown chain length and sequence were screened for their ability to inhibit the valine-tRNA synthetase system. Those copolymers containing certain ratios of AC, AU, and CU competitively inhibit the incorporation of valine into valine-specific tRNA.

Additional studies are in progress to determine the chain length and nucleotide sequence of the copolymers required to produce inhibition. Information is also sought as to the identity (or discreteness) of the amino acid acceptor site on the tRNA and its messenger RNA coding site.

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[1]F. H. Bergmann, P. Berg, and M. Dieckmann, *J. Biol. Chem.* **236**, 1735 (1961).

[2]J. Tarres-Gallardo and M. Kern, *Proc. Natl. Acad. Sci. U.S.* **53**, 91 (1965).

Biophysics

Radiation Biophysics (19.1–19.6)

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Molecular Biophysics (19.7–19.16)

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Participation in Cooperative Programs:
AEC-NIH Chemical Carcinogenesis (23.8–23.10)

19.1 THYMINE DIMER FORMATION IN UV-IRRADIATED DNA AT 77°K vs ROOM TEMPERATURE

R. O. Rahn

Introduction. – Thymine dimers between adjacent thymine residues in DNA are formed relatively easily upon 2600-A irradiation of DNA in solution at room temperature. However, thymine alone in solution dimerizes upon irradiation only when frozen. Presumably, freezing produces aggregates of thymine in which the bases are suitably arranged to form dimers. An examination of a DNA model suggests that the geometrical requirements for thymine dimer formation in DNA are met only upon rotation of the bases with destruction of the Watson and Crick base pairing. In order to determine whether rotational freedom was necessary for thymine dimerization in DNA, the irradiation of DNA was carried out at 77°K, at which temperature the bases are essentially frozen into position and any motion of one base relative to another is inhibited. The results obtained indicated that thymine dimer formation is reduced by more than an order of magnitude at 77°K.

Results. – *Escherichia coli* DNA labeled with ^{3}H-thymine was dissolved in either water or a 1:1 water–ethylene glycol mixture (EG-H_2O), which forms a glass at 77°K. The percentage of thymine dimers was determined from the radioactivity of the isolated dimer fraction. At room temperature, a dose of 3×10^4 ergs/mm^2 at 2804 A gave 8% and 19% thymine dimer formation for DNA dissolved in H_2O and EG-H_2O respectively. At 77°K, the same dose converted only 1% of the thymine in both systems to dimers (Fig. 19.1.1). Denaturation of the DNA enhanced dimer formation only slightly in ice, but in the glass the yield went up by a factor of 5. Inhibition of thymine dimer formation at 77° was also observed for 2537-A irradiation.

The loss of transforming activity of *Hemophilus influenzae* DNA upon 2804-A irradiation was found to be several orders of magnitude lower at 77°K

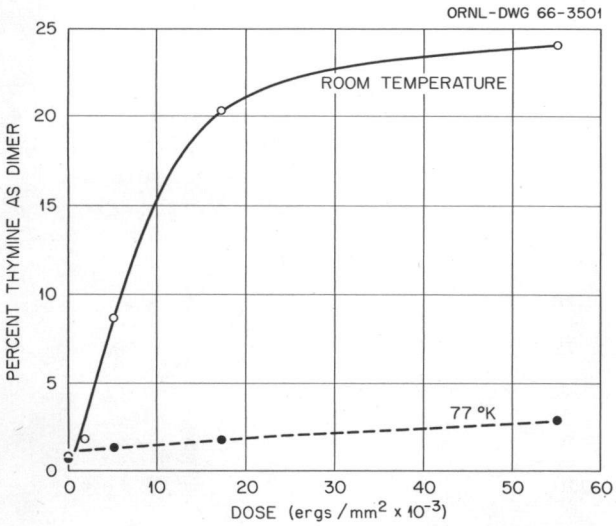

Fig. 19.1.1. Dose Curve for Thymine Dimer Formation in *E. coli* DNA Irradiated at 2804 A.

than at room temperature. Furthermore, the sensitivity of the transforming DNA at room temperature was greater in EG-H_2O than in H_2O. We conclude that the results obtained with transforming DNA reflect the decrease in dimer formation at 77°K shown for *E. coli* DNA. This change in quantum yield appears to be a function of the ease with which two neighboring thymine residues can form the proper geometry for dimer formation.

19.2 ENDOTHERMIC DECAY OF LONG-LIVED FREE RADICALS INDUCED IN AMINO ACIDS BY X RAYS

M. L. Randolph

Introduction. – Many electron spin resonance (ESR) studies have shown that subjecting crystalline amino acids to ionizing radiations induces free radicals with very long lifetimes even in air at 25°C. From measurements of radical concentrations and ESR line shapes as functions of postirradiation storage times at different temperatures in air and in vacuo, we attempt to determine multiple decay kinetics, energy barriers between free radicals and recombination products, and the molecular products. This report extends earlier work.[1]

Methods and Results. – Four amino acids and glycylglycine were heavily irradiated with x rays in air. Radical concentrations were measured up to one year postirradiation by double integration of ESR spectra with daily normalization. Line shapes were checked visually and by a width parameter defined as the square root of (area under absorption curve)/(height of derivative curve). Decay in air or in vacuo was comparable, except that leucine decay was much slower in vacuo. The concentrations $C(t)$ at times t at each temperature θ were empirically fitted to multiple regressions of the form

$$C(t) = \sum_{i=1}^{m} \frac{C_i(0)}{\{1 + K_i(\theta)[C_i(0)]^{n-1}t\}^{1-n}} ,$$

where i designates sets of recombining radicals, $n \geqq 2$ is the kinetic order of the reaction, and $K_i(\theta)$ is the rate coefficient at θ. Values of m and $C_i(0)$ were checked against plots of the width parameter vs concentration. From Arrhenius plots of the K_i's, energy barriers were estimated. Table 19.2.1 gives results. The decay reaction of the dominant alanine radical may be:

$$2(CH_3\dot{C}HCOOH) \longrightarrow \begin{array}{c} CH_3-C-COOH \\ \| \\ CH_3-C-COOH \end{array} + 2\dot{H} ,$$

which should be endothermic by 28 to 32 kcal/mole, judging by bond energies. This would be measured as the loss of two radicals since $\dot{H}$ is not detected.

Discussion. – Although empirical fits seem as good as the data warrant, and there are several reasons for using multiple decay reactions, present understanding of this problem is incomplete. Assignment of radical species is but tentative. Only for one radical do we suggest decay products fitting our estimated energy barrier. A greater difficulty with this presentation is, why should radical sets react independently of each other? Perhaps the truth is not our simple interpretation that mutually exclusive independent sets of radicals are coexistent, but that the sets overlap in the inclusion of radicals. Another difficulty is, why should all observed reactions be endothermic (e.g., why don't $\dot{N}H_2$ radicals recombine exothermically, since 40 kcal/mole is required to dissociate N_2H_4 into $2\dot{N}H_2$)? Two general

Table 19.2.1. Long-Term Decay of Radiation-Induced Free Radicals in Amino Acids Stored at Various Temperatures

Material	Radicals	Range of Analysis (°C)	$C(0)$ (spins/g)	E (kcal/mole)	Kinetic Order	Approximate Rate Coefficient, K, at 25° (hr)
			$\times 10^{17}$			$\times 10^{-4}$
Glycine	$\dot{N}H_2$		30			Very high
	$\overset{+}{H}_3N\dot{C}HCO_2^-$	20–90	75	19	2	60
	$\dot{C}H_2COOH$	50–120	28	19	2	1
Alanine	$\dot{N}H_2$?	25–50	~50	~21	2	~80
	$CH_3\dot{C}HCOOH$	50–150	220	29	2	0.5
		120–150	7	~112	2	3×10^{-9}
Glutamic acid		25–70	100	21	2	10
		70–90	80	~34	2	1.6×10^{-5}
Leucine		4–25	27	~10	2	6.4
		4–90	10	17	2	60
Glycylglycine	$\overset{+}{N}H_3CH_2CONH_2\dot{C}HCO_2^-$	25–120	490	21.4	4	9[a]
	$\overset{+}{N}H_3\dot{C}HCONH_2CH_2COO^-$?	120–150	120	60?	2?	$\sim 1.5 \times 10^{-8}$

[a]Not directly comparable with other values because of different-order kinetics.

arguments, although neither seems directly applicable to $\dot{N}H_2$, are evident: (1) Most possible exothermic reactions may have occurred during the prolonged irradiation at room temperature. (2) Steric considerations may exclude otherwise likely reactions.

References

[1]M. L. Randolph and M. G. Miller, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964*, ORNL-3601, p. 217.

19.3 REPAIR OF UV DAMAGE IN DIFFERENTIATING BACTERIAL SPORES

J. E. Donnellan, Jr. R. S. Stafford

Introduction. – Vegetative cells of *B. megaterium* react to ultraviolet (uv) radiation in the same manner as the vegetative organism *E. coli* – first in the production of thymine dimers in their DNA and second by removal of these lesions by excision during dark repair or by "splitting" during photoreactivation. In spores of this organism, on the other hand, thymine dimers are not produced by uv, but another photoproduct is found. In an attempt to compare the repair mechanisms in bacterial spores with those of vegetative cells, we have studied the fate of this photoproduct during differentiation of spores of *B. megaterium* into vegetative cells.

Results and Discussion. – Spores labeled with ^{3}H-thymidine were exposed to monochromatic uv light and inoculated into a medium containing small amounts of L-alanine, glucose, magnesium sulfate, and phosphate buffer. After various times, aliquots were removed and dried, and the amounts of radioactive photoproduct were determined by acid hydrolysis followed by paper chromatography.

The photoproduct disappeared from regions of the chromatogram it normally occupied. The time

required for 50% of the original photoproduct to disappear was approximately 20 min, although the decay was not exponential. It is clear that the spore photoproduct is altered during differentiation, but whether this alteration is back to normal thymine or to some other compound cannot be stated.

It is possible to implicate the spore photoproduct in spore death by making use of the fact that alteration of the sporulation medium can result in spores having altered resistance to various physical agents. We produced spores in our normal liver extract medium or in this medium supplemented with $MnSO_4$. It can be seen (Fig. 19.3.1) that the slopes of the survival curves and the slopes of the dose-response curves for photoproduct production differ by the same factor in their response to added manganous ion.

Conclusions. – These studies show that the spore photoproduct is involved in the same mechanism which causes spore death. In addition, the spores contain a repair mechanism for removal of the photoproduct from the bacterial genome. The repair mechanism appears different from that found in vegetative cells of several organisms in that the spore photoproduct is changed to a

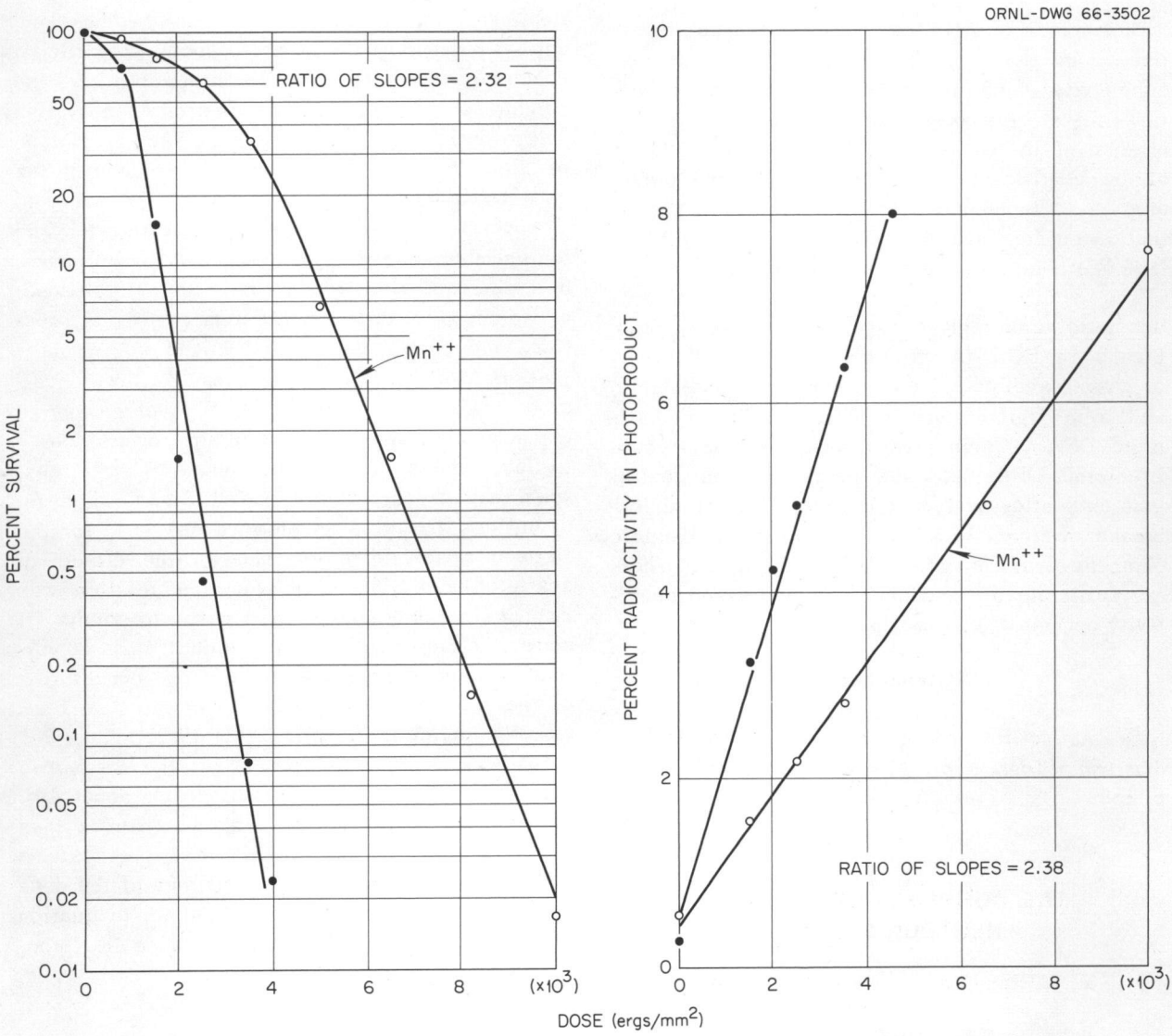

Fig. 19.3.1. Effect of Sporulation Medium on Survival and Photoproduct Production in *B. megaterium* Spores.

different compound rather than being removed by simple excision and resynthesis. It is possible that the photoproduct is excised before alteration, but we have never been able to find it in the acid-soluble fraction of extracts of differentiating spores, as in the case for thymine-dimer excision in vegetative cells.

19.4 UV-INDUCED BREAKS IN THE BROMOURACIL-SUBSTITUTED DNA OF PHAGE T3

M. B. Lion

Bromouracil-substituted phage T3 is more sensitive to uv than normal phage, but the increased sensitivity is abolished if the irradiation is carried out in the presence of cysteamine. The effects of uv on phage DNA have been looked at by centrifugation in alkaline sucrose gradients[1] – a technique that measures chain breaks that arise from the direct action of radiation or from alkali-labile photochemical changes.

The sedimentation profiles indicate that, at doses in the biological range, there are many chain breaks in BU-DNA, few breaks in the BU-DNA of phage irradiated in the presence of cysteamine, and a negligible number of breaks in unsubstituted DNA. Chain breaks were not observed in irradiated DNA that was centrifuged in neutral gradients after heat denaturation. Therefore, the breaks observed in alkali arise from alkali-labile photochemical changes in BU-DNA whose numbers are sufficient to account for the increased sensitivity compared to normal phage.

References

[1]R. A. McGrath and R. W. Williams, *Biol. Div. Semiann. Progr. Rept. July 31, 1965,* ORNL-3853, p. 158.

19.5 DOSIMETRY OF CHRONIC NEUTRON SOURCE

M. L. Randolph Marvin Slater[1]

Introduction. – Several years ago[2] a Po-Be fast-neutron facility was established for the chronic irradiation of mice at various low dose rates, from which irradiation the neutron-dose-rate dependence of various late effects might be evaluated. Prior to the experiment, extensive dosimetric measurements were made, and irradiation protocols were drawn up in collaboration with the Physiology group. During the experiment, checks were made of the source decay. Now, after the termination of the experiment, all the dosimetric data have been reassembled and thoroughly reappraised in the light of more precise basic constants and understanding of some of the detectors.

Results. – Seven at least semi-independent dosimetry systems were employed: polystyrene-polyethylene (PS-PE) ion chambers yielded neutron, gamma, and total doses from volume and current measurements; a hydrogenous proportional counter gave neutron dose by integration of pulse heights; a long counter gave neutron dose by flux determination (from calibrations at National Bureau of Standards and Los Alamos) and dose-to-flux conversion and checked decay of the source;[3] a special combination proportional counter and ion chamber gave total, neutron, and gamma doses; a tissue-equivalent ion chamber gave total dose; a two-chemical dosimeter system gave total, neutron, and gamma doses; and initial source calibration gave total dose via dose-to-flux conversion. The latest values of W (average energy expended per ion pair), stopping powers, dose-to-flux conversion, etc., were used. Corrections, when significant, were applied to the data for deviations from the inverse square law, for the finite size of detectors, and for the effects of scattered radiation. A plausible relation was empirically determined to fit measurements of scattered radiation in this nonideal case at various distances from the source. A compilation of the results for the initial dose rates at 1 cm from the source is given in Table 19.5.1.

Summary. – Reevaluation of all the dosimetric data yielded neutron and total doses about 10% less than and gamma dose about 4% less than the initial values. From the multiple systems used, rough estimates of the accuracy of the dose components were made. A method for evaluation of scattering as a function of distance in a non-idealized situation was devised.

References

[1]Deceased.

Table 19.5.1. Intercomparison of Mixed Neutron and Gamma-Ray Dosimetry for Chronic Po-Be Source

Detector and Its Calibration	Estimated Accuracy (%)	Initial Dose Rates at 1 cm			
		D_n (rads/hr)	D_γ (rads/hr)	D_T (rads/hr)	D_γ/D_T (%)
PS-PE ion chamber	5–10	331	66	393	17 ± 8
Proportional counter	5–10	324			
Long counter NBS – std $t_{1/2}$	5–10	352			
NBS – our $t_{1/2}$	5–10	345			
LA – std $t_{1/2}$	5–10	332			
LA – our $t_{1/2}$	5–10	310			
Counter chamber – std $t_{1/2}$	10–20	305	16	321	5 ± 10
		299	16	315	
Tissue-equivalent chamber	10–20			400	
Chemical dosimeters	10–20	333	24	357	7 ± 5
Source calibration (Mound Lab)	10–20	298			
Best estimates with rough 95% confidence interval		324 ± 20	46 ± 20	370 ± 35	13 ± 6
Initial estimates primarily from PS-PE data		363	48	411	12

[2]*Biol. Div. Semiann. Progr. Rept. Feb. 15, 1956*, ORNL-2060, p. 58.

[3]M. L. Randolph, H. G. Jones, and D. L. Parrish, *Health Phys.* **5**, 45–49 (1961).

19.6 RELATIVE RESPONSE OF NaI(Tl) AND ANTHRACENE TO ^{32}P BETA RAYS UNDER CONDITIONS OF PARTIAL ABSORPTION

J. L. Hosszu

Introduction. – A basic problem of radiation dosimetry is the resolution of an incident mixed radiation into its various components. A pure beta emitter such as ^{32}P presents a characteristic spectrum when observed directly. The same source, when shielded with material thicker than 808 mg/cm^2, the maximum range of the beta particles, presents a spectrum of x rays and bremsstrahlung which originate in the absorber. At intermediate thicknesses, the radiation spectrum represents beta particles, x rays, and bremsstrahlung. The dosimetry becomes more difficult if the absorber is composed of several layers of the same or different materials.

Since dose and biological effect depend on the kind, as well as the magnitude, of incident radiation, measurement of the separate contributions of the total dose is necessary. For single-detector systems, pulse-shape or pulse-height discrimination is widely used. Alternatively, multidetector systems are used with separate detectors selected for greater sensitivity to particular types of radiation anticipated. We here use the second approach.

Results. – The radiation spectra transmitted through C, Al, Cu, Ni, Zr, In, Pt, W, Pb, and several plastic absorbers were observed with 1 × 1 in. and 1 × 1½ in. NaI(Tl) and anthracene scintillation detectors and a multichannel analyzer. The integral transmission spectra as functions of absorber thickness and the differential transmission spectra as functions of energy for single and multiple absorbers were compared.

Because of the relative insensitivity of anthracene to bremsstrahlung and *K* x rays, the transmission spectra could be separated into beta-particle and photon components. As a check, Kurie plots of the beta-particle spectra were constructed and found to be essentially linear.

From measurements of the ^{32}P spectrum with minimum source structure (less than 1 mg/cm^2) and no absorber, the relative efficiency of NaI(Tl) to that of anthracene for 0.5-, 1.0-, and 1.5-Mev electrons was estimated as 1.416, 0.532, and 0.097.

Conclusion. – The use of interchangeable NaI(Tl) and anthracene crystal heads with a photomultiplier and multichannel analyzer permits one to evaluate both the beta and the photon components in a mixed radiation field.

19.7 THE ATTACHMENT OF f1 BACTERIOPHAGE TO MALE STRAINS OF *ESCHERICHIA COLI*

L. G. Caro Maria Schnös

Introduction. – The male-specific, DNA-containing bacteriophage f1[1] has a filamentous shape, approximately 8500 × 63 A. In some cases an axial core, approximately 20 A in diameter, is visible in the electron microscope in negatively

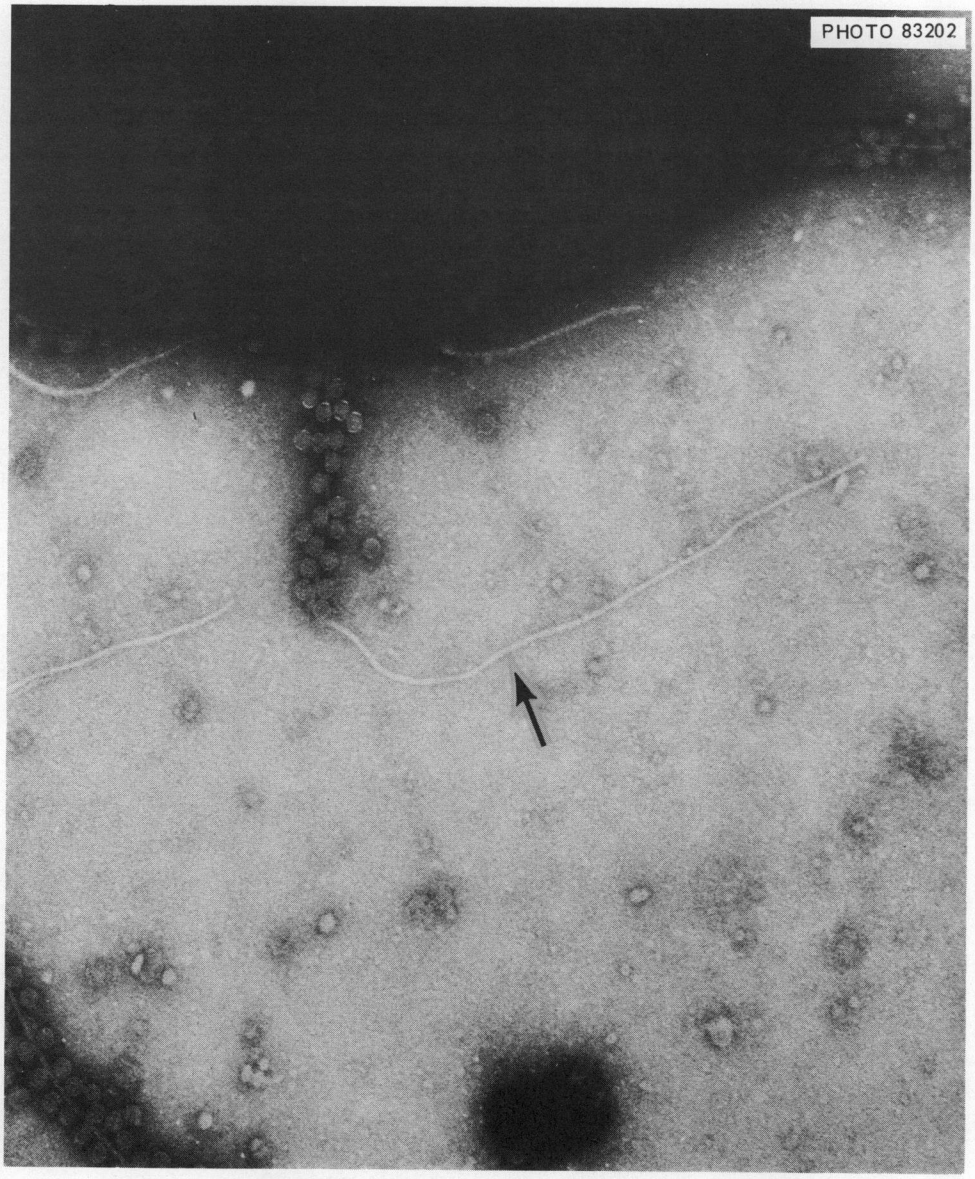

Fig. 19.7.1. Attachment of an f1 Phage (Arrow) to an Hfr Cell. The F pilus is coated with spherical RNA phages, while the f1 bacteriophage, similar in structure to the pili, does not adsorb the RNA phage. 100,000×.

stained preparations. This phage differs strikingly from the RNA male-specific bacteriophages, which are spherical with a diameter of 225 A. RNA bacteriophages have been found to bind specifically to F pili, filamentous structures present only in cells containing the sex factor.[2] We have studied by electron microscopy the attachment of f1 phage to Hfr strains of *E. coli* K12.

Results. – The f1 bacteriophage was added to exponentially growing cultures of Hfr cells in presence of 4×10^{-2} *M* KCN. (The KCN does not stop attachment, but prevents development of the phage in the infected cells.) After 10 min the RNA phage MS-2 was added to permit identification of F pili. In electron micrographs f1 was seen to attach by one end to the tip of F pili (Fig. 19.7.1).

The bacteriophage f1 can also attach to F pili that have been isolated by breaking them with a homogenizer (Fig. 19.7.2). It was found that

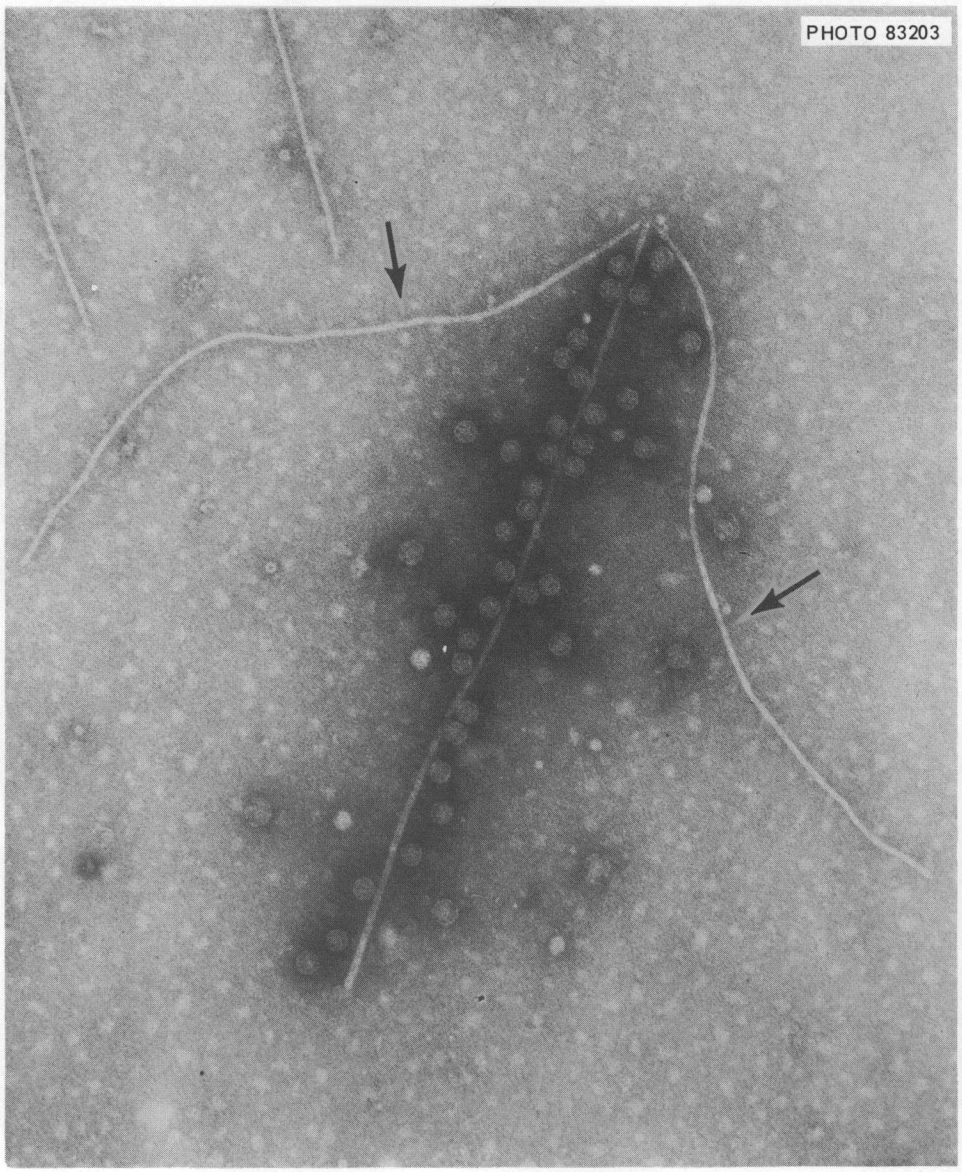

Fig. 19.7.2. Attachment of Two f1 Phages (Arrow) to End of a Fragment of F Pilus. The other end is free. 120,000×.

such F-pilus fragments are polarized with respect to phage attachment: only one of the two ends of the fragment has an attachment site. That end contains more than one site, since two phages can adsorb to it. The attachment of one phage at the tip of an F pilus does, however, reduce considerably the probability of attachment of a second phage.

Conclusion. – The specificity of f1 for strains of *E. coli* containing the sex factor F resides most probably in its unique mode of attachment. The possibility that injection of the phage DNA takes place through the F pilus raises the question of possible analogies with the mechanism of DNA transfer in bacterial mating (see Curtiss and Caro, p. 95).

References

[1]N. D. Zinder *et al.*, *Virology* **20**, 638 (1963).

[2]C. C. Brinton, Jr., P. Gemski, Jr., and J. Carnahan, *Proc. Natl. Acad. Sci. U.S.* **52**, 776 (1964).

19.8 THE TRANSDUCING BACTERIOPHAGE P1kc

L. G. Caro Claire M. Berg[1]
Maria Schnös

Introduction. – The generalized transducing phage P1kc can transfer genetic markers from the cells in which it grew to a suitable recipient. In so doing, it presumably samples the chromosome of the infected cell and can thus be used to study the state of replication of that chromosome and other related questions.[2]

Results. – *Structure of P1kc (Figs. 19.8.1–19.8.3).* – No base plate is visible; the tail fibers seem to be attached directly to the sheath. The periodicity of rows of protein subunits in the tail sheath is 38 A in the extended position and 33 A in the contracted, analogous to the dimensions observed in T4. There are four to six tail fibers visible.

The DNA of P1kc. – The DNA of P1kc, released by osmotic shock and adsorbed to a unimolecular film of cytochrome C, was examined in the electron microscope. The DNA content of the phage

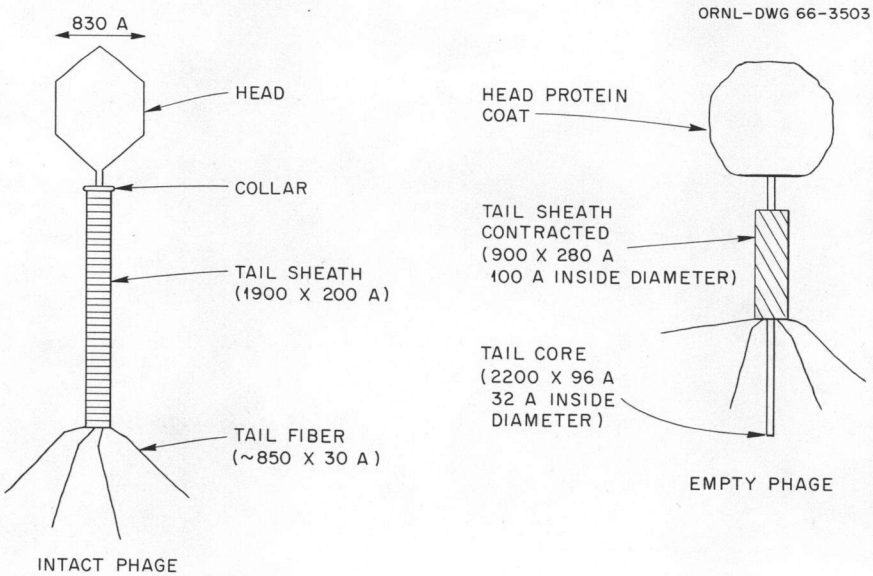

Fig. 19.8.1. Important Dimensions of P1kc.

seems to be one single molecule of approximate length 39 μ, corresponding to a molecular weight of 7.5×10^7 daltons for the sodium salt (in reasonable agreement with the value of 6×10^7 obtained from sedimentation velocity data by Ikeda and Tomizawa[3]). In many instances partial ejection of the DNA had taken place, and the passage of DNA through the tail core was clearly demonstrated (Fig. 19.8.4).

The DNA of Transducing Particles. – P1kc was grown on bacteria whose DNA had been labeled for various lengths of time with ^{3}H-bromouracil. By analyzing the density of the resulting P1 bacteriophages and transducing particles it was determined that (1) transducing particles contain bacterial DNA with little or no phage DNA and each such particle carries, therefore, a segment equivalent to ~3.5% of the bacterial chromosome and (2) the transducing particles pick up only DNA made before infection; this implies that infection with P1kc stops bacterial DNA replication and is at variance with results obtained by Ikeda and Tomizawa.[3]

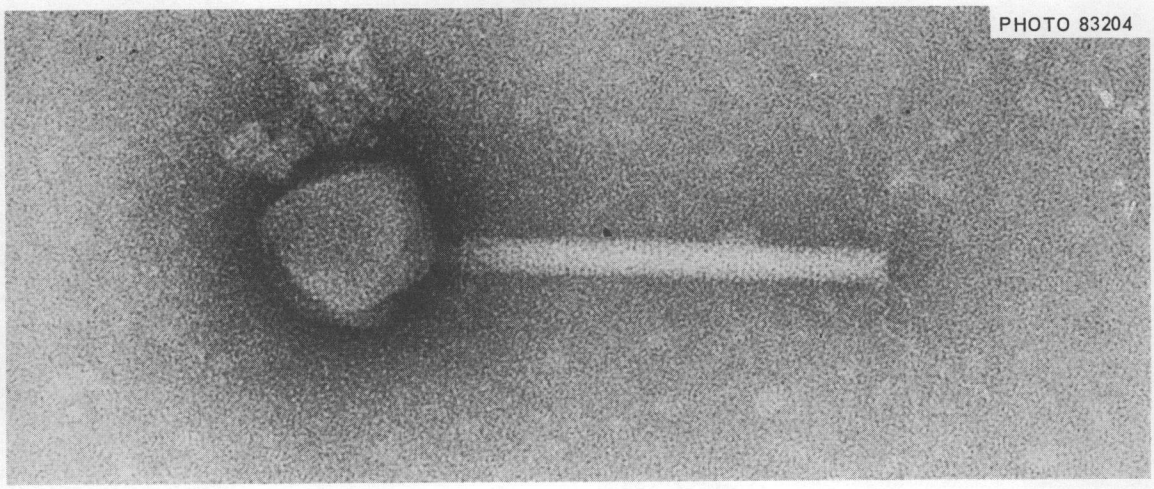

Fig. 19.8.2. Electron Micrograph of P1kc. 280,000×.

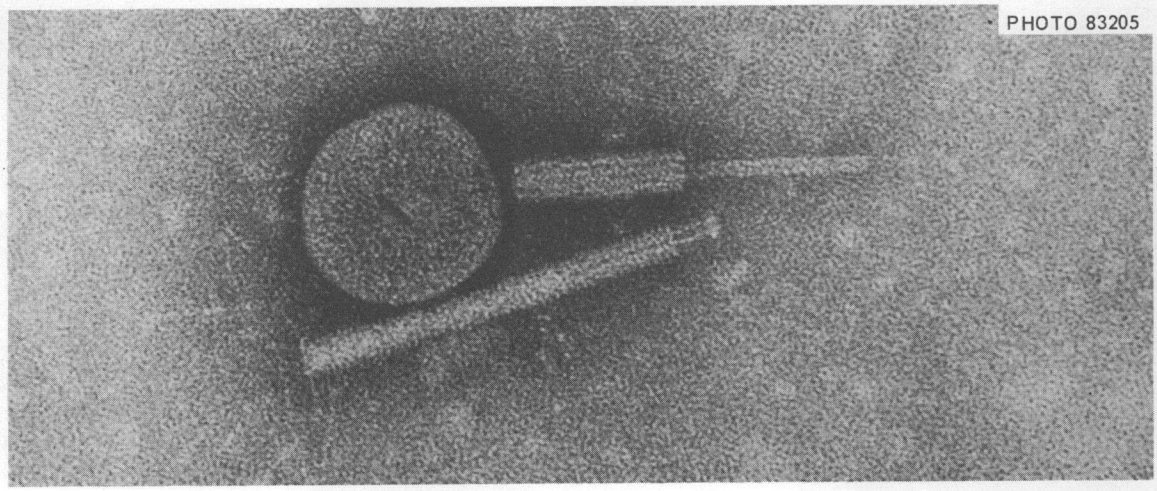

Fig. 19.8.3. An Isolated Tail of P1kc with Extended Sheath and an Empty Phage with Contracted Sheath. 280,000×.

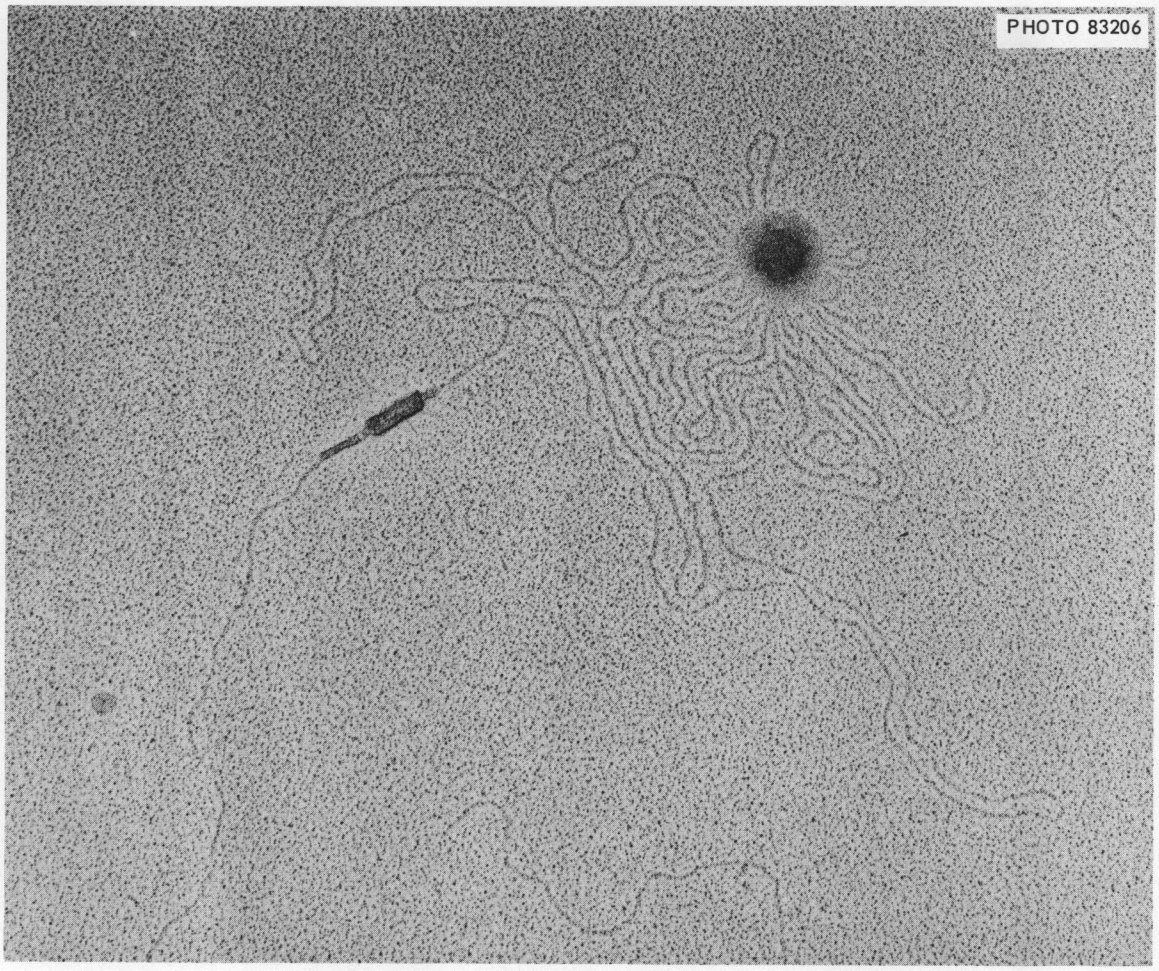

Fig. 19.8.4. DNA of P1kc. The DNA molecule is threaded through the tail core and contracted sheath. 90,000×.

References

[1]Radiation Microbiology section.

[2]C. M. Berg, *Biol. Div. Semiann. Progr. Rept. July 31, 1965*, ORNL-3853, p. 91.

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19.9 DNA TRANSFER IN BACTERIAL CONJUGATION

L. G. Caro J. D. Gross[1]

Introduction. – We have concluded a series of experiments in which the relation between the replication of DNA and the transfer of the bacterial chromosome from Hfr to F^- cells of *Escherichia coli* K12 was examined.

Results. – Chromosome transfer from Hfr to F^- cells was analyzed by labeling the DNA of the Hfr cells with ^{3}H-thymine prior to mating and/or by labeling any DNA made during mating. The F^- cells had been either starved of adenine or heavily irradiated with ultraviolet light, with the aim of preventing them from synthesizing DNA. The appearance of labeled DNA in the F^- cells was determined by quantitative autoradiography, taking advantage of a morphological difference between the Hfr and the F^- cells. The results indicate that: (1) the DNA accumulating in F^- cells during mating contains equal amounts of

label in "old" DNA (made before mating) and in "new" DNA (made during mating); (2) the template used for synthesis of new DNA is Hfr DNA; (3) the synthesis is simultaneous with transfer; (4) only one strand of old DNA enters the F^- cell; (5) the thymine incorporated into the new DNA appearing in F^- cells is derived mainly or exclusively from the precursor pool of the Hfr cells.

Conclusion. – The DNA transferred during conjugation appears to be a semiconservative replica of the Hfr chromosome synthesized concurrently with, and at the same rate as, its transfer to the F^- cell. These experiments thus verify one aspect of the "replicon" theory of chromosome replication proposed by Jacob and Brenner.[2]

References

[1]Microbial Genetics Research Unit, Medical Research Council, Hammersmith Hospital, London.

[2]F. Jacob, S. Brenner, and F. Cuzin, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 329 (1963).

19.10 NEW APPROACHES TO THE PURIFICATION OF NUCLEIC ACID POLYMERASES

B. K. Zimmerman Patricia N. Howard

The enzymes involved in nucleic acid synthesis have, in general, two probably related properties which make their purification somewhat more difficult than the purification of most enzymes: They are large molecules (~100,000 daltons) and are rather unstable. DNA polymerase from *Micrococcus lysodeikticus*, for example, loses activity rapidly and irreversibly upon freezing and thawing, exposure to low ionic strength, adsorption on DEAE cellulose, and exposure to room temperature. While the enzyme may be obtained at a high degree of purity (1500-fold over crude lysates), the yield by standard purification procedures is poor.

However, it was found, not unexpectedly, that DNA, and to some extent RNA, will stabilize DNA polymerase activity indefinitely, even at elevated temperatures. This fact makes it possible to utilize chromatography on DEAE cellulose as a purification step for DNA polymerase. If nucleic acids are digested by nucleases and removed by dialysis or gel filtration at an early stage in the purification of DNA polymerase, subsequent chromatography on DEAE cellulose results in a 95% loss of activity. If the nucleic acids (mostly RNA) are not digested, 25 to 50% of the activity is recovered. If a DEAE column is first saturated with DNA before chromatography, nearly 100% of the activity is recovered. In the latter case, purification is substantially better than in the other two due to the lowered affinity of many proteins which are normally strongly bound to the positively charged DEAE groups and lack any specific interaction with polynucleotides. Techniques which involve adsorption and chromatography of proteins on DNA trapped in inert media (rather than an ion exchange resin) are now under study.

Another approach which has been found to be highly useful in stabilizing DNA polymerase in solution is to use, instead of primarily aqueous solvents, a solution containing up to 50% of a nonpolar liquid such as glycerol or ethylene glycol. The latter is preferred because of its relatively low viscosity at low temperatures. In 30 to 50% ethylene glycol buffers, DNA polymerase may be dialyzed into low-ionic-strength solutions and be stored at –20°C without the loss of activity. Ion exchange and adsorption chromatography may also be successfully carried out in such solvents.

The precise manner in which stabilization is achieved by such solvents is not thoroughly understood. However, the structure of water in the immediate vicinity of the protein molecule would be greatly disrupted, an effect which would be expected to weaken certain types of bonds (hydrophobic bonds) but stabilize others (hydrogen bonds and ionic bonds). It is also well known that not all types of proteins are stabilized by glycols. Thus, the effect may well depend on the local environment to which the enzyme is subjected in a highly compartmented cell. That is, enzymes which are normally bound to membranes or polymers may well not be surrounded by water molecules in vivo as they would be in aqueous solution. Hence, it is conceivable that for such proteins a strictly aqueous medium would constitute a denaturing solvent.

19.11 DNA POLYMERASE ACTIVITY IN PUROMYCIN-TREATED SYNCHRONIZED MAMMALIAN CELLS

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In order to determine whether or not turnover of DNA polymerase exists in mammalian cells and the position(s) in the cell-division cycle at which polymerase synthesis and/or breakdown occur, Chinese hamster cells in vitro, previously synchronized with 5-aminouracil, were treated with puromycin at a concentration which normally blocks protein synthesis. Puromycin was added at the time when the cells were removed from 5AU-containing medium (early S phase) in one series and at 2, 4, and 6 hr after removal of 5AU in other series. DNA polymerase activity, protein, DNA, and cell number were determined periodically in each series until well after mitosis.

The results demonstrated that puromycin addition at the beginning of, or during, the S period had no effect on DNA synthesis, DNA polymerase content, or cell division. These observations are consistent with the hypothesis that all of the apparatus necessary for DNA synthesis and cytokinesis exists before the beginning of S and does not require additional protein synthesis. If breakdown and resynthesis of DNA polymerase does take place, it must do so in middle or late G_1, unless, as seems unlikely, DNA polymerase synthesis is insensitive to puromycin. All evidence obtained thus far indicates that the level of DNA polymerase in mammalian cells is maintained at a constant level throughout the entire cell cycle.

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19.12 DIMER FORMATION AND MONOMERIZATION IN THE PRESENCE OF PROFLAVINE

R. B. Setlow W. L. Carrier

It is known that proflavine inhibits the formation of thymine dimers in DNA by uv irradiation and that any dimers that are present in a DNA are destroyed by irradiation in the presence of proflavine.[1] We have investigated the effects of proflavine on dimer formation as a function of wavelength for ^{3}H-thymine DNA of *E. coli* and ^{3}H-cytosine dI:dC and have shown for the latter polymer that (1) dimer destruction is monomerization, (2) proflavine does not affect the rate of monomerization, and (3) proflavine only inhibits dimer formation. Therefore, we infer that at any wavelength

$$-\mathrm{PyPy}- \underset{}{\overset{\lambda}{\rightleftharpoons}} -\widehat{\mathrm{PyPy}}- \underset{\text{proflavine}}{\overset{\lambda}{\rightleftharpoons}} -\mathrm{PyPy}- .$$

Table 19.12.1 gives 1/e doses for monomerization, in the presence of proflavine, of $\widehat{TT}$ and $\widehat{CT}$ at several wavelengths. It is obvious that at longer wavelengths it is possible to monomerize an appreciable fraction of $\widehat{CT}$ without affecting $\widehat{TT}$. This fact was used to identify cytosine-containing dimers as contributing to the biological inactivation of transforming DNA.[2]

Table 19.12.1. 1/e Doses for the Monomerization of $\widehat{TT}$ and $\widehat{CT}$ in *E. coli* DNA

Wavelength (mμ)	1/e Dose (ergs/mm^2)	
	$\widehat{TT}$	$\widehat{CT}$
280	67×10^4	2.2×10^4
265	4.8	0.66
239	0.86	0.56

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19.13 IN VITRO EXCISION OF PYRIMIDINE DIMERS

W. L. Carrier R. B. Setlow

Introduction. – Many biological systems can recover in the dark from damage produced by uv

radiation, and recent evidence has pointed toward uv-induced pyrimidine dimers in DNA as important biological lesions. Such dimers are excised in vivo from the DNA of radiation-resistant cells, but are not excised by sensitive mutants of *E. coli*. It is presumed that excision is one of the steps in processes that repair damaged DNA. Several workers[1,2] have shown that cell-free extracts of *M. lysodeikticus* are able to restore, in the dark, the biological activity of uv-irradiated DNA. We have investigated the ability of such extracts to excise pyrimidine dimers from uv-irradiated DNA.

Results. – Extracts of *M. lysodeikticus* are able to excise thymine-thymine and cytosine-thymine dimers from uv-irradiated DNA's. The excision rate is greater than the rate of hydrolysis of the DNA's. Dimers excised from DNA appear as parts of acid-soluble oligonucleotides which seem to have one terminal phosphate group. The in vitro excision is similar to the reaction observed in vivo in resistant cells and may be one of the steps in the dark repair, in vitro, of the biological activity of irradiated DNA.

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19.14 REPAIR OF UV DAMAGE IN T3 PHAGE BY *ESCHERICHIA COLI* B/r

B. S. Baker R. B. Setlow

Introduction. – The survival of uv-inactivated T3 phage is higher when assayed on *E. coli* B/r than when assayed on *E. coli* B_{s-1}. Thus the uv-resistant strain B/r is described as being able to reactivate some inactivated phages by a process of host-cell reactivation (hcr). The hcr mechanism is thought to work by the excision of pyrimidine dimers from phage DNA since cells that do hcr can excise dimers from their own DNA.

Results. – Phage T3 was labeled with ^{3}H-thymine, irradiated with 265 mμ (~5 mean lethal hits), and adsorbed at a multiplicity of ~5 to either *E. coli* B/r or B_{s-1}. At various times during the latent period, the infected complexes were collected by centrifugation and analyzed for thymine and thymine-containing dimers in DNA and in oligonucleotides.

During growth of phage on B_{s-1} there was no change in the ratio $\widehat{TT}$/T in phage DNA, but during growth on B/r, the host-cell-reactivating strain, $\widehat{TT}$/T for DNA decreased by over twofold. Thus it seems probable that hcr involves excision of dimers from phage DNA by bacterial enzymes.

19.15 TRANSFER OF THYMINE DIMERS FROM PARENT TO PROGENY PHAGE T4 v$^-$

B. S. Baker R. B. Setlow

Introduction. – Hershey and Burgi[1] showed that, under conditions of multiple infection, DNA from irradiated phage can appear in progeny particles but that many of the progeny are not viable. It has been hypothesized that multiplicity reactivation (the ability of several uv-killed phage to give rise to viable progeny) is a recombination-like process that occurs in a way to exclude uv lesions from the DNA that is encapsulated.[2] It should be possible to put this hypothesis to a test by determining the transfer of specific uv lesions from parent to *viable* progeny. We report here the first part of this experiment – the transfer of dimers to progeny particles.

Methods. – Phage T4 v$^-$, a mutant unable to excise dimers, was labeled with ^{3}H-thymine and irradiated about five mean lethal doses of 265 mμ radiation. Irradiated phage were used to infect *E. coli* B_{s-1} (a mutant that cannot excise its own or phage dimers) at a multiplicity of 5. Infected complexes were grown until lysis was complete; the progeny particles were purified by differential centrifugation, and the amounts of thymine and thymine dimers were determined in the progeny and parental particles.

Results. – The results of two typical experiments are shown in Table 19.15.1. Though a marked decrease (~50%) in the dimer-to-thymine ratio occurs during multiplicity reactivation, the exclusion of dimers from the phage DNA that is encapsulated is not complete. We have not yet determined the transfer of dimers to viable progeny phage.

Table 19.15.1. Transfer of Thymine and Thymine Dimers from Irradiated Parental T4 v⁻ Phage to Progeny Particles

Experiment	Counts per Minute		Percent Transfer	
	T	$\widehat{TT}$	T	$\widehat{TT}$
1. Parental	44,500	31		
Progeny	22,800	6	51	19
2. Parental	138,200	137		
Progeny	44,500	28	32	20

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19.16 BIOLOGICAL PARAMETERS INVOLVED IN REPAIR OF X-RAY DAMAGE

R. A. McGrath R. W. Williams

Introduction. – Centrifugation of DNA from radiation-sensitive (B_{s-1}) and radiation-resistant (B/r) strains of *E. coli* in alkaline sucrose density gradients indicated that x-ray-induced breaks in the DNA were "repaired" in B/r, but not in B_{s-1}.[1] The calculations led to the conclusion that 3 kr (a mean lethal dose for B_{s-1}) was sufficient to produce one break in each strand of the bacterial chromosome and suggested that four or five breaks were repaired for each break left unrepaired in B/r. In order to assess the biological parameters involved in repair, we have used split x-ray exposures and measured survival, based on colony formation, of the two strains, a technique similar to that devised for studying mammalian chromosome breaks. Results indicate that the damage produced by 1 kr to B_{s-1} is left essentially unrepaired, whereas, within 15 min after exposure, the damage produced by 5 kr is corrected in B/r.

Methods and Results. – Exponentially growing cultures of *E. coli* strains B/r or B_{s-1} were exposed to 5 or 1 kr of x rays respectively. They were incubated at 37°C for different periods of time, exposed again to 5 kr (B/r) or 1 kr (B_{s-1}), diluted, and plated on nutrient agar to measure surviving colony formation. Theoretically, if correction of the damage caused by the first x-ray exposure had occurred during the incubation period between the first and second exposure, the second irradiation should lower survival to a level observed after only one exposure. If no repair had occurred, the two exposures should affect survival in an additive manner.

In order to distinguish between these alternatives, the ratio of survivals observed,

$$\frac{\text{first exposure} + \text{second exposure}}{\text{first exposure}} = R ,$$

was plotted. Since the first and second exposures were equal, it is clear that repair of damage produced by the first exposure gives an $R = 1.0$, whereas nonrepair would give values of R less than 1.0. Results indicate that B_{s-1} yields approximately 60% survival after 1 + 1 or 2 kr total exposure and that incubation between exposures does not change the survival ratio significantly. B/r, on the other hand, gives about 60% survival after 5 + 5 or a total exposure of 10 kr when no intervening incubation occurs between exposures. Within 15 min after the first 5 kr, however, the survival ratios rise to near 1.0, indicating that during this time the damage produced initially has been corrected or circumvented. One can also argue that growth between x-ray exposures results in formation of additional target sites, thus changing the cells' sensitivity to irradiation. We are unable to distinguish between these alternatives at this time.

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Plant Physiology and Photosynthesis

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Plant Physiology (20.1–20.6)

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20.1 EVIDENCE FOR DIFFERENT TYPES OF RADIATION-INDUCED ELECTRON SPIN RESONANCES BY SEED HYDRATION AND PHYSICAL CHARACTERISTICS

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Introduction. – Irradiation of seed with x or gamma rays induces an electron spin resonance (ESR) signal in addition to that originally present in unirradiated seeds. In seed irradiation studies it is usually assumed that the radiation-induced signals are eliminated by seed hydration.[2,3] In the course of studies with lettuce seeds, we tested the extent to which the preceding generalization is true. The results led to the finding, here reported, of a seed-hydration-resistant component of the radiation-induced ESR signal. Also reported are physical studies characterizing this component of the radiation-induced signal.

Results and Discussion. – When gamma-irradiated seeds were completely wetted and redried, most, but not all, of the radiation-induced signal was eliminated. The radiation-induced signal that persisted after wetting and redrying was approximately 8% of the radiation-induced signal in unwetted seeds. The signals induced by 5 megarads, both before and after wetting and redrying, had a line width of about 7.4 gauss, whereas the line width of the signals in unirradiated controls was about 6.5 gauss. For doses less than 1 megarad, the radiation-induced signals were no wider than the endogenous signals. No differences in *g* values (2.006 to 2.007) were detected resulting from either irradiation or from wetting and redrying. Studies of power saturation, however, did indicate a difference between the radiation- (5 megarads)-induced resonances that persisted after wetting and redrying vs the radiation-induced resonances that were present in unwetted controls. Relative

to the radiation-induced signal in unwetted control seeds, the radiation-induced signal that persists after hydration and redrying has a maximal signal height at a slightly greater microwave power. In contrast to the radiation-induced signal in unwetted controls, the radiation-induced signal that resists seed hydration also fails to decrease appreciably with increasing superoptimal power levels up to 400 mw. These differences in power saturation cannot be explained as artifacts resulting from the wetting-redrying procedure, because for unirradiated seeds the power saturation curves of wetted-redried and unwetted control seeds are similar, both resembling the radiation-induced signal in unwetted seeds. These results indicate a difference in the molecular environment of the radiation-induced unpaired electrons that persist after hydration vs the molecular environment of radiation-induced unpaired electrons in unwetted seeds. Reirradiation of seeds that had been wetted and redried after receiving 8 megarads was equally effective in inducing resonances as was irradiation of control seeds that had not been irradiated before having been wetted and redried. This indicates that the radiation-induced resonances that persist after hydration and redrying do not alter the subsequent induction of resonances by irradiation. This last finding also suggests that the radiation-induced signal that disappears on seed hydration can be reversibly eliminated and induced by hydration and reirradiation.

In summary, these results with heavily irradiated lettuce seeds provide evidence for two different types of radiation-induced free radicals: (1) a group that can be reversibly eliminated and reinduced by seed hydration and reirradiation, respectively, and (2) a group that persists after seed hydration and redrying and differs from the former group also in characteristics of power saturation.

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20.2 DEVELOPMENTAL STUDIES OF AUXIN EFFECTS ON WHEAT PARENCHYMA GROWING WITHOUT CELL DIVISION

D. E. Foard Sarah A. Angel

Introduction. – The auxin indoleacetic acid (IAA) has been implicated in certain disorganized growths of plants.[1,2] The rapid increase in size of typical plant tumors or calluses is usually accompanied by many mitoses and randomly oriented cell divisions. We are studying the effects of high concentrations of auxin on parenchyma – the main, if not sole, tissue in which plant tumors arise – in order to investigate possible relations between cell divisions and disorganized growth. Thus far we have used parenchyma from two structures in wheat embryos: (1) the epiblast-coleorhiza, which is all parenchyma except for a single layer of epidermis and which normally grows without cell divisions; (2) the cortical parenchyma of the primary root in "gamma-plantlets," seedlings which grow without divisions after exposure of the air-dry grain to large doses of gamma irradiation.

Results and Discussion. – When either irradiated or unirradiated wheat grains are sown on 10^{-3} *M* IAA, the parenchyma of the epiblast-coleorhiza in both cases grows in an excessive, disorganized fashion. Cells of the parenchyma become greatly elongate, and the long axis of the cells appears to be randomly oriented. The cells, moreover, become loosely connected, and in sectioned material appear to float free from one another. The tumor-like condition in the epiblast-coleorhiza can also be induced by auxin after the normal growth of the epiblast-coleorhiza during germination has ceased. The inducibility of this disorganized growth after normal growth has ceased thus strengthens the analogy to tumor induction in plants.[3]

In gamma-plantlets growing on 10^{-3} *M* IAA, the cortical parenchyma of the primary root growing without concurrent cell divisions has features identical with those described for the epiblast-coleorhiza parenchyma. In the cases studied thus far, then, when parenchyma grows in the presence of high auxin concentrations without cell divisions, either naturally as in the epiblast-coleorhiza

or after irradiation as in the root cortical parenchyma, it appears to have similar characteristics of excessive, disorganized growth. Thus, it may be suggested that in typical callus or tumorous growth, the numerous cell divisions and their random orientations are a consequence rather than a cause of the excessive, disorganized growth. Continued cell divisions are necessary for continued abnormal growth as they are for normal growth. The similarity in response to high concentrations of auxin of the epiblast-coleorhiza and the cortex of the primary root growing without cell division suggests a similarity in the morphologic nature of these two structures. This suggestion is in agreement with the interpretation of Pashkov,[4] who postulates that the coleorhiza of the grasses is the true primary root, which has lost its vascular cylinder during the evolution of the group.

Cortical parenchyma in the primary root growing from irradiated grain sown on auxin does not show the disorganized features mentioned above. The cortical parenchyma is indeed intact; however, the long axis of the cells is not parallel to the long axis of the root, as is normal, but perpendicular to it. This is one of the few cases thus far observed of a difference in growth responses between gamma-plantlets and seedlings growing from unirradiated grain. The vascular cylinder in such roots is about twice the diameter of that of roots in untreated plants, and the initiation of lateral roots is frequent.

We have been looking for excessive, disorganized growth of shoot parts and have recently observed macroscopically what we believe to be tumor-like growths of the coleoptile when it is treated with very high concentrations of auxins other than IAA. We plan anatomic investigations of this phenomenon in the near future.

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20.3 BASE COMPOSITION OF NUCLEIC ACID FRACTIONS IN ROOT CELLS OF *VICIA FABA*

John McLeish

Introduction. – Owing to a lack of suitable techniques for the isolation of nuclear and cytoplasmic components from higher plant cells, our knowledge of the nucleic acid base composition in such components is still fragmentary. Recently, however, it has been found possible to obtain cytoplasmic, nuclear, nucleolar, and chromosomal fractions from root tips of *Vicia faba*, and the base composition of the nucleic acids extracted from these has now been studied.

Methods. – Nuclear and cytoplasmic fractions were isolated from the apical 2-mm segments of lateral roots by a method described elsewhere.[1] Some of the isolated nuclei obtained were washed in 0.01 *M* Tris buffer at pH 7.0, transferred to 1.5 m*M* $CaCl_2$, and disrupted by the use of an ultrasonic probe. Under these conditions the extranucleolar chromosomal material disintegrated and dispersed while the nucleoli themselves remained undamaged. Chromosomal and nucleolar fractions could then be separated by appropriate centrifugation.

The DNA, extracted by a method essentially similar to that described by Marmur,[2] was hydrolyzed in formic acid, and the bases were separated by paper chromatography. The acid-insoluble RNA fractions were extracted and hydrolyzed in 0.3 *N* KOH, and the resulting mononucleotides, after purification by precipitation as Ba salts, were separated by paper electrophoresis.

Results. – The nucleolar DNA, identified as such in a previous study,[3] was found to closely resemble the chromosomal DNA in having a high adenine and thymine content and 5-methyl cytosine present as a molar proportion of 5%. The nucleolar, cytoplasmic, and chromosomal fractions of RNA resembled one another in their high guanine and cytosine content, but both the nucleolar and chromosomal fractions were alike in having adenine : uracil ratios which were significantly lower than those found in the cytoplasm.

Discussion. – The fact that the base ratios of the DNA and the chromosomal RNA differ from one another may be of little significance. In *Vicia*, as in many other species of higher plants,

the DNA: nuclear RNA ratio is high,[1] and it is not yet known to what extent, if at all, the base composition of the total DNA is representative of the synthetically active DNA. Similarities in base composition between nucleolar and cytoplasmic RNA fractions have also been reported in some animal cells,[4,5] and these similarities have been widely quoted in support of the theory that ribosomal RNA is made in the nucleolus. However, in making such comparisons, it should be borne in mind that in these animal cells, as in the *Vicia*, the nucleolar and cytoplasmic fractions do differ in certain other respects, and such differences have yet to be explained.

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20.4 SOME EFFECTS OF TEMPERATURE ON THE RNA CONTENT OF *VICIA FABA* ROOT-MERISTEM CELLS

John McLeish

When seedlings of *Vicia faba* are grown at different temperatures, differences in the amounts of RNA can be detected in the cells, nuclei, and nucleoli of the lateral root meristems.

Plants brought to a suitable stage of growth at 20°C were transferred to the 10, 20, and 25°C growth chambers for an additional five days, and then the apical 2-mm root segments were harvested for examination. The amounts of RNA in homogenates from known numbers of whole roots could be expressed on a per cell basis after hemacytometer cell counts had been made on chromic acid macerates of replicate root samples. The methods used in the isolation and counting of nuclei and nucleoli have been described elsewhere[1] and the amounts of RNA in them, as well as the amounts in the whole roots, were determined by a modification of the Schmidt-Thannhauser procedure.[2]

The results, summarized in Table 20.4.1, show that there are disproportionate decreases in RNA content with increased temperature in the three types of sample studied. The figures suggest that changes in temperature may have differential effects on the rates of RNA synthesis or turnover in different regions of the cell, and, in order to test this possibility, the following autoradiographic experiment has been carried out.

Roots labeled for 1 hr in ^{3}H cytidine (3 μc/ml; specific activity of 2 curies/millimole) at the three temperatures shown, were transferred to media containing excess unlabeled cytidine at the same three temperatures. Fixations were then made at intervals up to 12 hr, autoradiographs of squashes and sections were prepared, and grain counts were made over cytoplasm, nuclei, and nucleoli.

At the two higher temperatures the changes in the distribution of grains with time followed the now well-established pattern suggesting a movement of RNA from nucleus to cytoplasm. The distributions with time at 10°C, however, differed in that there was no early peak in the nuclear RNA labeling preceding the rise in the cytoplasmic labeling. This could have been due merely to the presence of a persistent radioactive pool which could not be diluted out effectively at the lower temperature. However, the possibility that there may have been some independent cytoplasmic RNA synthesis has not been entirely ruled out.

Table 20.4.1. Effects of Temperature on RNA Content in *Vicia faba*

Temperature (°C)	Average Amounts of RNA per Cell (g)		
	Total	Nuclear	Nucleolar
	$\times 10^{-12}$	$\times 10^{-12}$	$\times 10^{-12}$
10	169	13	10
20	155	14	6
25	149	6	5

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20.5 MICROSPECTROPHOTOMETRY OF WHEAT NUCLEI

John McLeish D. E. Foard
Helen J. Luippold

Introduction. – Wheat grains that have received heavy doses of gamma radiation can germinate and develop without concurrent cell divisions. Although there is no mitosis in these "gamma-plantlets," certain other types of nuclear behavior characteristic of unirradiated seedlings are found, and autoradiographic studies have revealed nuclear RNA synthesis in roots lacking DNA synthesis. Since the irradiation does not appear to have inactivated all nuclear functions, further investigations of nuclei at various stages of development might be profitable. We have decided to study the quantitative relations between DNA and nuclear histone and have begun exploring the use of microspectrophotometry for this purpose. Thus far we have directed our attention solely to standardizing the techniques of Feulgen and fast-green staining and measurement that would be required.

Results and Discussion. – For test material we have used the lower seminal root primordia from unirradiated wheat embryos and the lower seminal root meristems from two-day-old wheat seedlings. Lower seminal root primordia were chosen since preliminary observations on sections suggested that they might contain a relatively uniform population of nuclei with which populations of nuclei in older roots could be compared. Meristems of the two-day-old roots were used to provide mitotic figures which could be measured in order to establish, for comparative purposes, the 2C and 4C levels of DNA and nuclear histone. Measurements of the amounts of Feulgen and fast-green stains were made in squash preparations with a Canalco ultra-microspectrophotometer using the two-wavelength method with technical aid of R. F. Kimball. Suitable wavelength pairs were selected (511 and 490 mμ for Feulgen and 630 and 582 mμ for the fast-green stain) on the basis of a series of measurements on homogeneous "plugs" of stained nuclei.

In the first series of tests, seeds were imbibed in 0.1 *M* sodium azide (pH 5.7) for 24 hr. The lower seminal root primordia were then dissected out of the embryo before fixing and staining. In confirmation of the preliminary observations, over 95% of the nuclei were found to be 2C with respect to both DNA and histone, the mean stain values corresponding with the mean telophase values obtained from meristems of two-day-old roots fixed and stained concurrently. The remainder fell into the 4C class and had the same average amount of stain as prophases and metaphases. It was possible to make the Feulgen and fast-green measurements sequentially on the same preparations. The Feulgen preparations were destained in hot 5% TCA and then restained with fast green. The same nuclei could then be relocated for the second series of measurements from previously prepared photographs. Unfortunately, during the tests we encountered a large amount of variation both within and between preparations, some of which appeared to be due to the method of fixation and some due to the staining and mounting techniques. These variations have not yet been eliminated and require further study before microspectrophotometry can be used profitably in this material.

20.6 STUDIES ON THE ENDOGENOUS ELECTRON SPIN RESONANCE SIGNAL IN LETTUCE SEEDS

Eddy Noerhadi A. H. Haber

Introduction. – Studies of radiation-induced electron spin resonances in seeds must take into account the occurrence of resonances that occur even in unirradiated seeds. This report deals with the question of whether the endogenous signal is related to the respiratory activity of the seeds previous to dehydration, since certain intermediates in the respiratory chain would be expected to have unpaired electrons. Also reported are some chemical modifications of the ESR signal in unirradiated seeds.

Results and Discussion. – The endogenous signal amounts to approximately 10^{13} spins per seed. Incubation of lettuce seeds for 6 hr in 0.1 *M* $HgCl_2$ reduces the Q_{O_2} from the normal value of about 0.64 μl·seed^{-1}·hr^{-1} to about 0.02 μl·

seed^{-1}·hr^{-1}. When these seeds were redried without washing, however, their endogenous ESR signal was not significantly different from the signal in controls that had not been subjected to $HgCl_2$. After wetted lettuce seeds are boiled, their oxygen consumption is completely eliminated; nevertheless, their endogenous ESR signal is unaffected by the boiling treatment. These results indicate that the endogenous ESR signal in lettuce seeds is not directly related to respiratory or other physiological activity.

Certain chemical treatments, however, were found to reduce the endogenous ESR signal to only about 10% of its original value. These included infiltration with solutions of KCN and K_2S followed by redrying, without washing, before making the ESR measurements. Similar results were found with solutions of KI but not with solutions of KF, KCl, or KBr. None of the salts mentioned in this study itself has an ESR signal when assayed in pure (solid) form in the regions where endogenous signals are normally found.[1] After washing the KI and KCN from the seeds and then redrying the seeds, the endogenous ESR signal returns to normal or near-normal levels. Although these results show that the magnitude of the endogenous ESR signal in lettuce seeds can be chemically altered, further work must be done before we can speculate on the chemical bases for these changes.

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20.7 PHOTOSYNTHESIS

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We are continuing the studies on the delayed light from green plants. Delayed light still seems to be one of the best ways to learn about the energy conversion steps in photosynthesis.

Dr. Bertsch has taken one of our phosphoroscopes to Hill's laboratory in England. He will make a study of the delayed light emitted by chloroplasts while various Hill reactions are taking place. It is too early to have any results.

Our other phosphoroscope is now being used to give emission spectra for both delayed light and fluorescence. The measurements are being made on green plants, blue-green algae, red algae, and on some of the photosynthetic mutants obtained by Dr. Bishop. Some of these spectra have been made before, but our new phosphoroscope gives so much more detail in the spectra that it seems worth while to repeat the older work.

We have completed a study of the decay of various parts of the emission spectrum of delayed light. No differences in the decay were found between one part of the spectrum and another for any of the plants used.

The study on the changes in fluorescence obtained by illuminating *Chlorella* with flashing light has been finished and is ready for publication.

References

[1]Instrumentation and Controls Division, ORNL.

Microbiology

Electron Transport Mechanisms in Microorganisms

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21.1 MALIC-LACTIC TRANSHYDROGENASE

M. I. Dolin

The properties of malic-lactic transhydrogenase[1,2] offer a unique opportunity for studying complex formation between substrate and enzyme-bound pyridine nucleotide. The transhydrogenase has been prepared in homogeneous form from two different species of *Micrococcus lactilyticus*.[3] These enzyme preparations are very similar with respect to the properties that have been examined.

The enzyme-bound NADH is approximately 20 times as fluorescent as free NADH. Preliminary experiments indicate a molecular weight of 115,000 at 3° with dissociation to a lower molecular weight at higher temperatures. Based on the total pyridine nucleotide content, the minimum molecular weight is 30,000 to 40,000. A variety of approaches demonstrate that the NADH is not on the surface of the enzyme, but is shielded in such a way that it is accessible only to substrates and to certain substrate analogs. When the enzyme unfolds in the presence of urea, the quantum yield for fluorescence decays at a measurable rate to that of free NADH, presumably because the NADH is "uncovered" and removed from the environment that causes fluorescence enhancement. The enzyme contains spectrophotometrically detectable phenylalanine, which suggests the presence of a hydrophobic region, but it is not known whether the phenylalanine is responsible for or contributes to the enhanced fluorescence of enzyme-bound NADH.

The fluorescence of the native enzyme is completely quenched on the addition of the oxidant (pyruvate); however, the reducing equivalents initially present in NADH remain bound to the enzyme in some form. Complex formation with pyruvate is completely reversed by dialysis, but the liberation of reducing equivalents from the enzyme takes place only when the oxidant and reductant are present simultaneously. Stopped-flow experiments are planned to determine whether the fluorescent enzyme (E · NADH) and the nonfluorescent complex (E · NADH · pyruvate) are catalytically competent species.

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[3]The author is indebted to E. F. Phares and M. V. Long for isolation and partial purification of the transhydrogenase used in recent work and to A. P. Pfuderer and D. R. Holladay for the analytical ultracentrifuge studies.

Biochemical Regulation

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22.1 PARTICIPATION OF PROTEIN HORMONES IN CONTROL OF THE SYNTHESIS OF A "STEROID INDUCIBLE" ENZYME IN LIVER

F. T. Kenney Joseph Kendrick
L. E. Roberson

Introduction. – Induction of the tyrosine transaminase of rat liver by adrenal glucocorticoids has been studied intensively in this and other laboratories for several years. Results of these studies can be summarized in a statement which appears to contain internal contradictions: the adrenal steroids promote a limited general increase in hepatic RNA and protein synthesis and large increases in the rate of synthesis of tyrosine transaminase and of a few other enzyme proteins. A major advance in resolving this apparent contradiction was made when Garren, Howell, Tomkins, and Crocco found evidence for the participation of specific repressors of the synthesis of those enzymes that are induced by glucocorticoids.[1] We have confirmed that a selective repression of tyrosine transaminase synthesis can be elicited and have found that initiation of this repression is a function of the hypophyseal growth hormone. The evidence thus far indicates that growth hormone acts on transcriptional processes to effect formation of a repressor which selectively inhibits transaminase synthesis at the translational level. In addition, we have found that tyrosine transaminase can be "induced" by yet another hormone, insulin.

Results and Discussion. – As described in a recent publication,[2] when adrenalectomized rats are subjected to severe stress, the hepatic tyrosine transaminase level falls with a half-life of 2.5 hr. Because this is the normal turnover time in maintenance of the steady-state level in vivo, the result suggested that transaminase synthesis had been reduced essentially to zero. This was confirmed by direct immunochemical assay, wherein the extent of ^{14}C-leucine incorporation into the enzyme during a brief pulse is measured by precipitating the enzyme with a specific antiserum. Stress is ineffective in hypophysectomized rats, implicating the pituitary in this repression. This implication was strengthened by the demonstration that stressing the pituitary-bearing members of parabiotic pairs caused repression in their livers and in the livers of joined hypophysectomized members. After some searching it was found that the pituitary growth hormone is the active principle in the initiation of the repression phenomenon. Fully active growth hormone preparations[3] are active in eliciting transaminase repression at doses as low as 40 μg/100 g, so there is little reason to suspect contamination. No other hormone tested, of pituitary or nonpituitary origin, is capable of causing transaminase repression.

When sufficient actinomycin D[3] is given to inhibit RNA synthesis 50 to 60% and to block transaminase induction by glucocorticoids almost completely, the transaminase level stays constant for at least 5 to 6 hr. Since the transaminase is known to be undergoing turnover with a half-life of $2\frac{1}{2}$ hr, this result indicates that this enzyme is synthesized on stable templates. This being so, the repression of enzyme synthesis that is controlled by growth hormone must take place at the translational level. However, the effect of growth hormone is itself blocked by actinomycin. We interpret this finding as indicating that growth hormone brings about an alteration of transcriptional events, resulting in formation of a substance (repressor) capable of selectively inhibiting transaminase synthesis at the ribosomal or translational level.

It is well established that, in overall physiological effects, the glucocorticoid hormones are catabolic and act in opposition to the anabolic growth hormone. In recent years it has become apparent that the roles of these hormones are reversed when their effects on various physiological parameters of liver metabolism are analyzed; that is, growth hormone is catabolic, and the glucocorticoids act anabolically, in liver. It is therefore particularly satisfying to see this antagonistic relationship operating in the control of the synthesis of liver enzymes, with growth hormone acting to repress the synthesis of the enzymes induced by glucocorticoids, thus blocking the growth response of the liver to the steroid hormones. Further experimentation will be required in order to determine if the selectivity of enzyme induction by the glucocorticoids reflects their capacity to overcome the selective repression controlled by growth hormone.

The repression phenomenon has been difficult to reproduce with regularity, even when administering growth hormone of known potency to fully adrenalectomized and hypophysectomized rats. After several potential variables were tested and rejected, it became apparent that an "inducing" capacity remains in the absence of adrenal steroids. Insulin secretion is known to be stimulated in growth-hormone-treated rats, and upon testing this hormone we found insulin to be an effective "inducer" of tyrosine transaminase. Transaminase levels are increased three- to fivefold in adrenalectomized-hypophysectomized rats given 1 unit or more of insulin, and the effects of growth hormone are not detectable when both hormones are given simultaneously. The effect of insulin is blocked by actinomycin, and thus the process appears to be a true induction, but we are now testing immunochemically to determine if enzyme synthesis is actually enhanced. Rats made diabetic by administering alloxan respond to growth hormone very well; that is, transaminase repression is immediate and complete and thus far appears to be completely reproducible. We are now hopeful that routine use of diabetic rats will provide us with an adequate experimental system in which the mechanism of the growth-hormone-initiated repression can be studied further.

The mechanism of "induction" by insulin also remains to be elucidated. Like the steroid induction, the insulin effect is blocked by actinomycin D and thus appears to involve transcriptional processes. However, the effects of insulin and of hydrocortisone are additive, and the kinetics of the response to each hormone differ significantly. From this we infer that the two hormones act by different mechanisms.

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22.2 HORMONAL CONTROL OF ENZYME LEVELS IN THE REUBER HEPATOMA IN VITRO

F. T. Kenney
R. L. Tyndall[1]
L. E. Roberson
Joseph Kendrick

Introduction. – As we have pointed out before,[2] the availability of relatively unaltered rat-liver cells which grow in culture and which retain the

ability to respond to hormones is of great significance in the study of normal regulatory mechanisms and of neoplasia. Insofar as these cells respond in a normal fashion, they offer a system in which the role of various hormones in regulating enzyme synthesis can be studied individually and independently of the enormously complex in vivo environment. On the other hand, when instances are found in which the response of the hepatoma cells is lacking or abnormal, the possibility exists that an alteration peculiar to the neoplastic state has been uncovered. With these aims we are continuing our introductory studies on the hormonal regulation of the synthesis of tyrosine-α-ketoglutarate transaminase in culture.

Results and Discussion. – Response of the culture cells to hydrocortisone (10^{-6} *M*) is like that of the normal liver, except in one important respect which will be discussed later. Addition of insulin to the culture medium (10 milliunits/ml) results in an elevation of the transaminase level that resembles the normal liver response in all respects. Thus, the hormonal effects that bring about a "growth response" appear to function normally in the hepatoma cells, and the in vitro culture system can be employed to study these responses without the complications that becloud in vivo experiments.

Opposing these growth-promoting effects of insulin and glucocorticoids in the normal liver is the capacity of growth hormone to initiate repression of enzyme synthesis. We have not yet been able to detect this effect of growth hormone in experiments with the cultured hepatoma cells. Inability to respond to growth hormone could be due to a number of factors, all of which will be examined in future experiments. The possibility exists that these results reflect an inability of the neoplastic cells to form repressors and to thus limit growth. This is strengthened by the observation that the high transaminase levels induced by hydrocortisone are maintained for at least 36 hr in the cultured hepatoma cells. In the normal liver, the enzyme level falls off after reaching a peak at 6 to 8 hr after hormone treatment; this rapid decline of the transaminase level in vivo has been attributed[3] to the action of a specific repressor.

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22.3 RNA SYNTHESIS IN FETAL RAT LIVER

W. D. Wicks

We have extended our studies on RNA synthesis in fetal rat liver by the use of the modified thermal phenol-extraction technique described previously.[1] Three fractions of RNA have been obtained from livers of 20-day fetuses using this procedure. The first fraction, obtained by extraction of the fetal liver with 0.14 *M* NaCl and phenol at 3°, contained better than 90% of the total liver RNA and exhibits all the properties of a mixture of ribosomal and transfer RNA's. Subfractionation by the selective precipitation of ribosomal RNA with 1 *M* NaCl yielded the same pattern observed previously with adult liver RNA.[2] Both subfractions exhibited only slight incorporation of ^{32}P after 15 min exposure to the isotope, but much more extensive labeling occurred after 30- and 60-min exposures. Base-composition analyses revealed a preponderance of isotope in GMP and CMP with little or no indication of turnover of the terminal adenylate of transfer RNA. Further studies are planned to verify this point. All the ^{32}P-RNA was located in the 4 S region of glycerol density gradients in both subfractions at 15 min, but the 18 S and 28 S ribosomal RNA components showed distinct labeling at 30 and 60 min after the injection of ^{32}P. This represents a more rapid appearance of the isotope in ribosomal RNA than has been seen in the adult.

The second RNA fraction was obtained by extraction at 45° with salt and phenol and contained only about 5.7% of the total RNA of fetal liver. In a glycerol gradient, two main RNA peaks at 18 S and 28 S were observed with a minor component at 4 S. This fraction incorporates a very large amount of isotope even at 15 min. In a gradient, a fairly sharp peak of ^{32}P-RNA occurred during labeling periods of from 15 to 60 min. Base-composition studies revealed that this fraction is almost exactly the same chemically and isotopically, being particularly rich in GMP and CMP. We take this to be the ribosomal RNA precursor fraction.

The third RNA fraction was obtained by extraction at 80° with 0.14 *M* NaCl, phenol, and 0.5% sodium dodecylsulfate. Less than 5% of the total liver RNA is found in this fraction, and it labels very heavily even after only a 15-min exposure to isotope. The RNA pattern in a gradient is heterogeneous in the very dense regions, with a small peak occurring at 18 S and a major one at 4 to 8 S. The profile of ^{32}P-RNA is very heterogeneous at 15 min with a peak at 4 to 10 S. At later intervals, a broad peak of isotope appeared in the 20- to 24-S region, with lesser amounts occurring in all other regions. This fraction is quite similar chemically and isotopically, being rich in AMP and UMP, with a nearly perfect DNA-like composition.

Further studies are planned to elucidate more clearly the kinds of RNA represented by the last two fractions. Also, the effect of gestational age on the observed patterns of labeling and other parameters is being investigated.

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22.4 CHARGING OF TRANSFER RNA'S WITH AMINO ACIDS IN ADULT AND FETAL RAT LIVER

W. D. Wicks

Because no significant turnover of the –CCA terminus of fetal transfer RNA (tRNA) was noted in recent experiments, it became of interest to examine the ability of adult and fetal tRNA preparations to become charged with certain amino acids. For this purpose partially purified tRNA was prepared from adult and fetal livers by a modification of the technique of Brunngraber.[1] Sephadex-treated 105,000 × *g* supernatant fractions were used as sources of amino-acid-activating enzymes.

When assayed with freshly prepared supernatants, there was no marked difference between the rate or extent of charging with leucine and phenylalanine by all four possible combinations of adult and fetal tRNA's and supernatants. Upon standing at –20°, however, the ability of the fetal supernatant enzymes to charge leucine with either adult or fetal tRNA was largely lost, while that obtained with the identically prepared and treated adult preparation was not changed. This large difference was not noted with phenylalanine, however, suggesting the existence of differences in the stability of adult and fetal leucyl synthetases on the one hand and between the fetal leucyl and phenylalanyl synthetases on the other. These data were not collected under the most ideal kinetic conditions, however, and similar experiments are now being conducted under more suitable circumstances to more clearly reveal these differences which have been noted. Further experiments are also planned to examine more closely the nature of these differences and their possible relationship to the process of development.

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22.5 THE MECHANISM OF THE IN VITRO INACTIVATION OF TYROSINE α-KETOGLUTARATE TRANSAMINASE FROM RAT LIVER

D. D. Holten F. T. Kenney

Introduction. – A rapid increase in the rate of synthesis of rat liver tyrosine α-ketoglutarate transaminase is induced by adrenal steroids, with a maximal level of enzyme being obtained 6 hr after injection of the steroid. From 6 to 12 hr after steroid injection, the enzyme undergoes degradation with a half-life of 2.5 hr. Any understanding of the control of enzyme levels in vivo must include knowledge of the mechanism of enzyme degradation as well as enzyme synthesis since the steady-state level of an enzyme is a function of both parameters.[1]

We have recently described an in vitro system obtained from the soluble fraction of rat liver[2,3] which shows an amino-acid-stimulated inactivation

of tyrosine α-ketoglutarate transaminase. Results with this system have shown that certain amino acids, which form complexes with pyridoxal phosphate, render the enzyme unstable to basic conditions at 37°C; this can be prevented by pyridoxal phosphate, binding to the catalytically active site of the transaminase. Results using partially purified transaminase containing ^{14}C-amino acids have shown that no trichloroacetic acid–soluble peptides result during the inactivation of the enzyme at pH 8.7 and 37°C, suggesting that the loss of activity under these conditions resulted from instability of the apotransaminase rather than degradation of the transaminase by proteolytic enzymes. Investigations into the mechanism of the pyridoxal phosphate stabilization of tyrosine transaminase have established the role of sulfhydryl groups on the enzyme in maintaining a stable protein in vitro and perhaps also in vivo.

Results and Discussion. – The rate of inactivation of apotyrosine transaminase at 37°C as a function of pH indicated that the ionization of an ionizable group on the enzyme with a p*K* value of 8.6 resulted in instability of the enzyme. The possibility that this was a sulfhydryl group[4] (p*K* 8.3 to 8.6) was tested by reacting the enzyme with *p*-hydroxymercuribenzoate (PHMB) in the presence and absence of pyridoxal phosphate. Pyridoxal phosphate was found to protect the enzyme from inhibition with PHMB just as this coenzyme protects the enzyme against inactivation at pH 8.7 and 37°C. These facts taken together indicate that the ionization of a sulfhydryl group on the transaminase results in the enzyme being unstable at 37°C unless pyridoxal phosphate is bound to the active site of the enzyme. The pyridoxal phosphate may maintain the protein in a stable conformation when the sulfhydryl group is ionized or may protect the sulfhydryl group from ionization by causing a conformational change which masks the group within the folds of the enzyme. Perhaps the most plausible explanation, however, is that the pyridoxal phosphate itself masks a sulfhydryl group on the active site of the enzyme. Inorganic phosphate (P_i), which is a competitive inhibitor for pyridoxal phosphate, does not protect the enzyme from inhibition by PHMB, but does protect the enzyme from inactivation at pH 8.7 and 37°C. This may indicate that P_i which is bound to the pyridoxal phosphate site on the enzyme does not have enough bulk to protect the sulfhydryl group from reacting with PHMB, but causes enough conformational change to stabilize the enzyme.

Tyrosine α-ketoglutarate transaminase is unique among transaminases in that it has a very short half-life and is only partially saturated with pyridoxal phosphate in vivo. The results reported here suggest that rapid turnover in vivo may be a consequence of the inability of this enzyme to bind pyridoxal phosphate as tightly as other transaminases can.

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22.6 STUDIES ON THE MECHANISM OF PYRIDOXINE INDUCTION OF TYROSINE α-KETOGLUTARATE TRANSAMINASE IN RAT LIVER

D. D. Holten F. T. Kenney

Introduction. – Greengard and Gordon[1] have reported a threefold increase in the level of tyrosine-α-ketoglutarate transaminase in the livers of adrenalectomized rats following the injection of pyridoxine. The increase in transaminase level was sensitive to puromycin, was insensitive to actinomycin D, and was preceded by an increase in the level of pyridoxal phosphate in the liver. The authors proposed that pyridoxal phosphate resulting from the injection of pyridoxine increased the rate of transaminase synthesis at the translational level. The report that a similar actinomycin-insensitive increase in tryptophan pyrrolase following tryptophan injection resulted from a protection of tryptophan pyrrolase from degradation[2] suggested to us that the increased levels of tyrosine transaminase following pyridoxine injection in adrenalectomized rats might result instead from a

protection by pyridoxal phosphate of the transaminase from degradation. This possibility was strengthened by the finding that pyridoxal phosphate protected the transaminase from inactivation in vitro.[3] However, as previously reported,[4] we found that the injection of pyridoxine did not stabilize the transaminase in vivo (the half-life of tyrosine transaminase was the usual 2.5 hr after pyridoxine injection). The increased levels of the transaminase due to pyridoxine were found to result from an increased rate of synthesis of the transaminase which was, contrary to the report of Greengard and Gordon, sensitive to actinomycin D. The increased rate of transaminase synthesis thus required RNA synthesis. This suggested that pyridoxine initiates events which increase the rate of transaminase template formation at the transcriptional level. A similar mechanism of transaminase induction has been proposed for hydrocortisone.

Results and Discussion. – The specificity of pyridoxine induction of rat-liver enzymes was examined by measuring the response to pyridoxine of other enzymes which respond rapidly to hydrocortisone but which do not require pyridoxal phosphate as a cofactor. One of those enzymes examined was phosphoenolpyruvate carboxykinase. This enzyme does not increase after the injection of pyridoxine. Results with a second enzyme, tryptophan pyrrolase, have been inconsistent due to the method of assay. However, a new method for assay for this enzyme is being employed in the hope that more consistent results will be obtained.

Current investigations in this laboratory (see Sect. 22.2, this report) show that insulin also causes increased levels of tyrosine transaminase in rat liver. These increases in transaminase levels were inhibited by actinomycin D and reached a maximum within 3 to 4 hr after insulin injection. These similarities between pyridoxine and insulin induction suggested that pyridoxine might increase the transaminase levels by causing the release of insulin from the pancreas, which in turn induces the formation of the enzyme. We have tested this possibility by injecting pyridoxine into adrenalectomized rats which had been made diabetic with alloxan. In these animals, pyridoxine did not induce the transaminase but rather decreased the level of the enzyme. This decrease could result if pyridoxine had caused a stress in these animals resulting in the release of growth hormone. Growth hormone has been shown to repress the formation of tyrosine transaminase. In the hypophysectomized rat, pyridoxine induced transaminase formation. Thus pyridoxine does not appear to act by interfering with the production or action of growth hormone. The failure of pyridoxine to induce the transaminase in diabetic rats suggests that insulin is required for the action of pyridoxine; this possibility is currently being investigated.

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Cooperative Programs

AEC-NIH CHEMICAL CARCINOGENESIS (23.1–23.21)

Biochemistry of Carcinogenesis (23.1)

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Enzymology of the Carcinogenic State (23.2–23.5)

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Mammalian Chemical Carcinogenesis

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Chromosomal Effects of Chemicals and Radiation (23.6)

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Effects of Radiation and Chemicals on Paramecium (23.7)

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Biophysics of Carcinogenesis (23.8–23.10)

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Chemical Mutagenesis in Microorganisms (23.11, 23.12)

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Inhalation Carcinogenesis (23.13–23.21)

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Mary Lou Williams

AEC-NIH ZONAL ULTRACENTRIFUGE DEVELOPMENT (23.22–23.25)

Tumor Virus Isolation Studies (NCI) (23.22, 23.23)

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Respiratory Virus and Viral Subunit Isolation (NIAID) (23.24, 23.25)

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AEC-NASA SPACE BIOLOGY (23.26–23.28)

Space Biology – High Energy Proton Studies

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Space Biology – Biosatellite (23.26, 23.27)

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Space Biology – Gemini Shots (23.28)

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AEC-NIH Chemical Carcinogenesis

23.1 STUDIES OF THE MECHANISM OF ACTION OF THE SHOPE PAPILLOMA VIRUS

Stanfield Rogers J. H. Evans

Metabolic comparisons of the tumors in wild and domestic rabbits induced by the wild-type virus and Shope's recoverable (domestic-rabbit) virus have been continued.

Histones. – It was previously reported[1] that the virus-induced tumors, in contrast to hyperplastic, normal skin, or coal-tar-induced papillomas, have a deficiency in arginine-rich histones, this deficiency being apparently related to the presence of large amounts of the specific virus-induced arginase in these papillomas. Histone extraction and polyacrylamide gel electrophoresis have recently demonstrated that, although normal rabbit liver and normal skin have three major histone bands, the histones extracted from the virus-induced papilloma have only two. The histone bands of rabbit skin are currently being isolated and chemically and immunologically characterized. Concurrently, rabbits inoculated with the virus, together with necessary controls, are being treated as indicated with supplemental parenterally injected arginine and canavanine, this latter an arginase antagonist. Such treatment has previously been shown to result in a cessation of growth of the tumors without demonstrable ill effects on the rabbits per se.[2] It is hoped to find whether the third histone band will then appear in the papillomas of the treated animals.

Threonine Aldolase. – It was reported in a previous progress report that Shope's recoverable-line virus obtained from domestic rabbits induces the enzyme threonine aldolase in wild rabbits, in contrast to the wild-type virus which does not induce this enzyme. This ability is lost usually upon one passage of the virus in the wild rabbit, as is its recoverability from domestic-rabbit tumors.[3] The purification of domestic-rabbit-liver threonine aldolase is under way as a prerequisite of the purification of wild-rabbit-papilloma threonine aldolase. This latter is to be purified to cast light on the question as to whether the enzyme induced is synthetically virus information, that of the wild-rabbit epithelium merely unmasked, or whether the enzyme is the result of domestic-rabbit genetic information perhaps transduced to the wild rabbit by the virus. A domestic-rabbit antigen has previously been found in wild-rabbit tumors induced with virus obtained from the domestic rabbit, that is, Shope's recoverable line.[4]

Efforts to Selectively Modify the Genetic Information Carried by Viruses. – Attempts thus far to

add polynucleotides to the Shope virus DNA, using Bollum's end terminal addition enzyme, have failed. This appears to be almost necessarily related to the fact that the Shope virus DNA is a continuous rather than a linear helix. Because of this, Ochoa's polynucleotide phosphorylase has been also concentrated and purified in order to make the attempt with an infectious RNA. It is planned to add polyuridine to the end of tobacco mosaic virus RNA or to poliovirus RNA, whichever of the two is first available, and hopefully both virus systems.

The Shope Papilloma Virus: A Passenger in Man. – This work, reported in the immediately previous progress report, is being continued with consistent results. In view of the nature of the material, we delayed definitive publication until the results could be reproduced elsewhere. Dr. John Davenport, of the John L. Smith Memorial for Cancer Research of the Charles Pfizer Company, under Public Health Service Contract No. PH 43-66-98, in a "blind" run confirmed our previous results. This was accomplished through the cooperation of Dr. C. A. Evans, University of Washington, Seattle, and Dr. W. R. Bryan, National Institutes of Health, Bethesda, Maryland. These results provide a model justifying extensive investigations of the use of passenger viruses to specifically treat genetic deficiency diseases. It had been hoped that the Shope virus could be used in the therapy of argininosuccinuria since it lowers the blood arginine both in a significant number of people who have worked with the virus and in rabbits upon intravenous injection. We have learned, although an arginine-deficient diet has been the empirical treatment of this disease, that the blood arginines are already on the low side.

Miscellaneous Problems. – In conjunction with John Trentin of the Baylor University School of Medicine, studies are being made of the mechanism of action of the adeno-12 virus. Both amino acid and nucleic acid precursor intermediary metabolism are under study. Efforts are also being made to find whether the papilloma virus or its infective DNA will infect HeLa cells. This is being done in conjunction with Alfred Hellman of the Biology Division's Inhalation Carcinogenesis group.

The comparative physical and chemical properties of wild-type and Shope's domestic-rabbit recoverable line are under study. The behavior of the two virus lines in cesium chloride is quite different. The protein coat of the recoverable line is stripped off by the concentrations of cesium chloride used to study the density of the wild-type virus. Electron photomicrographs made by Miss Frances Ball and Mr. Harris of the Biological Separations group at K-25 of pelleted wild-type and recoverable viruses have revealed no resemblance in structure. Their pictures of wild-type virus are identical with those of the literature. On the other hand, no detectable capsomeres were found among the population of particles, most of which were roughly of a size similar to wild type, in the pellets derived from recoverable-line material. Efforts are currently being made to purify the recoverable line, using glycerine gradients proven to be effective for wild type. Following this, the characteristics of the DNA's and peptide analysis of the protein coats of the virus lines will be compared.

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23.2 A SOLUBLE AMINO-ACID-INCORPORATING SYSTEM FROM TROUT LIVER

Lawrence Rosen G. David Novelli

Introduction. – In vitro studies of amino acid incorporation into protein have shown that a high-speed supernatant (S_{200}) and a particulate fraction are needed. However, anomalous incorporations have been noted that do not require particulate fractions. Such systems are of two types: (1) those that require an intermediate aminoacyl-tRNA and have been reported in *E. coli*[1] and rat liver,[2] and (2) one which does not require an intermediate aminoacyl-tRNA and is found in *A. faecalis*.[3] The physiological significance of these systems has not been demonstrated.

It has been found that certain amino acids are incorporated into a hot-TCA-stable fraction by the S_{200} fraction derived from trout liver. The incorporation of phenylalanine in this system was further investigated.

Results and Discussion. – The incorporation of phenylalanine requires ATP and is insensitive to RNase since incorporation is not through the intermediate formation of phenylalanyl-tRNA. Other differences demonstrated by this system from the typical particle-dependent system are the following: (1) no sharp Mg^{2+} optimum, (2) no requirement for transfer enzymes, and (3) relative temperature independence from 20 to 37°. The latter difference is probably related to the second difference since the transfer enzymes are rapidly inactivated at 37°.

These findings indicate that the present system may be related to the one reported for *A. faecalis*, and further investigations are being carried out.

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23.3 STUDIES CONCERNING PROTEIN SYNTHESIS IN NORMAL AND CANCEROUS TROUT LIVER

L. C. Waters[1] E. L. Murray
G. David Novelli

Introduction. – The capacity for incorporating amino acids into protein in the absence of ribosomes has been demonstrated in microorganisms[2,3] and rat liver.[4] Experiments designed to ascertain the presence or absence of such a system in trout liver were made. In addition to providing for a study of its biological significance, such a system would also provide an additional parameter for comparing trout hepatomas.

A preliminary study investigating the role of methylation of nucleic acids (tRNA in particular) as possible control mechanisms in the regulation of protein synthesis in normal and cancerous trout livers was begun.

Results and Discussion. – In the absence of ribosomes, ^{14}C-labeled amino acids were incorporated into protein-like material at a very low level. In the trout liver system, as in the other known soluble incorporating system, there was a degree of specificity toward certain amino acids. Phenylalanine, tyrosine, and proline were incorporated at a significantly higher level than others tested.

Unlike the other systems the fish system was not stimulated by RNA preparations; nor was it sensitive to RNase. Treatment of a complete amino-acid-incorporating system (i.e., plus ribosomes) with RNase reduced the incorporation to a level identical to that of the soluble system alone.

There appeared to be no significant differences between normal and hepatomatous livers with regard to their soluble amino-acid-incorporating ability. A marked lack of stability in the trout liver system makes it a poor choice for investigation of the biological role(s) for such systems.

In vivo labeling experiments with ^{14}C-methionine (methyl labeled) suggested that the predominant nucleic acid species being methylated in normal trout liver was tRNA. However, even after 48 hr of labeling, the radioactivity was at such a low level as to make valid comparison of normal vs cancerous tissue not feasible.

Another approach to the problem is now under way. Using reverse-phase chromatography as developed by Kelmers *et al.*,[5] we are now attempting to separate the precharged ^{14}C-aminoacyl-tRNA's, as there is evidence[6] that this column is capable of separating sRNA's differing possibly only in the extent of methylation. Preliminary results indicate that, at room temperature and at a pH of 4.5, phenylalanyl-tRNA can be clearly resolved from a mixture of aminoacyl-tRNA's. However, others such as tyrosyl-, prolyl-, threonyl-, and lysyl-tRNA's are poorly resolved from each other. It is anticipated that, by altering the chromatographic conditions, a better resolution can be obtained and a comparative study between normal and cancerous trout livers can be made.

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23.4 STUDIES ON HEPATOMATOUS TROUT

E. L. Murray G. David Novelli

Introduction. – After studying the various characteristics of control fish, it was hoped that we could experiment with hepatomatous fish and find basic biochemical differences between the two types of fish.

Results and Discussions. – We needed a "marker" that would distinguish between the hepatomatous and control fish – and not kill the fish. The obvious start was with serum protein concentrations. However, the levels varied in different fish. The level in the hepatomatous fish was only slightly higher than that of the control, and this was not consistent. Gel-electrophoresis patterns were different in that two bands were more concentrated in the hepatomatous fish, but we had no consistent standard to check against.

Scarpelli *et al.*[1] found tremendous differences in lactic acid dehydrogenase (LDH) activities. There was a 300-fold increase in activity in the hepatomatous fish. We tried this assay several times and could find absolutely no differences between the fish. Our values were reproducible, and the control values were much higher than the control values of Scarpelli.

The gel-electrophoresis patterns of the LDH were quite interesting in that the most active bands seemed to be the opposite of the active bands in mice for several organs. No differences in the electrophoretic patterns of control and hepatomatous fish could be found.

Scarpelli also found that the demethylase activity increased a great deal in hepatomatous fish. Once again, we did not find this to be the case.

We cannot explain the fact that our data differ so significantly from those of Scarpelli's. Perhaps the control fish were different in some aspect – maybe age. Or, it could be that we were using fish with advanced and less active tumors, whereas Scarpelli was using fish with initial but rapidly developing tumors. There is the added possibility, since these fish had a kidney disease and had been treated with all sorts of antibiotics, that the metabolism of the tumors had been altered.

Currently we are exploring the acute effects of dimethyl and diethyl nitrosamines on liver metabolism.

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23.5 DEVELOPMENT OF TISSUE CULTURE LINES FOR STUDIES ON CHEMICAL CARCINOGENESIS

Kiki B. Hellman G. David Novelli

Introduction. – This report represents the initiation of a new laboratory and another approach in research in the Chemical Carcinogenesis Program. Endeavors of the past three months have seen the establishment of a cell and organ culture laboratory. The general areas of biochemistry dealing with normal and tumor tissue maintained and propagated in vitro will be investigated. Tissues from teleost sources, as well as those of mammalian origin, will be of particular interest in this study.

Results and Discussion. – One phase of the study dealing with the propagation of stable cell lines for subsequent biochemical evaluation is currently in progress. The L-M cell,[1] derived from L strain clone 929 (a fibroblast of mouse origin),[2,3] is being routinely maintained in medium 199 of Morgan *et al.*,[4] supplemented with 0.5% peptone (medium 199–peptone). This cell strain can also be propagated in a simple, chemically defined medium.[5] Addition of 0.12% methyl cellulose to medium 199–peptone permits the growth of cells in suspension as monodisperse cultures. This attribute, as well as the ability to grow in serum-free media, thereby utilizing a defined substrate, makes this an ideal cell system, particularly suited to metabolic studies since the evaluation of data derived will be greatly simplified. An area of

particular interest deals with the possible alterations in RNA, as well as in other nucleic acids, due to the effect of tumor-inducing agents such as 20-methylcholanthrene.

Studies will not only be limited to the L-M cell system but, due to certain biological attributes, will also include the KB cell line. This epithelial cell was originally isolated by Eagle[6] from a human epidermoid carcinoma of the mouth and is currently being established in this laboratory. It would be of interest to compare and/or contrast studies undertaken, utilizing these cell systems under controlled conditions.

The other phase of this study deals with the development of hepatoma in the rainbow trout and the elucidation of the possible biochemical changes taking place in the normal trout liver which may lead to its development. The growth in vitro of cells from both normal liver, as well as those of the hepatoma, would greatly simplify such a study. Therefore, attempts will be made to initiate cell lines from primary sources and to maintain and propagate the cell lines established as stock cultures in the laboratory. These cultures will then be utilized for comparative biochemical evaluation and analogy with in vivo studies.

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23.6 AMINOACYL-sRNA SYNTHETASES FROM NORMAL AND NEOPLASTIC HUMAN CELLS

J. D. Regan

Human neoplasms exhibit elevated rates of growth and protein synthesis. Multiple synthetase–multiple sRNA systems have recently been demonstrated in microbial systems.[1] It is possible that a comparative study of synthetases and sRNA's from normal and carcinomatous human cells may reveal differences in the ability of synthetases to recognize specific sRNA's; one aspect of the "dedifferentiation" of the cancer cell could conceivably be manifested in this fashion.

Synthetase and sRNA preparations have been made from the HeLa[2] cell grown in suspension (without antibiotics) and from human liver. The ability of these synthetases to charge homologous sRNA and heterologous sRNA's from *E. coli, Neurospora,* yeast, rat liver, and *Tetrahymena* with several amino acids has been demonstrated.

A quantity of human liver sRNA sufficient for fractionation into specific sRNA's has been prepared. The HeLa system has been less amenable to quantity sRNA isolation. However, in collaboration with Alfred Hellman, HeLa is now propagated in mass cultures to yield several hundred grams of HeLa cells sufficient for fractionation of sRNA's.

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23.7 MITOSIS AND MUTATION INDUCTION BY TEM[1]

R. F. Kimball — Barbara R. Bussell
E. G. Bailiff — Stella W. Perdue[2]

A previous report[3] has shown that premutational damage induced in postduplication interphase (G_2) cells of *Paramecium aurelia* remains reparable through mitosis. No evidence for the fixation of any damage during mitosis was found. A further exploration of the mitotic period was carried out by treating synchronized groups of cells with TEM at various closely spaced intervals throughout the period from late G_2 through division into early G_1. Samples of each group were fixed at the time of the TEM treatment for determination of the nuclear stage. The data show that there is an approximately linear increase in mutation yield from G_2 through mitosis into G_1. Earlier data[4] had shown that the mutation yield with this same mutagen

increases throughout G_1 until S, when a maximum is reached.

These data are entirely consistent with the hypothesis that TEM produces a single kind of premutational damage that is subject to repair at a constant or nearly constant rate throughout the cell cycle and is fixed as mutation at the time of DNA synthesis and at no other time. Some small portion of the mutation yield might result from initial damage with other properties, for example, chromosomal breaks rejoining at times other than S to form aberrations, but the contribution from such other forms of damage must be small. This uniformity of the premutational damage induced by this chemical mutagen contrasts with the behavior of x-ray-induced premutational damage discussed elsewhere in this report.[5]

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23.8 A POSSIBLE METHOD FOR SOLVING PROTEIN STRUCTURES[1]

J. R. Einstein

The use of uranium as a heavy-atom anomalous scatterer is suggested as the basis of a method for solving protein structures. This method would require only one crystal form. The Hönl corrections for uranium are very large for Cu $K\alpha$ and Cr $K\alpha$, with an unusually large difference between the two $\Delta f'$ values. If uranium were present in relative proportion suitable for the heavy-atom method itself and if data were collected for *hkl* and $\overline{hkl}$ with both wavelengths, there would, in general, be very large differences between the four amplitudes. Phase determination would then be powerful, as required for data of (usually) only moderate accuracy and of limited resolution. Probability functions for a "best Fourier" may be derived from a combination of the heavy-atom and the anomalous-scattering methods and from consideration of the measurement errors.

In order to determine the positions of a number of heavy atoms in the presence of a great many light atoms, a Patterson function for the heavy atoms *alone* may be calculated. The four measured amplitudes depend on only three independent parameters: the structure amplitudes for protein and for uranium (uncorrected), F_P and F_U, and their relative phase. There is a twofold ambiguity in the determination of F_U from the four amplitudes. However, it may be shown that one value is, in general, much more probable than the other, so that the ambiguity is not serious. In order to minimize the effects of this ambiguity and of measurement errors, a "best Patterson" (analogous to the "best Fourier" for electron density) may be calculated. With relatively few peaks within a large unit-cell volume, it should be possible to solve the Patterson for the uranium positions; this implies a limitation on the size of the protein molecule.

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23.9 COMBINATION OF ISOMORPHOUS REPLACEMENT AND ANOMALOUS SCATTERING DATA IN PHASE DETERMINATION FOR PROTEIN CRYSTALS

J. R. Einstein

The probability distribution for the structure factor of the parent crystal is derived more rigorously than in earlier work.[1] Only independent probabilities are multiplied, while probabilities related to mutually dependent errors are convoluted. Use of the new expression in calculation of the centroid may give rise to significant differences.

Consider a parent crystal isomorphous with a series of derivatives differing by the addition or substitution of heavy atoms or groups, where N derivatives are assumed to contain only normal scatterers and N' derivatives contain heavy-atom anomalous scatterers. The pertinent quantities for one reflection include (1) for the parent: the measurement F_0 of the structure amplitude; (2) for each (jth) "normal" derivative: $\mathbf{f}_j$, the calculated contribution of the heavy group to the structure factor, and F_j, the measured structure amplitude; (3) for each (kth) "anomalous" derivative: $\mathbf{f}_k$, the calculated contribution of the heavy group, uncorrected for anomalous scattering, and F_k^+, F_k^-, the measured amplitudes for hkl and $\overline{hkl}$. Probability distributions are defined as two-dimensional functions over the complex plane, not just on the circle $F = F_0$. The resultant distribution $p(F, \phi)$ is given by the product

$$p_0 \left(\prod_1^N p_j \right) \left(\prod_1^{N'} p_k' \right),$$

where p_0 (for the parent) relates to the error in F_0, p_j (for a "normal" derivative) relates only to errors in $\mathbf{f}_j$ and F_j, and p_k' (for an "anomalous" derivative) relates only to errors in $\mathbf{f}_k$, F_k^+, and F_k^-. In the earlier treatments the probability distribution for the parent effectively multiplies that for *each* derivative rather than appearing only once, as above. An unduly large weight is thereby given to the measurement F_0 (ref. 2). In the present treatment for an "anomalous" derivative, the distribution related to errors in $\mathbf{f}_k$ is convoluted with, rather than multiplied by, the distribution related to errors in F_k^+ and F_k^-. The computation of the centroid may be reduced to one-dimensional integrations and is then not much more time consuming than with earlier probability expressions.

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23.10 AN ANALYTICAL METHOD FOR SINGLE-CRYSTAL EQUATORIAL-PLANE DIFFRACTOMETRY WITH A PLANE MONOCHROMATOR

J. R. Einstein

With the usual experimental conditions for neutron diffraction, reflections tend to be poorly resolved for crystals with large unit cells (cell molecular weights on the order of several thousand and up), despite the use of a plane monochromator. The feasibility of determining structures of molecules of biological interest such as large peptides and proteins by neutron diffraction depends on a proper analysis of the methods of diffractometry. The factors which determine the spread and intensity distribution of the diffracted beam include crystal size, shape, and mosaicity, spectral dispersion of the source, and size, shape, and mosaicity of the monochromator crystal. The problem is considered first in two dimensions, disregarding the direction perpendicular to the equatorial plane, in which diffraction from both the monochromator and specimen crystals is assumed to take place. An analytical method was previously developed for the case with no monochromator.[1] This method has now been adapted and extended so as to take into account the monochromator and its parameters. With the use of this method it is possible to predict (for an idealized case) the fraction of the integrated intensity measured with any experimental procedure (any scan range, etc.) and also the fraction of the integrated intensity of any neighboring reflection measured simultaneously, that is, the degree of overlap.

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23.11 IDENTIFICATION OF THE GENETIC ALTERATIONS IN ETHYL METHANESULFONATE–INDUCED *ad-3B* MUTANTS OF *NEUROSPORA*

H. V. Malling F. J. de Serres

Ethyl methanesulfonate (EMS) is a potent mutagen in procaryotes, and studies in T4 bacteriophage have shown that EMS reacts preferentially

with guanine to produce (1) transitions via errors in replication, (2) transversions by deletion of the ethylated purines and replacement by either cytosine or thymine, or (3) single base-pair deletions resulting from copy errors where the ethylated purines have led to "depurination."[1] EMS is also a potent mutagen in eucaryotes, and is potentially useful for both applied and theoretical studies because mutations can be obtained without any appreciable inactivation. However, the genetic alterations in EMS-induced mutations in eucaryotes have not been identified at the molecular level. Studies on nitrous acid–induced mutants in *Neurospora* showed that this can be accomplished readily among *ad-3B* mutants in tests for allelic complementation and specific reversibility.

Results. – Earlier attempts to characterize EMS-induced mutations in *Neurospora* showed that about 53% (50/94) of the *ad-3B* mutants showed allelic complementation[2] and that 5/17 *ad-3B* mutants revert after treatment with nitrous acid (NA).[3] In the present experiments, specific reversibility was studied with O-methylhydroxylamine (Me-O-HA), nitrous acid (NA), ethyl methanesulfonate (EMS), and a monofunctional quinacrine mustard (ICR-170) (Table 23.11.1). Mutants classified as base-pair substitutions reverted after treatment with at least one of the first three compounds, whereas mutants classified as base-pair insertions and deletions reverted only after treatment with ICR-170 or, in some cases, also after treatment with EMS.

By comparison of the reversion frequencies induced by HA and Me-O-HA in the same mutants,[4] the conclusion was reached[4] that Me-O-HA, like HA, preferentially induces reversions in those mutants which revert by a GC-AT transition. Since the same doses of Me-O-HA are not as toxic as HA, higher doses can be used, and the reversion frequencies can be more accurately determined. Mutations resulting from base-pair substitutions can be characterized with greater accuracy as to whether they revert by a GC-AT or an AT-GC transition. Fifty-nine percent (55/93) of the EMS-induced mutants have been identified as base-pair substitutions (BPS), and 62% of the BPS (34/55) revert by an AT-GC transition. In phage these same percentages were 70 and 80%, respectively, and are in good agreement with the present data.

In *Neurospora* the EMS-induced base-pair transition mutants differ markedly from those induced

Table 23.11.1. Total Number of Mutants and the Percentage Distribution of the Different Genetic Alterations Induced by EMS Leading to Mutants with Polarized (P) and Nonpolarized (NP) Complementation Patterns and Noncomplementing Mutants (NC)

Complementation Pattern	Genetic Alterations								
	Identified			Unknown					Total
	Transitions		Insertions or Deletions	Revert Only Spontaneously	Non-revertible	Only Revertible with EMS	High Spontaneous Reversion Rate	Not Tested	
	AT at Mutant Site	GC at Mutant Site							
	Number of Mutants								
NP	7	12	0	8	2	1	2	7	39
P	5	0	1	2	1	1	2	0	12
NC	22	9	4	2	3	1	1	0	42
Total	34	21	5	12	6	3	5	7	93
	Percentage of Mutants								
NP	18	31	0	20	5	3	5	18	100
P	42	0	8	17	8	8	17	0	100
NC	53	21	10	5	7	2	2	0	100
Mean percentage	37	23	5	13	6	3	5	8	100

by NA; in the latter sample only 15% have an AT pair at the mutant site.[4] In addition, a lower percentage of complementing mutants was found among EMS-induced mutants (53%) than among the NA-induced mutants (82%). These two observations agree with the hypothesis[5] that an AT pair at the mutant site gives rise to mutants with polarized complementation patterns or to noncomplementing mutants more often than a GC pair at the mutant site does.

Only 5% of the mutants have been identified as base-pair insertions and deletions. These may result from a deletion of the ethylated purines from the DNA by hydrolysis. During replication this gap may then lead to a loss of a single base pair.

Thirteen percent of the mutants revert only spontaneously, and the highest percentage of these are found among mutants with nonpolarized complementation patterns. This suggests that this class of mutants results from base-pair substitutions.[2] Transitions are easily characterized by their ability to revert by NA, EMS, or HA, but transversions cannot as yet be identified. On the basis of present data it seems that mutants which only revert spontaneously in the present tests are more likely base-pair substitutions resulting from transversions than other types of genetic alterations.

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23.12 MUTAGENICITY OF TWO POTENT CARCINOGENS: DIMETHYLNITROSAMINE AND DIETHYLNITROSAMINE IN *NEUROSPORA CRASSA*

H. V. Malling

Dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) possess potent carcinogenic activities in many rodents. The carcinogenicity of the compounds is organ and species specific and depends on whether they are administered orally or whether they are injected.[1] DEN and DMN are presumably metabolized, with the intracellular formation of a methylating and an ethylating agent, respectively, before becoming tumorgenic. DMN and DEN are mutagenic in *Drosophila*[2] but are without mutagenic activity in bacteria, and, until recently, were believed to be nonmutagenic in *Neurospora.*[3]

All the suggestions[4] about the active metabolic product involve an initial enzymic oxidative N-dialkylation of the nitrosamine. The lack of the mutagenicity in bacteria and in *Neurospora* may be because these organisms are not able to carry out this oxidation. This oxidation process[5] can, however, be carried out in an enzyme-free hydroxylase model[6] consisting of ascorbic acid (20 m*M*), EDTA (sodium salt) (2 m*M*), and Fe^{2+} as $FeSO_4$ (1 m*M*), all of which were dissolved in phosphate buffer at pH 7.4 (0.06 *M*). A direct approach to determine whether mutagenic compounds are formed during this oxidation would be to study the mutagenicity of DEN and DMN dissolved in this hydroxylase-model system. Since the oxidation should occur in an atmosphere of oxygen,[5] the effect of the nitrosamines and the buffer alone can be studied by bubbling through with nitrogen instead of oxygen. By comparison of oxygen and nitrogen bubbling, we should be able to study the mutagenicity of the oxidation products.

The mutagenicity was measured as the ability to induce reversions in a nitrous acid–induced *ad-3B* strain. This strain was selected among 13 NA-induced mutants which revert after treatment with NA; the mutant which reverted with the highest frequency after a 2-hr treatment with Udenfriend[6] buffer and 0.1 *M* DEN at 36°C was chosen for this study (mutant No. 2-17-68).

Oxygen markedly increases the toxicity of DMN, whereas the same effect is absent for DEN. Treatment with DEN in an oxygen atmosphere gives a slightly lower survival than the same treatment in a nitrogen atmosphere in this and repeated experiments. Treatment with DMN + oxygen gives the highest toxicity of all the treatments, suggesting that methylation occurs at a higher rate than the ethylation.

DEN treatment of *Neurospora* conidia in oxygen showed a marked increase in the frequency of reverse mutation (Fig. 23.12.1), whereas the increase obtained with oxygen is much lower but is

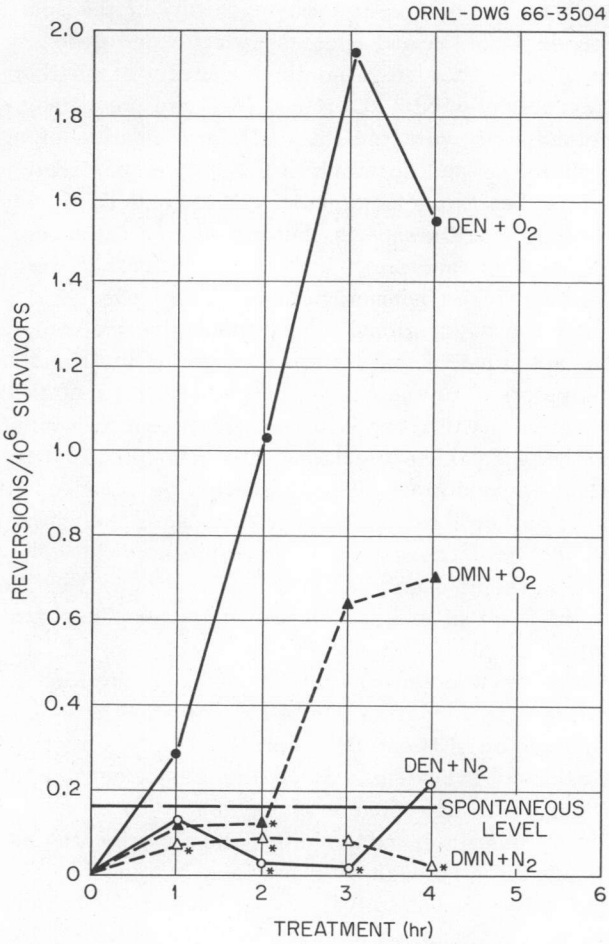

Fig. 23.12.1. Effect of Dimethylnitrosamine (DMN) and Diethylnitrosamine (DEN) with Bubbling of Either Oxygen or Nitrogen Through the Reaction Mixture on the Reversion Frequency of *Neurospora* Conidia. The reaction mixture contains 0.1 mole per milliliter of either DEN or DMN, dissolved in Udenfriend's[6] hydroxylase model system. Points in which the reversion frequency was not significantly higher than the control on the 5% level are marked by asterisks. The statistical test is done according to Birnbaum.

still significantly higher than the control (spontaneous reversion frequency: 0.15 per 10^6). However, since the number of reversions per initial number of conidia only exceeded the number of spontaneous reversions by 26%, an increase in reversion frequency after treatment with DMN due to selectively killing cannot be completely discounted.

With this reservation in mind, it would appear from these data that DEN and very probably DMN are mutagenic in *Neurospora*, but only under conditions which are believed[5] to give rise to carcinogenic metabolic products by enzymatic action in the liver of both rats and mice.

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[3]H. Marquardt, R. Schwaier, and F. Zimmermann, *Naturwissenschaften* **50**, 135 (1963).

[4]K. Y. Lee, W. Lijinsky, and P. N. Magee, *J. Natl. Cancer Inst.* **32**, 65–76 (1964).

[5]R. Preussmann, *Arzneimittel-Forsch.* **14**, 769–74 (1964).

[6]S. Udenfriend *et al.*, *J. Biol. Chem.* **208**, 731 (1954).

23.13 INHALATION CARCINOGENESIS

D. G. Doherty
M. G. Hanna, Jr.
Alfred Hellman
Paul Nettesheim
R. F. Newell

The various parameters essential to inhalation chamber operation such as mouse strain, bacteriological and viral background, viral infection techniques, radiation exposure, and dust and chemical dispersion in the chambers have all been established in the previous report.[1] Our current efforts are being directed toward loading the chambers and beginning the exposure to the inhalants according to the protocol for the experiment. Three chambers, containing initially 540 $BC3F_1$ mice each, are currently being exposed to chromic oxide dust, ozonized gasoline, and washed air respectively. Half the animals in each chamber have been subjected to 100 r of x radiation four weeks prior to exposure to the inhalants. Appropriate bacteriological, virological, and pathological samples have been taken from all chambers at the time of startup. The total exposure in the chambers is shown in Table 23.13.1.

Table 23.13.1. Inhalation Chamber Exposure Conditions and Time

Chamber	Treatment	Concentration (ppm)	Hours of Exposure
I	100 r of x ray		52
II	100 r of x ray + Cr_2O_3	53	129
		27	138
III	100 r of x ray + ozonized gasoline	Ozone, 1; gasoline, 30	154

In a preliminary experiment, mice exposed to chromic oxide dust for 200 hr at 50 ppm showed severe epilation. The dust level was reduced to 27 ppm, and the mice are now showing signs of recovery. Similar epilation was observed in the ozonized-gasoline chamber and, to a much lesser extent, in the washed-air chamber. Comparable control animals living in plastic pans show no signs of hair loss, so that the mice must be reacting to the stress of being in the chambers for 5 hr a day. This hair loss is aggravated by the presence of chemicals in the environment, and, for this reason, the concentration of dust in chamber II was cut to 27 ppm. Within the next three months, mice that have received radiation and viral pretreatment will have been placed in chambers and their exposure to environmental agents will have begun.

References

[1]D. G. Doherty *et al.*, *Biol. Div. Semiann. Progr. Rept. July 31, 1965,* ORNL-3853, p. 196.

23.14 MORPHOLOGIC CHANGES IN SPLEEN LYMPHATIC TISSUE DURING ANTIBODY PRODUCTION

M. G. Hanna, Jr. D. C. Swartzendruber
C. C Congdon[1]

Histologic and autoradiographic studies on spleen lymphatic tissue demonstrate progressive shifts of affected cell populations in response to antigen stimulation. An early and characteristic feature of this reaction is the occurrence of large pyroninophilic cells throughout the spleen. The realization that these cells originate in the white pulp of the spleen and subsequently migrate to the red pulp has provided a perspective for studying the kinetics of their rate of growth and their transformations.

Autoradiographic studies in mice injected with sheep erythrocyte antigen have shown that an early response within the spleen white pulp is increased proliferation of cells in germinal centers. A dissociation or loss of germinal centers during the first 24 hr after antigen, resulting in a migration of large pyroninophilic cells from the center throughout the white pulp, has also been described. It is agreed by most investigators that these large pyroninophilic cells are the precursors of plasma cells.

The cellular proliferation and differentiation in lymphatic tissue before and during productive antibody response provide an excellent experimental system for study on an ultrastructural level. Several investigators have examined antigenically stimulated lymphatic tissue with the electron microscope and described the fine structural changes during maturation of the plasma cells. However, very little consideration has been given to alterations in germinal centers, and the emphasis has not been on the primary events during the first 24 hr or the inductive phase of the immune reaction.

It is the purpose of these studies to correlate electron-microscopic studies with histologic observations of mouse spleen lymphatic tissue during the inductive phase, as well as the antibody-productive phase, of the immune response.

Materials and Methods. – Male $BC3F_1$ mice were injected intravenously with 1 ml of 10% sheep erythrocyte suspension. Different groups of mice were killed at 2-hr intervals during the first day and at days 2, 3, 4, 5, and 6 after antigen injection.

Spleens were removed and bisected. One half of each spleen was fixed in Bouin's solution for hematoxylin and eosin staining, and the other half was fixed in Carnoy's solution for methyl green–pyronin staining. Spleens to be studied with the electron microscope were fixed with either acetate Veronal–buffered 2% OsO_4 (pH 7.2) or 3% glutaraldehyde buffered with cacodylate, pH 7.2, and postfixed in OsO_4. Fixed tissue was embedded in Epon. Alternate thick and thin sections were cut from the Epon blocks. The thick sections were stained with methylene blue and used for gross reference in a light microscope. Thin sections were stained in aqueous uranyl acetate prior to examination in a Siemens Elmiskop I electron microscope.

Discussion. – Histologic and autoradiographic studies suggest that germinal centers in normal animals are in a steady state in which cells are entering and leaving each compartment at a constant rate. An intravenous dose of sheep erythrocyte antigen above a concentration of 1% results in a marked disruption of the steady state, with an increased number of dividing cells and an increase in the number of cells migrating from the germinal centers into the associated lymphocyte mass and red pulp. This has been termed germinal center dissociation, resulting in a dissociative growth of these cells outside the centers. This results in a temporary atrophy or loss of germinal centers during the first 24 hr after antigen injection, with recovery and hyperplasia of the centers occurring at later intervals. We have also shown that the dissociation of germinal centers is antigen dose dependent, with a direct relation existing between duration of germinal center loss or atrophy and increasing antigen dose. Atrophy is only achieved at higher SRBC-antigen exposures (> 0.1% SRBC suspension), which are capable of drastically altering the flux of cells from the centers. With doses lower than 0.1% SRBC suspension, germinal centers respond by hyperplasia alone.

In the present study the predominant, large blast cell type in the white pulp 24 hr after antigen administration is like the cell observed in the germinal centers at 4 hr after antigen. Both these cell types are characterized by a paucity of cytoplasmic structures and an abundance of free ribosomes. However, the large cells observed at the periphery of the white pulp at day 2 are characterized by their content of polyribosomes. The fact that the large cells at day 2 after antigen have a remarkable development of polysomes may be a demonstration of an intermediate step in the differentiation of immunologically competent cells prior to the extensive development of endoplasmic reticulum. Thus it can be speculated that the responsive cells characterized by abundant, freely dispersed ribosomes are in an early phase of protein synthesis which would result in the production of specific protein (antibodies) exemplified by the development of highly organized polysomes in the cytoplasm. Concurrent with the development of a highly organized system of rough-surfaced endoplasmic reticulum in large numbers of plasma cells at day 3, the serum antibody titer is in the log phase of production.

We feel that our previous autoradiographic and histologic studies suggest that in the mouse spleen lymphatic tissue reaction to SRBC antigen there are both a dynamic and a morphologic relation between the cells of spleen white pulp germinal centers and the large pyronin-staining cells which proliferate in the lymphatic nodules in response to antigen. This relationship is further supported by the fact that the cells in the secondarily hyperplastic centers beginning on day 3 after antigen more closely resemble the large cells that were present in the lymphatic nodules at day 2 than the original primitive cells that were observed in the centers 4 hr after antigen. The significance of this finding needs further investigation. However, by the criteria presented in this report, these cells are more differentiated than primitive in that they have a more highly organized cytoplasm than the large cells observed in the germinal centers 4 hr after antigen injection, and perhaps represent committed cells. In this sense, it is possible to envisage the cells of the secondarily hyperplastic centers as "memory" cells which retain the machinery for protein synthesis and stock the germinal center with cells which react quickly to subsequent encounters with antigen.

References

[1]Mammalian Recovery section.

23.15 PHYSIOLOGY OF THE VIRUS-HOST INTERACTION

Alfred Hellman D. H. Martin
J. G. Farrelly

The therapeutic properties, as well as the purported penetration capabilities, bestowed on dimethyl sulfoxide (DMSO) were studied by us in an effort to further delineate factors associated with viral infection as reflected by the physiological status of the host. The host system for the three types of attenuated, as well as three types of virulent, polioviruses was a strain of the Gey HeLa cell line. We have studied factors associated with DMSO's cytocidal property and its influence on virus synthesis. At low concentrations (1% or higher), DMSO is cytocidal and appreciably reduces RNA synthesis. However, it required greater than 60% DMSO to directly inactivate poliovirus. We were able to distinguish between the attenuated and virulent strains of polioviruses, taking advantage of the more rapid synthesis of the latter prior to the cytotoxic effects of DMSO on the host system. We were unable to demonstrate any enhancement of viral adsorption, penetration, or synthesis in the presence of DMSO or in cells previously exposed to DMSO. The opposite was observed; DMSO tends to be inhibitory to poliovirus synthesis.

In a related study, the influence of constant ionic environment in an in vitro host system is continuing to be investigated in an effort to determine the validity of existing viral data obtained in vitro and then related to intact animals. We have determined, using a variety of parameters previously described, that a constant ionic environment of the host does alter one of these parameters. Though we have not been able to demonstrate any significant direct influence of pH differences from 6.8 to 7.6, we have observed that the maintenance of a constant pH, irrespective of the range thus far studied, in fact does influence total RNA synthesized over one growth cycle of the cell population. The type of RNA so influenced will be investigated, as well as the influence of constant pH on the parameters leading to virus release.

We are continuing our study relating the influence of age and radiation dose on virus infection and pathogenesis.[1]

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[1]A. Hellman, D. H. Martin, and J. G. Farrelly, *Biol. Div. Semiann. Progr. Rept. July 31, 1965*, ORNL-3853, p. 203.

23.16 AMINOACYL-sRNA SYNTHETASE SYSTEMS OF VIRAL-INFECTED CELL SYSTEMS

Alfred Hellman J. D. Regan

Possible alterations induced by cytopathic and noncytopathic viruses in several tissue aminoacyl-sRNA synthetase systems are currently under investigation. Synthetase and sRNA preparations have been made from uninfected HeLa and polio-infected HeLa cell cultures at various time intervals after viral infection. The ability of these synthetase preparations to charge homologous and several species of heterologous sRNA's has been demonstrated.

Preliminary results of particular interest are: (1) a considerably greater charging activity of the HeLa cell synthetase with polio-infected HeLa sRNA's than that found with HeLa cell sRNA's and (2) an apparent higher nuclease activity present in the polio-infected HeLa cell synthetase preparation than that found in similar synthetase preparations of uninfected HeLa cells.

Since it is possible that viral infections may induce new sRNA,[1] we are currently propagating HeLa cells in mass culture; these will be subsequently infected with poliovirus so that sRNA's of such viral-infected cells may be isolated and fractionated.

To determine possible biochemical alterations induced by noncytopathic viral infections, we are also investigating synthetase and sRNA's of normal and oncogenic virus-infected tissues.

References

[1]N. Sueoka and T. Sueoka, *Proc. Natl. Acad. Sci. U.S.* **52**, 1535–40 (1964).

23.17 IMPROVEMENT IN THE DIFFUSION CHAMBER TECHNIQUE FOR CYTOKINETIC STUDIES OF ANTIBODY RESPONSE

Paul Nettesheim Carol J. Chadwick[1]

Introduction. – The Millipore diffusion chamber technique modified by Capalbo *et al.*[2] for studies of antibody response proved to be of great value in the cytokinetic analysis of antibody response. However, unbiased cell sampling from the chambers was difficult because of the clot that regularly forms in the chambers. Previous attempts to dissolve the clot with various enzymes were unsuccessful.

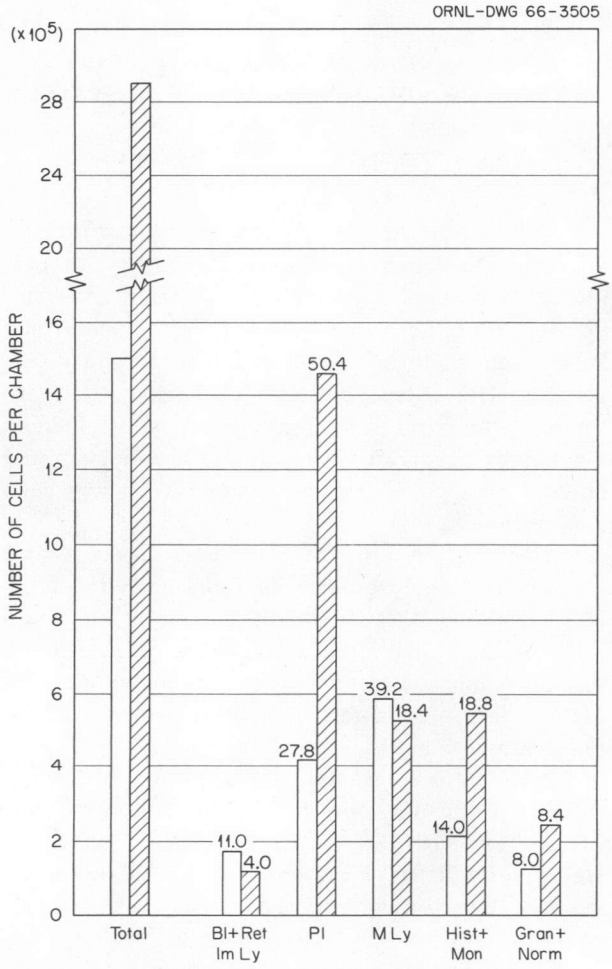

Fig. 23.17.1. Effect of Pronase Treatment on Cell Yield, and Differential Cell Count of 8-day Chamber Cultures. Each chamber was inoculated with 25×10^6 primed spleen cells and 2.5×10^6 rat RBC. Chambers were immersed either in PBS □ or in pronase ▨ for 1 hr at room temperature under constant shaking. Bl = blast cells; Ret = reticulum cells; Im Ly = immature lymphocytes; Pl = plasma cells; M Ly = mature lymphocytes; Hist = histiocytes; Mon = monocytes; Gran = granulocytes; Norm = normoblasts. Numbers on top of columns are percentages.

Methods and Results. – Encouraged by reports from various laboratories of the successful use of pronase in tissue culture, we tried to dissolve the chamber clot with this enzyme. Chambers containing 25×10^6 primed spleen cells and the test antigen were implanted into the peritoneal cavity of sublethally irradiated isologous recipients. On day 8 after culture the chambers were removed from the recipients. Half of them were incubated in phosphate-buffered saline (PBS) and the other half in 0.5% pronase for 1 hr at room temperature. Pronase treatment resulted in digestion of the chamber clot. The total number of cells and the number of different cell types per chamber were determined, and the results of one such experiment are summarized in Fig. 23.17.1. The total number of morphologically intact cells recovered per chamber was doubled by pronase treatment, the number of plasma cells was tripled, and the number of histiomonocytes, normoblasts, and granulocytes was doubled. Only an insignificant number of cells was detected on the inner surface of the chamber membranes after pronase treatment.

Summary. – Cell recovery was almost completed with the use of pronase. Thus, for the first time, this permits us to make unbiased samplings of the chamber cultures.

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23.18 DOES BENZO(*a*)PYRENE BIND TO BACTERIAL DNA IN VIVO? IN VITRO?

A. P. Harrison, Jr.

Ts'o and Lu[1] described in vitro covalent bonding of benzo(*a*)pyrene (BP) with thymus DNA as a result of photoradiation at 320 to 390 mμ. The

irradiation was carried out under nitrogen to prevent damage to DNA which occurs in the presence of atmospheric oxygen (photodynamic effect). It would be of interest to know whether similar binding can be observed in vivo. I have attempted to ascertain this with *Escherichia coli*.

One hundred milliliters of menstruum containing 10^{12} bacterial cells, their protein labeled with ^{14}C-leucine, was exposed to irradiation at approximately 360 mμ for 2 hr. The incident dose rate was 150 ergs mm^{-2} sec^{-1}. The suspension was bubbled with oxygen-free nitrogen for $\frac{1}{2}$ hr prior to irradiation, and bubbling was continued during irradiation. (The menstruum was 0.03 *M* phosphate buffer, pH 7.1, containing 1 μg/ml of ^{3}H-BP in colloidal suspension.) An identical suspension was held in the dark to serve as a control. The cells were harvested, washed with 75% ethanol-ether to remove as much BP as possible, and were lysed with Duponol. The extracted DNA was purified and deproteinized as described by Marmur.[2] Each time the DNA was spooled, it was passed through three changes of ethanol-ether prior to subsequent solubilization and deproteinization. Moreover, prior to each deproteinization the solubilized DNA was assayed for DNA (Dische test), for protein (^{14}C label), and for BP (^{3}H label). The results from these assays were plotted as counts in 50 min (as ordinates) vs the number of deproteinizations (as abscissas).

Observe in Fig. 23.18.1 that the ^{3}H label is lost at the same rate as the ^{14}C label and at a much faster rate than DNA loss. From this and other experiments, I concluded that BP is preferentially bound to protein and that this protein must hinder binding to DNA. We deal here, of course, with gross association of hydrocarbon with macromolecules; a proportionately small, though biologically important, binding with DNA would not be detectable in such an experiment.

In vitro experiments were carried out as follows: DNA was extracted from ^{14}C-labeled cells. It was purified to the extent of removing RNA and most protein, but some ^{14}C label was left on it, so that after irradiation at 360 mμ with ^{3}H-BP, both the ^{14}C and ^{3}H labels could be monitored after a series of deproteinizations just as in the in vivo experiments. The results were the same as in the in vivo experiments – a number of deproteinizations sufficient to decrease the ^{14}C to background reduced the ^{3}H label to background and at the same rate, although DNA was still

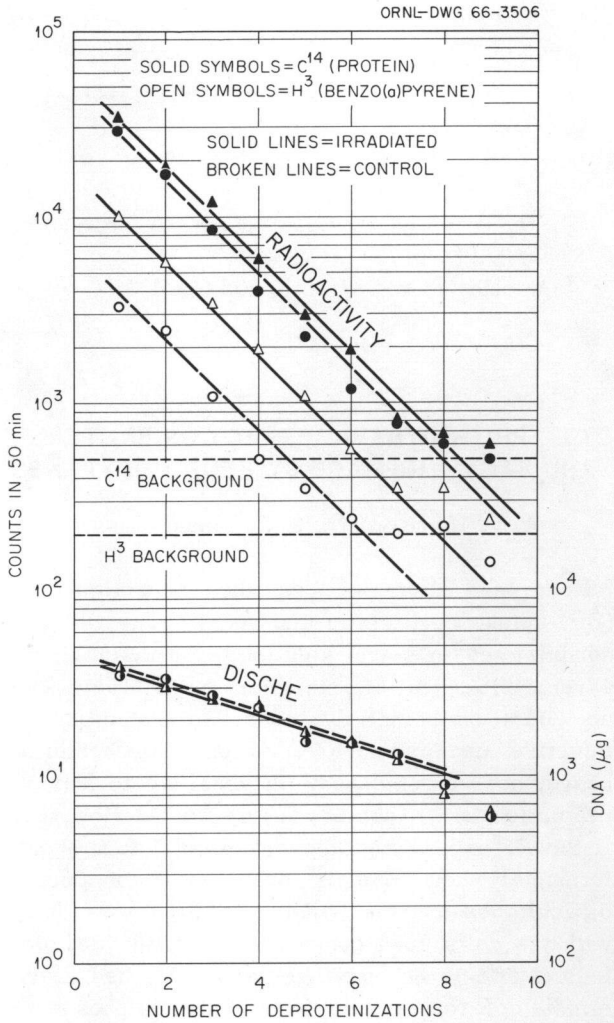

Fig. 23.18.1. Loss of Benzo(*a*)pyrene Concomitant with Deproteinization of DNA. The small DNA loss at each deproteinization is due largely to manipulation, imperfect pipet recovery at the interface after centrifugation.

available. Apparently, BP has a strong affinity for protein. Thus, binding of BP to bacterial DNA could not be demonstrated in these experiments. However, binding to protein was observed, and photoradiation increased this binding (Fig. 23.18.1).

These results do not necessarily conflict with those of Ts'o and Lu. Their thymus DNA was reported to be protein free at the outset, and they dealt with highly denatured DNA. However, it remains to be demonstrated whether in vivo biological effects brought about by BP with near uv

are due to *direct* action on DNA or are due to indirect action on DNA through the agency of the associated protein.

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23.19 PHOTODYNAMIC EFFECT ON BACTERIA AND BACTERIOPHAGE BY BENZO(*a*)PYRENE

A. P. Harrison, Jr. C. Dianne Cass

Moore and Harrison[1] described benzo(*a*)pyrene (BP) uptake by bacteria and yeast. The BP was administered in serum solution. Surprisingly, no adverse biological effects were noted: There was no effect on bacterial growth, no diminution in infective centers upon infection with bacteriophage, nor were the bacteria sensitive to 360 mμ irradiation in air (photodynamic effect). However, if the BP is administered in colloidal form – unfortunately this renders quantitation of uptake difficult because the particulate BP comes down with the cells upon centrifugation – adverse biological effects become detectable. Washed *Escherichia coli* from exponential cultures in phosphate buffer, pH 7.1, containing 1 μg/ml of BP lose viability in a characteristic way. No loss of viability occurs if either the BP or the irradiation is omitted.

Observe in Fig. 23.19.1 that the bacterial survival curve has a distinct "shoulder" of $1\frac{1}{2}$ hr duration. Cells from this shoulder show increasing lags when subcultured in fresh medium. Thus we seek to determine whether the shoulder is a manifestation of a cellular repair mechanism and if so, the nature of this repair. Observe also in the figure that decline in free phage and infective centers is identical and that the latter occurs "on the shoulder" of the bacterial survival curve. The following questions arise: Is the photodynamic effect on free phage the result of damage to the DNA injection mechanism or the result of direct action on phage DNA? If the latter is the case, does the destruction of infective centers represent action by BP already within the host

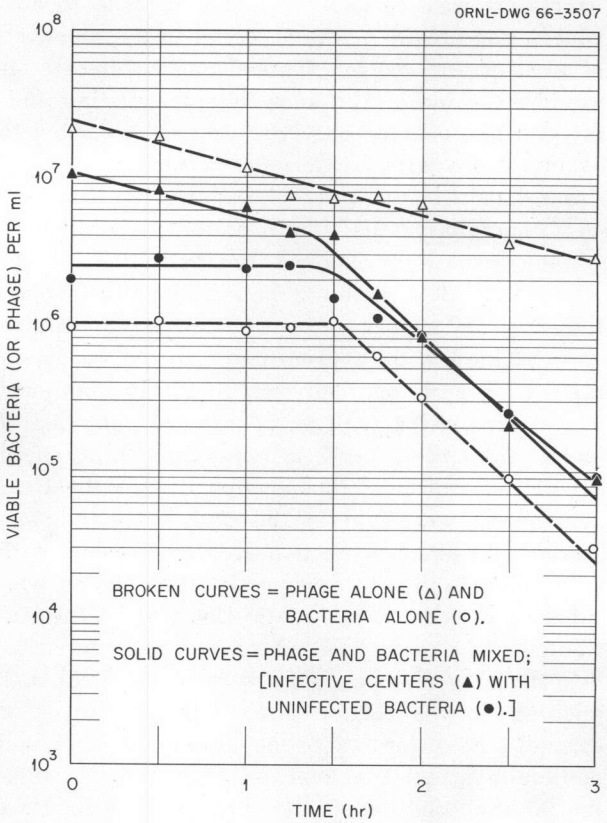

Fig. 23.19.1. Survival of Bacteria and Bacteriophage upon Photoradiation at 360 mμ at 50 ergs mm^{-2} sec^{-1} in Presence of Benzo(*a*)pyrene.

cell or does infection render the bacteria more permeable to BP? This question, in turn, relates to the nature of the shoulder of the bacterial survival curve. We seek also to determine whether there is a correlation between carcinogenicity of a hydrocarbon and its potency as a photodynamic agent.

References

[1]B. G. Moore and A. P. Harrison, Jr., *J. Bacteriol.* **90**, 989 (1965).

23.20 CONTINUING STUDIES WITH THYMINELESS BACTERIA

A. P. Harrison, Jr. Vivian E. Raabe

Harrison[1] has described the thymineless bacteria derived by the aminopterin method[2] and has compared them with their double mutants and with

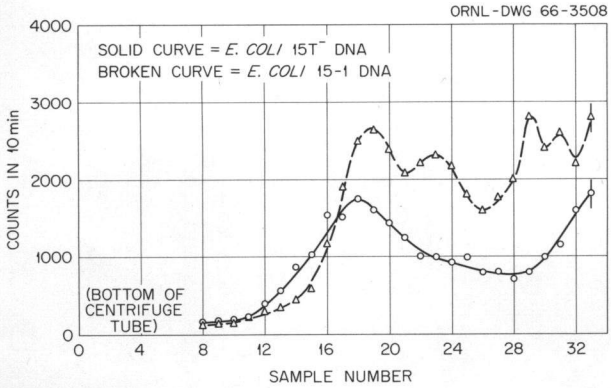

Fig. 23.20.1. DNA (and Derivatives Therefrom) from Thymineless Bacteria as Determined in Sucrose Gradients. Centrifugation was at 30,000 rpm for 90 min at 5°C.

Escherichia coli strain 15T⁻. Two characteristics of the aminopterin-derived auxotrophs are a 20-fold higher thymine requirement than 15T⁻ and diphasic growth. The DNA formed by the thymineless bacteria is being investigated with the aid of sucrose gradients.[3] The bacteria were cultivated in ^{3}H-thymine to label their DNA.

Observe in Fig. 23.20.1 that whereas strain 15T⁻ forms one band (normal DNA) in the sucrose gradient, strain 15-1 (an aminopterin-derived high thymine requirer) forms additional bands nearer the top of the gradient. We wish to determine the nature and origin of these additional bands.

References

[1]A. P. Harrison, Jr., *J. Gen. Microbiol.* **41**, 321 (1965).

[2]T. Okada, K. Yanagisawa, and F. J. Ryan, *J. Bacteriol.* **84**, 602 (1962).

[3]We are grateful to R. B. Setlow and members of his group, R. A. McGrath, R. W. Williams, and J. E. Donnellan, Jr., for their instruction in the preparation and use of sucrose gradients.

23.21 METABOLIC STUDIES ON 1-(4-DIMETHYLAMINOBENZYLIDENE)INDENE

C. T. Bahner D. G. Doherty

1-(4-Dimethylaminobenzylidene)indene (**I**) has been found to be effective against Walker 256 tumors in rats and is closely related in structure to the radiomimetic compound 4-(4-dimethylaminostyryl)quinoline and the carcinogen 4-dimethylaminostilbene, but its metabolic fate in rats has not been reported. **I** labeled by a ^{14}C methyl group on the nitrogen has been prepared, and a study of its fate in vivo begun. A solution in peanut oil was not readily absorbed when administered subcutaneously. When injected intraperitoneally, the rate of absorption varied widely. Some rats excreted 30 to 50% of the injected dose in the feces within 24 to 48 hr, while in other cases part of the unabsorbed oil solution was visible at autopsy a week or longer after administration. By column chromatography applied to the extracts of feces from ten rats which received repeated doses of the drug, it was possible to identify the chief radioactive material in the feces as **I** itself. The identification was confirmed by the mixed melting point method. There was evidence of at least two other radioactive compounds in the feces and three radioactive compounds in the urine, one of which appeared to be urea. This finding supports the hypothesis that a part of the administered **I** is demethylated in the same way as 3′-methyl-4-dimethylaminoazobenzene and that the methyl groups are converted to carbon dioxide and urea.

AEC-NIH Zonal Ultracentrifuge Development

23.22 NEW GRADIENT SEPARATION METHODS

N. G. Anderson

Introduction. – In addition to the rate- and isopycnic-zonal separations of particles previously described, several other interesting separations may be accomplished in liquid density gradients in centrifugal fields. These possibilities arise from the following considerations. In density gradients, reagents of low molecular weight may be arranged in discrete zones in such a manner

that larger particles sediment through them. A sedimenting particle may be sequentially dissected during sedimentation, leaving behind its low-molecular constituents in zones. A subcellular particulate may thus be successively exposed to various enzymes, detergents, salts, or changes in pH. A particle may also be immobilized at its isopycnic point in a gradient together with reagents which will dissociate it. If the dissociated constituents differ in density from the parent particle, they then reband at another density level. This technique is applicable to the separation of nucleic acid and protein components of viruses, for example.

Macromolecular substances, including proteins and nucleic acids, may be introduced as solutions near the top of a gradient in such a manner that precipitating substances diffuse in from above and below, causing the macromolecules to flocculate. As the floc moves out in the density gradient, which contains a decreasing gradient of the precipitant, it reaches a level where it may go into true solution and cease to sediment at an appreciable rate. This method, termed gradient resolubilization, has many variants involving both aqueous and nonaqueous solvents. Isoelectric precipitation may be used to precipitate a colloid at one position in a gradient and to dissolve it in another. Low-molecular substances may also be separated on the basis of the density of either their crystals or precipitates. Organic substances insoluble in water dissolved in a water-miscible solvent may be layered over an aqueous density gradient. As diffusion between the organic solvent and the aqueous solution occurs, the organic solute will precipitate and be recovered as a band at its isopycnic point in the density gradient. (The situation may be reversed with a water-soluble solute layered over a gradient prepared from organic solvents.)

Experimental. – Orienting experiments have demonstrated the feasibility of these concepts. One example is presented here. A fraction of the soluble cytoplasmic proteins of all tissues thus far examined are isoelectrically precipitated near pH 5.0.

Rat liver soluble phase was layered over a sucrose pH gradient made from 10% sucrose containing 0.1 *M* acetic acid brought to pH 4.8 with Tris base and 20% sucrose containing 0.1 *M* Tris hydroxide brought to pH 7.5 with acetic acid.

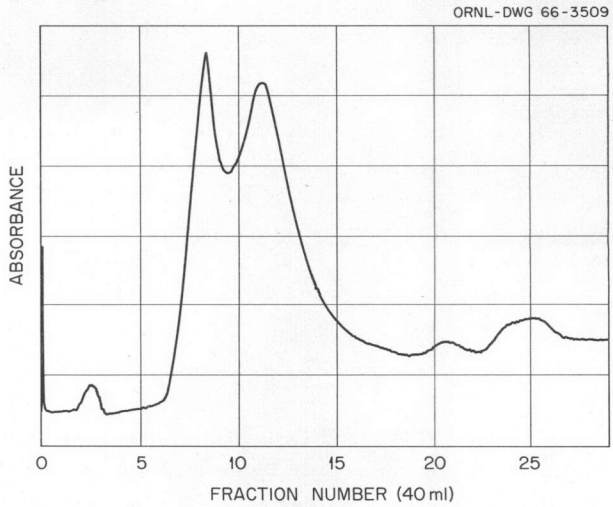

Fig. 23.22.1. Absorbance Diagram (260 mμ) of Recovered pH Gradient from B-XV Zonal Centrifuge. Sample zone is in fraction 8. Remaining 40-ml fractions (9–28) contain buffer of increasing pH.

After the gradient was introduced into the B-XV zonal centrifuge at 3000 rpm, 20 ml of the soluble phase was introduced centripetal to the gradient and followed with an overlay of pH 4.8 buffer without sucrose. As the buffer diffused into the sample layer and lowered its pH, isoelectric precipitation occurred. The precipitate was then sedimented through the pH gradient until it went back into solution at approximately the pH 6.0 level. The absorbance of the recovered gradient is shown in Fig. 23.22.1.

Discussion. – A number of new separation techniques involving manipulations of density gradients have been devised. Of these, one using isoelectric precipitation and resolubilization in a pH gradient is presented in detail.

23.23 SEPARATION OF RAT LIVER CELL COMPONENTS IN THE B-XV ZONAL CENTRIFUGE ROTOR

J. R. Corbett

Introduction. – A new rotor (model B-XV), with a radius of 8.9 cm and a capacity of 1700 ml, has been designed for zonal centrifugation.

Methods and Results. – The B-XV has been used for two separations: (1) Lysosomes were separated from mitochondria on a rate basis. Nitrocatechol sulfatase (EC 3.1.6.1), *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30), *p*-nitrophenyl-β-D-galactopyranosidase (EC 3.2.1.23), *p*-nitrophenyl phosphatase (EC 3.1.3.2), and phenolphthalein glucuronidase (EC 3.2.1.31) were used as lysosomal markers; cytochrome oxidase (EC 1.9.3.1) was used as a mitochondrial marker. (2) The soluble phase, lysosomes, mitochondria, and nuclei were separated in one run with the rotor. The criteria for the identification of these fractions were (1) the enzymes mentioned above, (2) uv light transmission and position in the rotor at the end of the run, and (3) light and electron microscopy.

Discussion. – This separation might be of use in determining the intracellular localization of enzymes, pesticides, etc.

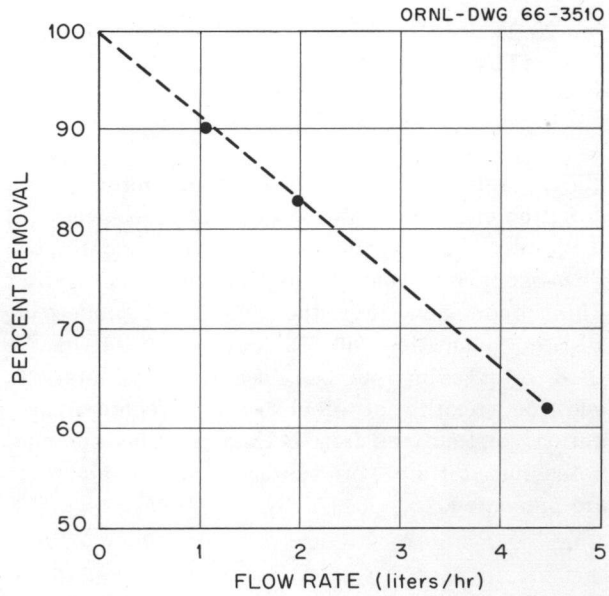

Fig. 23.24.1. Removal of T3 Bacteriophage from a Flow-Through Stream in the K-1 Zonal Rotor as a Function of Flow-Through Rate. Rotor speed was 50,000 rpm.

23.24 SERIES K-1 ZONAL ROTOR EVALUATION

G. B. Cline N. G. Anderson

The second generation of zonal rotors begins with the K series, which are designed to operate in the conventional Sharples air-driven centrifuges. The first rotor of the K series, called the K-1 rotor, has been built and tested for gradient recovery and bacteriophage T3 isolation properties. This report briefly describes the operation of the K-1 rotor and results of initial biological studies.

Results and Discussion. – The K-1 zonal rotor is the production counterpart of the B-IX zonal rotor used in the Spinco zonal ultracentrifuge. The K-1 rotor itself is not designed for use in production laboratories but is used primarily for proof of principle. This rotor has the same overall configuration as the conventional Sharples centrifuge head, but the core and flow-through path have been altered to permit solutions passing through the rotor to pass over a density gradient. The gradient is subsequently recovered from the spinning rotor. The K-1 rotor is fabricated of titanium and will spin to 60,000 rpm with a 225-ml cesium chloride density gradient. Results reported here were obtained using a 150-ml gradient of maximum density 1.6 at a rotor speed of 50,000 rpm. Cleanout of the type 3 bacteriophage is described in terms of the percent of the virus removed from the flow-through stream by the rotor. The percent cleanout is based on plaque assay of samples of effluent using the soft-agar overlay technique. Figure 23.24.1 shows the results of one cleanout experiment in which 2.8 liters of crude lysate was passed through the K-1 rotor at the flow rates indicated. A flow rate of 1.0 liter/hr gave a cleanout of about 90%. By comparison, cleanout at a similar flow rate in the B-IX zonal rotor at top operating speed (with cesium chloride gradient, top speed is 35,000 rpm) is better than 99%. The higher efficiency of cleanout in the B-IX rotor is due in part to a larger radius of rotor and a longer residence time of the flow-through solution in the rotor. The K-1 rotor can be effectively used for continuous-flow experiments using a density gradient to simultaneously band the trapped particles. However, for greater usefulness as a production type of rotor, changes in size and rotating speed are being made. The revised rotor, called K-2, is also designed to be driven with a Sharples air-driven turbine.

23.25 RESPIRATORY SYNCYTIAL VIRUS STUDIES IN THE B-IX ZONAL ROTOR

G. B. Cline L. H. Elrod

The usefulness of the B-IX zonal rotor for the isolation and concentration of respiratory syncytial (RS) virus (Long strain) by combined continuous flow–isopycnic banding has been reported earlier. This report describes the results of processing 10-liter quantities of RS harvest fluid in the B-IX rotor using sucrose density gradients. In addition, results of electron microscopic examination[1] of fractions from B-IX runs using sucrose, potassium citrate, or cesium chloride gradients are presented.

Results and Discussion. – Figure 23.25.1 shows a composite of the data obtained from one 10.4-liter RS run using a sucrose density gradient. The gradient stream from the rotor was monitored at 260 mμ and is denoted as the absorbance curve. The highest peak of absorbance is centered in fraction 13. Coincident with the absorbance peak is the infectivity, which titered 2.1×10^9 plaque-forming units per milliliter of gradient.[2] The starting RS harvest fluid titered $10^{6.4}$. The major amount of the complement-fixing (CF) antigen was found to be coincident with the infectivity peak. Some fractions were not assayed for CF activity in this experiment. All fractions normally contain measurable CF activity. Flow-through rate was 4 liters/hr, with 99.6% of the infectious units

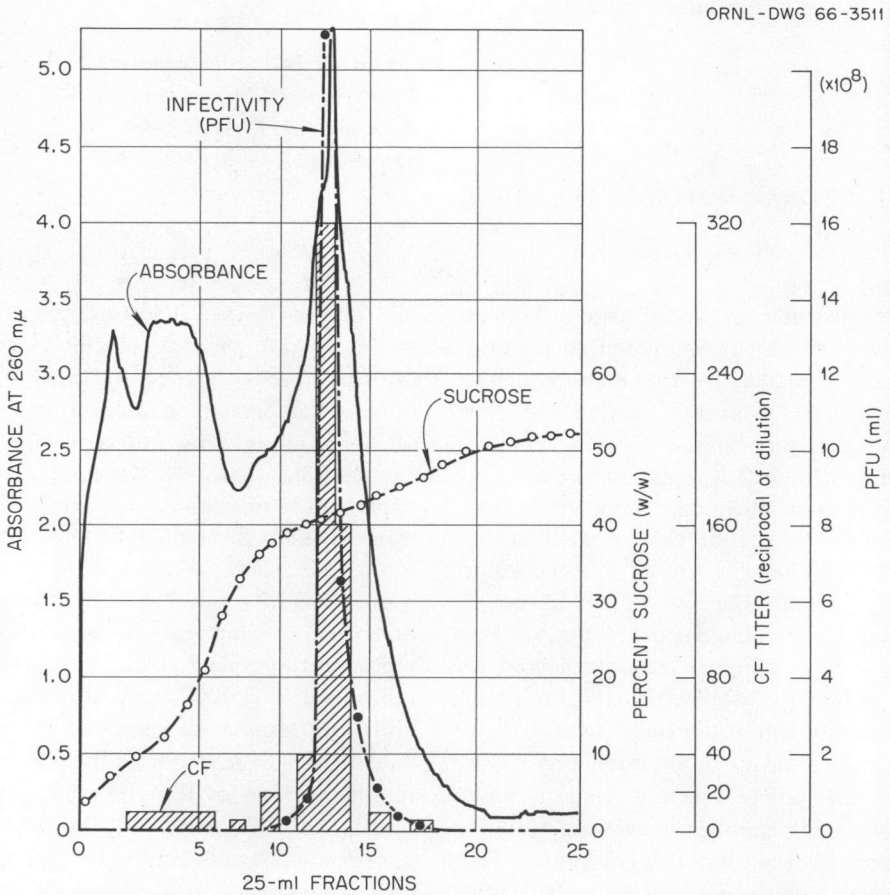

Fig. 23.25.1. Absorbance Profile at 260 mμ of a B-IX Rotor Gradient over Which 10.4 Liters of Respiratory Syncytial Virus Harvest Fluid Had Been Passed. Ninety-two percent of the infectious particles were found to be isopycnically banded in 40 to 42% sucrose and were coincident with an absorbance peak and the peak of CF activity. Flow-through rate was 4 liters/hr at a rotor speed of 40,000 rpm. The sucrose gradient was made up in phosphate saline buffered at pH 7.5.

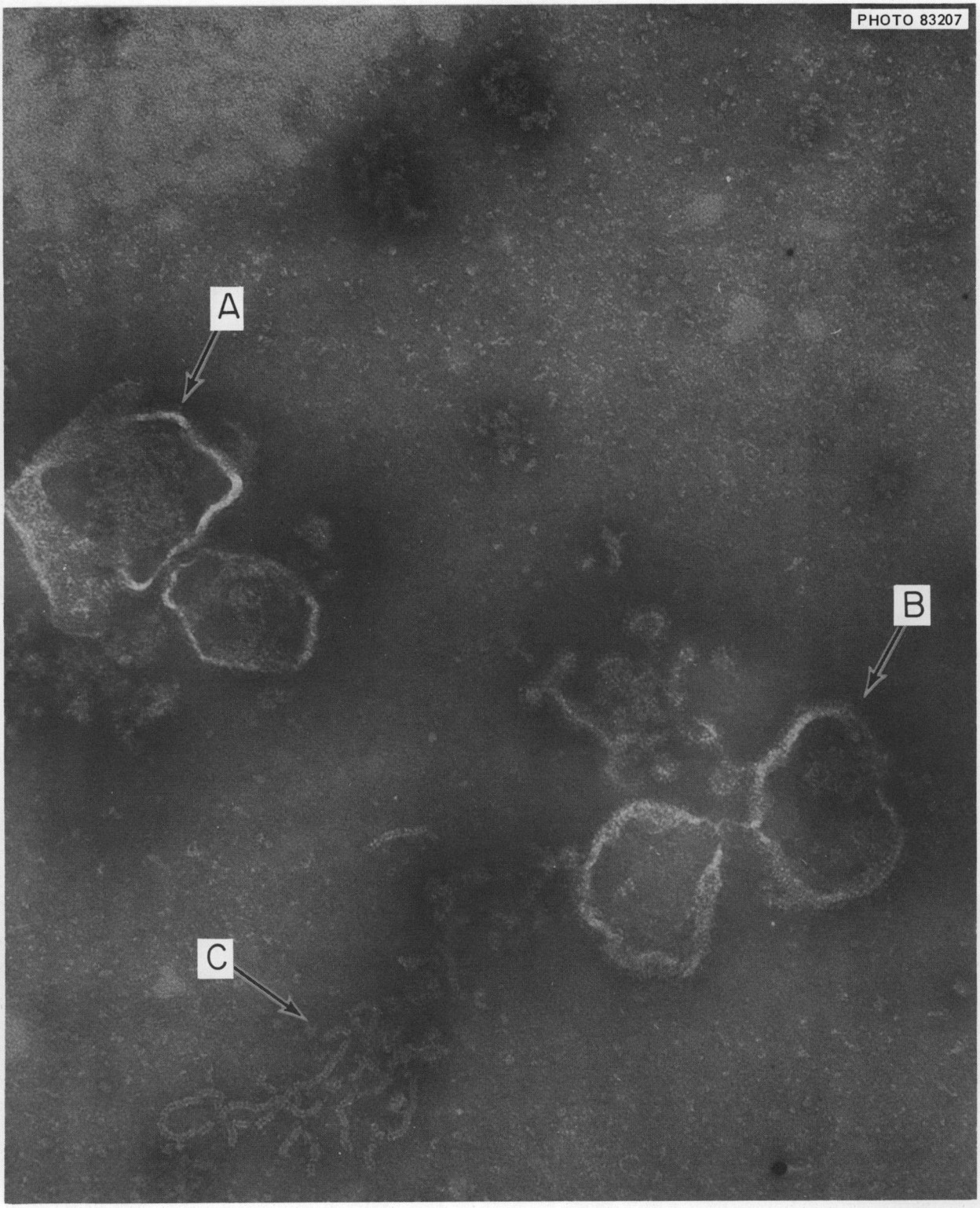

Fig. 23.25.2. Electron Micrograph of Phosphotungstic Acid Negatively Stained Respiratory Syncytial Virus in Various Stages of Breakdown. Arrow *A* points to one virus particle containing coiled inner component. Arrow *B* points to a particle which has lost most of its inner component. The surface components are still present. Arrow *C* points to inner component which has become unwound. The strands appear to be made up of a single-stranded helix, 17 mμ in width. 80,000×.

trapped by the rotor. The infectivity zone in the rotor covered 60 ml and contained 92% of the infectious units trapped by the rotor. The ratio of infectivity/CF was $10^{7 \cdot 6}$.

Similar experiments using potassium citrate or cesium chloride as the density gradient material indicated that these materials could also be used in the B-IX rotor for RS isolation. However, some loss of infectivity was noted when compared with sucrose gradients. A comparison of all fractions from three identical experiments using sucrose, potassium citrate, and cesium chloride indicated that

1. intact RS particles were concentrated in fractions containing a high titer of plaque-forming units,
2. some intact RS particles could be found in every centrifuge fraction,
3. virus zone width was narrowest in sucrose and widest in cesium chloride,
4. morphology of the particles was apparently retained best in sucrose,
5. the isopycnic banding density of the virions was 1.2 in sucrose and 1.22 in potassium citrate and cesium chloride.

The inner component of the RS virus is a strand of material resembling a single coil of rope 17 mμ in width. Figure 23.25.2 is an electron micrograph of phosphotungstate-stained RS virus preparation from a sucrose density gradient showing a virus containing coiled inner component (*A*), empty virus particles (*B*), and an unwound inner component (*C*).

References

[1]Electron microscopy was graciously performed by Frances Ball, Oak Ridge Gaseous Diffusion Plant.

[2]Infectivity assays and antigenicity values determined by NIAID.

AEC-NASA Space Biology

23.26 BIOCOMPATIBILITY TESTS OF THE PROTOTYPE PACKAGES FOR THE *NEUROSPORA* BIOSATELLITE EXPERIMENT UNDER SIMULATED FLIGHT CONDITIONS

F. J. de Serres B. B. Webber

New information has made it possible for NASA to provide random vibration spectra for each package in the *Neurospora* experiment which are generally less intense and which more closely approximate actual flight profiles than those tested in April 1965.[1] In September 1965 a testing program was planned for retesting the flight hardware and for assaying the biological effects of the new acceleration, impact, and vibration profiles with and without ionizing radiation. In these tests *Neurospora* conidia were subjected to (1) simulated flight profile alone, (2) radiation profile alone, and (3) simulated flight profile in combination with radiation profile, and these were compared with (4) untreated controls. Assay for a genetic effect consisted in determining the frequencies of forward mutation in the *ad-3* region.

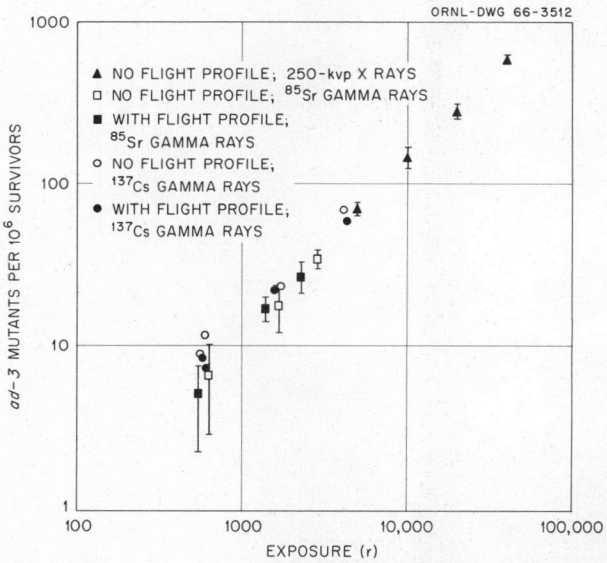

Fig. 23.26.1. Frequencies of Forward Mutations in the *ad-3* Region of *Neurospora crassa* After Exposure to Various Ionizing Radiations Alone or in Combination with Simulated Flight Profiles.

Gamma rays from a ^{137}Cs source were administered continuously during an interval of approximately 60 hr. Conidia subjected to (3) received total exposures of 4370, 1580, 620, and 590 r, and conidia subjected to (2) received total exposures of 4170, 1740, 600, and 588 r. The present tests also represent the first in a series of biological tests of the prototype packages before their final acceptance as actual flight hardware.

Results and Conclusion. – In Fig. 23.26.1 the mutation frequencies are plotted against radiation exposures for (1) the September 1965 ^{137}Cs experiment, (2) the April 1965 ^{85}Sr experiment,[1] and (3) an experiment performed at ORNL with 250-kvp x rays at 10 r/min.[2] The data show that the dose-effect curves are identical for these three types of ionizing radiation. If the simulated flight profiles in the present tests are a valid approximation of the actual flight profiles, these data suggest that any unusual effect encountered as a result of flight can be attributed to radiation in combination with weightlessness rather than radiation in combination with flight profile.

References

[1]F. J. de Serres and B. B. Webber, *Biol. Div. Semiann. Progr. Rept. July 31, 1965,* ORNL-3853, pp. 108–9.

[2]F. J. de Serres, H. V. Malling, and B. B. Webber, *Biol. Div. Semiann. Progr. Rept. July 31, 1965,* ORNL-3853, pp. 54–57.

23.27 A SIMULATED SPACE FLIGHT TEST FOR *HABROBRACON*

R. C. von Borstel Diane J. Goins

Introduction. – The purpose of this experiment was to test the radiation subassembly and prototype packages with *Habrobracon* under two separate conditions. These conditions were: the simulated flight profile with different exposures of radiation including nominal zero dose and the different exposures of radiation including nominal zero dose but without the simulated flight profile. Even though the packages in the test were the same packages used in the April 13 and 19, 1965, tests,[1] the simulated flight profile had been changed to what is believed to be a more realistic test of vibration parameters.

Experimental Conditions. – The test was begun on August 20, 1965, and ended on August 23. A ^{137}Ce source was used for the gamma radiation, and the irradiation time was 66 hr 10 min. Ten wasps were placed in each of the modules, except for a newly designed three-layered module for the controls (designed by Nakwon Cho, Technical Division of ORGDP), where 12 wasps were placed in each layer. Facing the packages from the radiation-source position, virgin females were placed in the upper right and lower left modules, and males were placed in the upper left and lower right modules. The females tested were of the *Raleigh* wild-type strain, and the males were of the *lemon* mutant strain.

Table 23.27.1. Dose Survival of Embryos After Exposure of Sperm to ^{137}Cs Radiation Source

Nominal Dose (rads)	Radiation Alone		Flight Profile and Radiation	
	Measured Dose (rads)	Embryo Survival	Measured Dose (rads)	Embryo Survival
0	0	0.99	0	1.00
500	500	0.79	505	0.73
1000	955	0.76	945	0.74
2000	1950	0.47	2110	0.46
4000	3755	0.16	3640	0.17

Results. – *Survival.* – In this test, none of the wasps were pulverized, as were some in the April test. Nevertheless, a few males died in almost all modules whether or not they had been taken through the simulated-flight profile. On the other hand, nearly all the females survived. Egg production of the females was unaffected.

Induced Lethality. – The summary data, analyzed by the method of von Borstel and Rekemeyer,[2] are shown in Table 23.27.1. The measured doses are those of J. E. Hewitt and M. G. Hershberger from Ames Research Center. The embryo survival at different exposures can be subtracted from the zero-dose controls to give the frequency of induced dominant lethality. As in the April test,[1] no synergistic effect was observed for the flight profile and radiation for induced dominant lethality in sperm.

References

[1]R. C. von Borstel *et al.*, *Biol. Div. Semiann. Progr. Rept. July 31, 1965,* ORNL-3853, pp. 109–10.

[2]R. C. von Borstel and M. L. Rekemeyer, *Genetics* **44,** 1053–74 (1959).

23.28 THE GEMINI-3 S-4 SPACE-FLIGHT–RADIATION INTERACTION EXPERIMENT

M. A Bender P. Carolyn Gooch Sohei Kondo

Introduction. – We have previously described the experimental hardware and the plan of an experiment to test whether a synergism exists between radiation and some space-flight parameter (e.g., weightlessness).[1] This experiment, known as S-4, was carried out in connection with the GT-3 manned orbital mission. The in-flight experimental device was activated at 00:50:18 Gemini elapsed time (g.e.t.) and deactivated at 01:10:18 g.e.t., thus starting and stopping the irradiation of a series of human blood samples by ^{32}P beta rays. The corresponding activation and deactivation of the duplicate (control) device on the ground occurred at 00:52:00 and 01:12:00 g.e.t. respectively. Blood samples were all in culture 6 hr after the spacecraft landed. All cultures were fixed after approximately 68 hr in culture.

Results. – The dosimeters and other instruments from the experimental devices, as well as records from the flight and the results of postflight determinations, all show that the doses received by the blood samples, as well as the temperatures, etc., were substantially the same for the in-flight and ground portions of the experiment.

The results of scoring the cells for chromosomal aberrations are shown in Table 23.28.1. The frequencies of chromatid aberrations and of cells with chromosome numbers other than $2n = 46$ are not different from those seen in other experiments; the average values in our laboratory are 1.3% chromatid aberrations and 4.9% cells with $2n \neq 46$. The two chromosome deletions seen in the samples from crew member B are not significant; in cells from normal control people the frequency of chromosome deletions is 0.5%. The two dicentric chromosomes seen in the samples from the same crew member appear to be identical. No acentric fragments accompanied either dicentric. It is clear that they have nothing to do with the flight.

The frequencies of chromosome-type aberrations in the control samples are quite consistent with the low dose they received (from beta-particle leakage and bremsstrahlung within the experimental devices). The frequencies of two-break ring and dicentric aberrations appear very similar; certainly there is no consistent difference between their frequencies in the series of irradiated samples. The frequencies of single-break chromosome deletions, on the other hand, do show a consistent difference when the in-flight and ground samples are compared: the chromosome deletion yields for the ground portion of the experiment are lower in all four dose groups. The data in Table 23.28.1 were fitted to linear (deletions) and dose-square (rings and dicentrics) models in order to determine the coefficients of aberration production. The results are shown in Table 23.28.2, together with those for a previous control experiment. It may be seen that the in-flight and ground coefficients for deletions are significantly different, though that for the ground portion is not different from that for the control experiment.

Discussion. – Even if extensive physical evidence did not rule out significant differences between the in-flight and ground portions of the experiment with respect to doses, temperatures, oxygen tensions, etc., the aberration results themselves would do so. A difference in any of

Table 23.28.1. Results of Experiment S-4 Chromosome Aberration Analyses

Subject	Sample	Cells Scored	Estimated Dose (rads)	$2n \neq 46$	Chromatid Deletions	Chromosome Deletions	Ring and Dicentric Chromosomes
				Crew			
A	Preflight	100		8	3	0	0
	Postflight	200		8	2	0	0
B	Preflight	100		4	0	1	1[a]
	Postflight	200		7	0	1	1[a]
				Experiment			
	Ground	400	4	31	3	3	1
	Flight	400		33	1	3	0
	Ground	400	49	20	1	6	5
	Flight	400		26	3	14	1
	Ground	400	94	22	5	13	13
	Flight	400		24	6	28	16
	Ground	400	141	20	6	32	43
	Flight	400		44	6	48	34
	Ground	400	185	38	6	45	36
	Flight	400		58	2	88	48

[a]These two dicentric chromosomes appear identical; both lacked acentric fragments.

these parameters would affect the two-break aberrations even more than the single-break aberrations. It thus appears very probable that there is, in fact, a synergism between radiation and some space-flight parameter, at least for human somatic chromosome aberration production. Further experiments will be necessary in order to confirm this unusual effect, to determine just what space-flight parameter is involved, and to elucidate the mechanism.

Table 23.28.2. Coefficients of Aberration Production for S-4 Experiment

	Deletions per Cell per Rad	Rings and Dicentrics per Cell per Rad^2
	$\times 10^{-4}$	$\times 10^{-6}$
Ground control	4.79 ± 0.72	3.23 ± 0.59
In flight	9.11 ± 1.02	3.48 ± 0.53
Run 5	5.36 ± 0.86	2.66 ± 0.60

References

[1]ORNL-TM-940 (1965).

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ORNL-3352	Period Ending August 15, 1962
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228. R. F. Loeb, College of Physicians and Surgeons, Columbia University, New York
229. Carl V. Moore, Washington University, St. Louis, Mo.
230. Russell S. Poor, Atomic Energy Commission, Washington
231. Robert Rabson, Atomic Energy Commission, Washington
232. H. M. Roth, Research and Development Division, AEC, OROO
233. M. B. Russell, University of Illinois, Urbana
234. C. S. Shoup, Atomic Energy Commission, Oak Ridge
235. James H. Sterner, Eastman Kodak Company, Rochester
236. John Totter, Atomic Energy Commission, Washington
237. Shields Warren, New England Deaconess Hospital, Boston
238. C. G. Zubrod, National Cancer Institute, Bethesda

239–242. Atomic Energy Commission, Washington
243–246. Battelle Northwest
247–248. New York Operations Office
249. Patent Branch, Washington
250–254. Division of Technical Information Extension, Oak Ridge