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BIOLOGY DIVISION
SEMIANNUAL PROGRESS REPORT
FOR PERIOD ENDING AUGUST 15, 1964

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BIOLOGY DIVISION
SEMIANNUAL PROGRESS REPORT
For Period Ending August 15, 1964

Alexander Hollaender, Director
Stanley F. Carson, Associate Director

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NOVEMBER 1964

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Publications and Lectures

PUBLICATIONS

A total of 362 papers, abstracts, book reviews, and other contributions to open literature have been published or gone to press during the reporting period February–August 1964. This compares with 337 for the same time span in 1963 and with 346 for the August 1963–February 1964 period. Listed below are the 114 published papers, reports, small conference booklets, and books edited by Division members for the current period – an increase of 31 items over the last two reporting periods, in each of which 83 published papers and reports were listed. The 61 published abstracts and 190 items in press are not listed.

The proceedings of the 1964 Gatlinburg Symposium, *The Molecular Action of Mutagenic and Carcinogenic Agents,* will be published by the Wistar Institute of Anatomy and Biology as an October supplement to the *Journal of Cellular and Comparative Physiology.*

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Adler, H. I., and Alice A. Hardigree	Analysis of a gene controlling cell division and sensitivity to radiation in *Escherichia coli*	*J. Bacteriol.* **87**, 720–26 (1964)
	Cell elongation in strains of *Escherichia coli*	*J. Bacteriol.* **87**, 1240–42 (1964)
Albright, J. F., Takashi Makinodan, and E. E. Capalbo	Factors regulating antibody production by spleen cells cultured *in vivo*	Pp. 301–8 in *Proceedings of the 9th Congress of the International Society of Blood Transfusion, Mexico, 1962,* Karger, Basel, Switzerland, 1964
Anderson, N. G. (prepared by)	The Joint National Institutes of Health–Atomic Energy Commission Zonal Centrifuge Development Program: semiannual report for period July–December 31, 1963	ORNL-3656
Barnett, W. E., and K. B. Jacobson	Evidence for degeneracy and ambiguity in interspecies aminoacyl-sRNA formation	*Proc. Natl. Acad. Sci. U.S.* **51**, 642–47 (1964)
Bell, Sandra, and Sheldon Wolff	Studies on the mechanism of the effect of fluorodeoxyuridine on chromosomes	*Proc. Natl. Acad. Sci. U.S.* **51**, 195–202 (1964)
Bender, M. A	Chromosome aberrations in irradiated human subjects	*Ann. N.Y. Acad. Sci.* **114**, 249–51 (1964)
Bollum, F. J.	Studies on the nature of calf thymus DNA-polymerase products	*Cold Spring Harbor Symp. Quant. Biol.* **28**, 21–26 (1963)

AUTHORS(S)	TITLE OF ARTICLE	PUBLICATION
Bollum, F. J., Eleanor Groeniger, and Masahiko Yoneda	Polydeoxyadenylic acid	*Proc. Natl. Acad. Sci. U.S.* **51**, 853–59 (1964)
Brewen, J. G.	Studies on the frequencies of chromatid aberrations induced by x rays at different times of the cell cycle of *Vicia faba*	*Genetics* **50**, 101–7 (1964)
Brockman, H. E.	Effects of amino acids on the utilization of tryptophan and indole for growth by a mutant of *Neurospora crassa*	*J. Gen. Microbiol.* **34**, 31–41 (1964)
Cameron, I. L., and Günter Cleffmann	Initiation of mitosis in relation to the cell cycle following feeding of starved chickens	*J. Cell Biol.* **21**, 169–74 (1964)
Capalbo, E. E., J. F. Albright, and W. E. Bennett	Evaluation of the diffusion chamber culture technique for study of the morphological and functional characteristics of lymphoid cells during antibody production	*J. Immunol.* **92**, 243–51 (1964)
Capalbo, E. E., and Takashi Makinodan	Doubling time of mouse spleen cells during the latent and log phases of primary antibody response	*J. Immunol.* **92**, 234–42 (1964)
Carlson, J. G., and Mary Esther Gaulden	Grasshopper neuroblast techniques	Pp. 229–76 in *Methods in Cell Physiology*, vol. 1 (ed. by D. M. Prescott), Academic, New York and London, 1964
Castellani, Amleto, John Jagger, and R. B. Setlow	Overlap of photoreactivation and liquid holding recovery in *Escherichia coli* B	*Science* **143**, 1170–71 (1964)
Chu, E. H. Y., J. H. Rubinstein, and Josef Warkany	Chromosome studies in a family with four cases of atypical Down's syndrome	Pp. 1169–75 in *Proceedings of the Second International Congress of Human Genetics (Rome, September 6–12, 1961)*, vol. II, Istituto G. Mendel, Rome, 1963
Chu, E. H. Y., H. C. Thuline, and D. E. Norby	Triploid-diploid chimerism in a male tortoiseshell cat	*Cytogenetics* **3**, 1–18 (1964)
Clayton, R. K.	Phototaxis in microorganisms	Pp. 51–77 in *Photophysiology: Vol. II – Action of Light on Animals and Microorganisms; Photobiochemical Mechanisms; Bioluminescence* (ed. by A. C. Giese), Academic, New York and London, 1964
	Physical aspects of the light reaction in photosynthesis	Pp. 155–97 in *Photophysiology: Vol. I – General Principles; Action of Light on Plants* (ed. by A. C. Giese), Academic, New York and London, 1964

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Cohn, W. E	Nouveaux nucléotides (in French)	*Bull. Soc. Chim. Biol.* **46**, 239–53 (1964)
Cohn, W. E (coeditor)	*Progress in Nucleic Acid Research*, vol. 2 (346 pp.)	Ed. by J. N. Davidson and W. E Cohn, Academic, New York and London, 1963
Congdon, C. C	Discussion of the Cronkite and Mathé papers	*Ann. N.Y. Acad. Sci.* **114**, 389–92 (1964)
	Summary and final discussion	Pp. 379–93 in *Control of Cell Division and the Induction of Cancer (International Symposium Held at Lima, Peru, and Cali, Colombia, July 1–6, 1963)* (ed. by C. C Congdon and Pablo Mori-Chavez), National Cancer Institute Monograph No. 14, U.S. Department of Health, Education, and Welfare, Bethesda, Md., 1964
Congdon, C. C (coeditor)	*Control of Cell Division and the Induction of Cancer (International Symposium Held at Lima, Peru, and Cali, Colombia, July 1–6, 1963)* (403 pp.)	Ed. by C. C Congdon and Pablo Mori-Chavez, National Cancer Institute Monograph No. 14, U.S. Department of Health, Education, and Welfare, Bethesda, Md., 1964
Congdon, C. C, Joan W. Goodman, and J. W. Ferrebee	Effect of thymus transplantation on radiation chimeras	*Ann. N.Y. Acad. Sci.* **114**, 460–67 (1964)
Cosgrove, G. E., B. M. Nelson, and A. W. Jones	*Spirura tamarini* sp. n. (Nematoda: Spiruidae) from an Amazonian primate, *Tamarinus nigricollis* (Spix, 1823)	*J. Parasitol.* **49**, 1010–13 (1963)
Cosgrove, G. E., A. C. Upton, C. C Congdon, D. G. Doherty, K. W. Christenberry, and D. G. Gosslee	Late somatic effects of x-radiation in mice treated with AET and isologous bone marrow	*Radiation Res.* **21**, 550–74 (1964)
Cudkowicz, Gustavo, Michael Bennett, and G. M. Shearer	Pluripotent stem cell function of the mouse marrow "lymphocyte"	*Science* **144**, 866–68 (1964)
Cudkowicz, Gustavo, and J. H. Stimpfling	Deficient growth of C57BL marrow cells transplanted in F_1 hybrid mice: association with the histocompatibility-2 locus	*Immunology* **7**, 291–306 (1964)
	Hybrid resistance to parental marrow grafts: association with the K region of H-2	*Science* **144**, 1339–40 (1964)
Cudkowicz, Gustavo, A. C. Upton, L. H. Smith, D. G. Gosslee, and W. L. Hughes	An approach to the characterization of stem cells in mouse bone marrow	*Ann. N.Y. Acad. Sci.* **114**, 571–82 (1964)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
de Serres, F. J.	Genetic analysis of the structure of the *ad-3* region of *Neurospora crassa* by means of irreparable recessive lethal mutations	*Genetics* **50**, 21–30 (1964)
Finamore, F. J.	The oligonucleotide nature and synthesis of acid-soluble ribonucleic acid in amphibian eggs and embryos	*J. Biol. Chem.* **239**, 1882–86 (1964)
Foreman, C. W.	Tryptic peptide patterns of some mammalian hemoglobins	*J. Cellular Comp. Physiol.* **63**, 1–6 (1964)
Fuccillo, D. A., Jr.	A survey of services offered authors by biological editors	*Bioscience* **14**, 23–24 (1964)
Giudice, Giovanni, F. T. Kenney, and G. D. Novelli	Effect of puromycin on deoxyribonucleic acid synthesis by regenerating rat liver	*Biochim. Biophys. Acta* **87**, 171–73 (1964)
Gooch, P. Carolyn, M. A Bender, and M. L. Randolph	Chromosome aberrations induced in human somatic cells by neutrons	Pp. 325–42 in *Biological Effects of Neutron and Proton Irradiations*, vol. I, International Atomic Energy Agency, Vienna, Austria, 1964
Goodman, Joan W.	Stem cells of the hematopoietic and lymphatic tissues	Pp. 151–68 in *Control of Cell Division and the Induction of Cancer (International Symposium Held at Lima, Peru, and Cali, Colombia, July 1–6, 1963)* (ed. by C. C Congdon and Pablo Mori-Chavez), National Cancer Institute Monograph No. 14, U.S. Department of Health, Education, and Welfare, Bethesda, Md., 1964
Goodman, Joan W., and M. A. Bender	Irradiation of donor bone marrow: effects on survival and on chromosomal aberrations in the marrow of recipient mice	*Transplantation* **2**, 334–42 (1964)
Greenman, D. L., and F. T. Kenney	Effects of alterations in hormonal status on ribosomes of rat uterus	*Arch. Biochem. Biophys.* **107**, 1–6 (1964)
Grell, Rhoda F.	Chromosome size at distributive pairing in *Drosophila melanogaster* females	*Genetics* **50**, 151–66 (1964)
Grell, Rhoda F., and J. I. Valencia	Distributive pairing and aneuploidy in man	*Science* **145**, 66–67 (1964)
Grumbach, M. M., Akira Morishima, and E. H. Y. Chu	An XXX/XX/XO sex chromosome constitution in gonadal dysgenesis and other examples of sex chromosome mosaicism in man	Pp. 1195–97 in *Proceedings of the Second International Congress of Human Genetics (Rome, September 6–12, 1961)*, vol. II, Istituto G. Mendel, Rome, 1963
Gude, W. D., V. K. Jenkins, A. C. Upton, and R. L. Tyndall	Glomerulosclerosis in mice with myeloid leukemia	*Proc. Soc. Exptl. Biol. Med.* **115**, 691–93 (1964)

AUTHORS(S)	TITLE OF ARTICLE	PUBLICATION
Gude, W. D., and A. C. Upton	Histologic study of spontaneous glomerular lesions in aging RF mice (abstracted from *Am. J. Pathol.* **40**, 699–709, June 1962)	Pp. 408–10 in *The Year Book of Veterinary Medicine*, vol. 1 – 1963 (ed. by W. F. Riley, Jr., K. W. Smith, and R. J. Flynn), Year Book Medical Publishers, Inc., Chicago, 1963
Haber, A. H., and D. E. Foard	Further studies of gamma-irradiated wheat and their relevance to use of mitotic inhibition for developmental studies	*Am. J. Botany* **51**, 151–59 (1964)
	Interpretations concerning cell division and growth	Pp. 491–503 in *Regulatéurs Naturels de la Croissance Végétale, Colloques Internationaux du Centre National de la Recherche Scientifique, No. 123*, Centre National de la Recherche Scientifique, Paris, 1964
Hanna, M. G., Jr.	An autoradiographic study of the germinal center in spleen white pulp during early intervals of the immune response	*Lab. Invest.* **13**, 95–104 (1964)
Hollaender, Alexander	Introduction to the International Symposium on the Control of Cell Division and the Induction of Cancer	Pp. 1–4 in *Control of Cell Division and the Induction of Cancer (International Symposium Held at Lima, Peru, and Cali, Colombia, July 1–6, 1963)* (ed. by C. C Congdon and Pablo Mori-Chavez), National Cancer Institute Monograph No. 14, U.S. Department of Health, Education, and Welfare, Bethesda, Md., 1964
Hollaender, Alexander, and S. F. Carson	Biology Division semiannual progress report for period ending February 15, 1964	ORNL-3601
Jacobson, K. B.	Chromatographic separation of nucleotides and nucleosides	*J. Chromatog.* **14**, 542–43 (1964)
Jacobson, K. B., and Susumu Nishimura	Chromatographic studies on aminoacyl-acceptor RNA using paper sheets of diethylaminoethyl-cellulose	*J. Chromatog.* **14**, 46–56 (1964)
Jagger, John, W. C. Wise, and R. S. Stafford	Delay in growth and division induced by near ultraviolet radiation in *Escherichia coli* B and its role in photoprotection and liquid holding recovery	*Photochem. Photobiol.* **3**, 11–24 (1964)
Kimball, R. F., and D. M. Prescott	RNA and protein synthesis in amacronucleate *Paramecium aurelia*	*J. Cell Biol.* **21**, 496–97 (1964)
Krieg, D. R.	Specificity of chemical mutagenesis	Pp. 125–68 in *Progress in Nucleic Acid Research*, vol. 2 (ed. by J. N. Davidson and W. E Cohn), Academic, New York and London, 1963

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Lesher, S., H. E. Walburg, Jr., and G. A. Sacher	Generation cycle in the duodenal crypt cells of germfree and conventional mice	*Nature* **202**, 884–86 (1964)
Levin, Gershon, R. B. Setlow, and G. B. Brown	Purine N-oxides. XIII. Kinetics of the photochemical alteration of adenine 1-N-oxide	*Biochemistry* **3**, 883–84 (1964)
Mans, R. J., and G. D. Novelli	Stabilization of the maize seedling amino acid incorporating system	*Biochim. Biophys. Acta* **80**, 127–36 (1964)
Mans, R. J., Caridad Maria Purcell, and G. D. Novelli	The involvement of soluble ribonucleic acid in amino acid incorporation by a maize system	*J. Biol. Chem.* **239**, 1762–68 (1964)
Marin, Guglielmo, and M. A Bender	A comparison of mammalian cell-killing by incorporated ^{3}H-thymidine and ^{3}H-uridine	*Intern. J. Radiation Biol.* **7**, 235–44 (1963)
	Survival kinetics of HeLa S-3 cells after incorporation of ^{3}H-thymidine or ^{3}H-uridine	*Intern. J. Radiation Biol.* **7**, 221–33 (1963)
Marin, Guglielmo, and D. M. Prescott	The frequency of sister chromatid exchanges following exposure to varying doses of H^3-thymidine or x-rays	*J. Cell Biol.* **21**, 159–67 (1964)
Mazur, Peter	Basic problems in cryobiology	Pp. 28–37 in *Advances in Cryogenic Engineering*, vol. 9 (ed. by K. D. Timmerhaus), Plenum, New York, 1964
McDonald, T. P., T. T. Odell, Jr., and D. G. Gosslee	Platelet size in relation to platelet age	*Proc. Soc. Exptl. Biol. Med.* **115**, 684–89 (1964)
Mead, C. G.	A deoxyribonucleic acid–associated ribonucleic acid from *Drosophila melanogaster*	*J. Biol. Chem.* **239**, 550–54 (1964)
Miller, Barbara A., and Rhoda F. Grell	Nonrandom assortment of chromosome 3 and a Y	*Drosophila Information Service* **38**, 65–66 (1963)
Miller, O. L., Jr., G. E. Stone, and D. M. Prescott	Autoradiography of water-soluble materials	Pp. 371–79 in *Methods in Cell Physiology*, vol. I (ed. by D. M. Prescott), Academic, New York and London, 1964
Nishimura, Susumu, and G. D. Novelli	Amino acid acceptor activity of enzymically altered soluble RNA from *Escherichia coli*	*Biochim. Biophys. Acta* **80**, 574–86 (1964)
	Species differences in soluble ribonucleic acids detected by differing ribonuclease sensitivity	*Biochim. Biophys. Acta* **80**, 340–42 (1964)
Oakberg, E. F., and Evelyn Clark	Species comparisons of radiation response of the gonads	Pp. 11–24 in *Effects of Ionizing Radiation on the Reproductive System, Proceedings of an International Symposium Held at Colorado State University, Fort Collins, Colorado* (ed. by W. D. Carlson and F. X. Gassner), Symposium Publications Division, Pergamon, Oxford, 1964

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Odell, T. T., Jr., and T. P. McDonald	Two mechanisms of sulfate-S^{35} uptake by blood platelets of rats	*Am. J. Physiol.* **206**, 580–84 (1964)
Pal, B. C., and C. A. Horton	The structure of 3-methyladenine, and methylation of 6-dimethylaminopurine	*J. Chem. Soc.* **71**, 400–405 (1964)
Perkins, E. H., and Takashi Makinodan	Relative pool size of potentially competent antibody-forming cells of primed and nonprimed spleen cells grown in *in vivo* culture	*J. Immunol.* **92**, 192–200 (1964)
Phares, E. F., Mary V. Long, and S. F. Carson	Negative and positive ion mass analysis of dicarboxylic acids in studies on the mechanism of the methylmalonyl isomerase reaction	*Ann. N.Y. Acad. Sci.* **112**, 680–83 (1964)
Popp, R. A.	Inheritance of factors affecting the ability of bone marrow of (C57BL × 101)F_2 mice to establish permanent grafts in irradiated F_1 mice	*J. Natl. Cancer Inst.* **33**, 7–14 (1964)
Popp, R. A., G. E. Cosgrove, and Diana M. Popp	Relative ability of parental marrows to repopulate lethally irradiated F_1 hybrids	*Ann. N.Y. Acad. Sci.* **114**, 538–48 (1964)
Prescott, D. M.	Autoradiography with liquid emulsion	Pp. 365–70 in *Methods in Cell Physiology*, vol. I (ed. by D. M. Prescott), Academic, New York and London, 1964
	Comments on the cell life cycle	Pp. 57–72 in *Control of Cell Division and the Induction of Cancer (International Symposium Held at Lima, Peru, and Cali, Colombia, July 1–6, 1963)* (ed. by C. C Congdon and Pablo Mori-Chavez), National Cancer Institute Monograph No. 14, U.S. Department of Health, Education, and Welfare, Bethesda, Md., 1964
	Turnover of chromosomal and nuclear proteins	Pp. 193–99 in *The Nucleohistones* (ed. by James Bonner and Paul Ts'o), Holden-Day, Inc., San Francisco, 1964
Prescott, D. M. (editor)	*Methods in Cell Physiology*, vol. 1 (465 pp.)	Ed. by D. M. Prescott, Academic, New York and London, 1964
Prescott, D. M., and M. A Bender	Preparation of mammalian metaphase chromosomes for autoradiography	Pp. 381–85 in *Methods in Cell Physiology*, vol. I (ed. by D. M. Prescott), Academic, New York and London, 1964
Prescott, D. M., and Romance F. Carrier	Experimental procedures and cultural methods for *Euplotes euroystomus* and *Amoeba proteus*	Pp. 85–95 in *Methods in Cell Physiology*, vol. I (ed. by D. M. Prescott), Academic, New York and London, 1964
Randolph, M. L.	Genetic damage as a function of LET	*Ann. N.Y. Acad. Sci.* **114**, 85–95 (1964)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Rinehart, R. R.	Influence of oxygen, helium, and metabolic inhibition on x-ray induced dominant lethality in stage 7 and stage 14 oocytes of *Drosophila melanogaster*	*Genetics* **49**, 855–63 (1964)
Rinehart, R. R., and R. M. Valencia	A method for pair mating Drosophila without etherization	*Drosophila Information Service* **38**, 101 (1963)
Rinehart, R. R., R. M. Valencia, and J. I. Valencia	Comparative lethal rates from x-irradiated *D. melanogaster* males and females mated together or to unirradiated partners	*Drosophila Information Service* **38**, 71–72 (1963)
Ritossa, F. M., and R. C. von Borstel	Chromosome puffs in *Drosophila* induced by ribonuclease	*Science* **145**, 513–14 (1964)
Roberts, Paul	Mosaics involving aristapedia, a homeotic mutant of *Drosophila melanogaster*	*Genetics* **49**, 593–98 (1964)
Russell, Liane B.	Another look at the single-active-X hypothesis	*Trans. N.Y. Acad. Sci.* **26**, 726–36 (1964)
Schuel, Herbert, and N. G. Anderson	Studies on isolated cell components. XVI. The distribution of acid phenyl phosphatase activities in rat liver brei fractionated in the zonal ultracentrifuge	*J. Cell Biol.* **21**, 309–23 (1964)
Schwartz, Drew	Genetic studies on mutant enzymes in maize. IV. Comparison of pH 7.5 esterases synthesized in seedling and endosperm	*Genetics* **49**, 373–77 (1964)
Setlow, R. B., and W. L. Carrier	The disappearance of thymine dimers from DNA: an error-correcting mechanism	*Proc. Natl. Acad. Sci. U.S.* **51**, 226–31 (1964)
Smith, L. H.	Marrow transplantation measured by uptake of Fe^{59} by spleen	*Am. J. Physiol.* **206**, 1244–50 (1964)
Speece, A. J.	Histochemical distribution of lysozyme activity in organs of normal mice and radiation chimeras	*J. Histochem. Cytochem.* **12**, 384–91 (1964)
Stone, G. E., and I. L. Cameron	Methods for using *Tetrahymena* in studies of the normal cell cycle	Pp. 127–40 in *Methods in Cell Physiology*, vol. I (ed. by D. M. Prescott), Academic, New York and London, (1964)
Stone, G. E., and D. M. Prescott	Cell division and DNA synthesis in *Tetrahymena pyriformis* deprived of essential amino acids	*J. Cell Biol.* **21**, 275–81 (1964)
Swenson, P. A., and Susumu Nishimura	Inactivation of S-RNA by ultraviolet radiations	*Photochem. Photobiol.* **3**, 85–90 (1964)
Takata, Chinami, J. F. Albright, and Tuneo Yamada	Lens antigens in a lens-regenerating system studied by the immunofluorescent technique	*Develop. Biol.* **9**, 385–97 (1964)
	Study of lens antigens in the developing newt lens with immunofluorescence	*Exptl. Cell Res.* **34**, 207–10 (1964)
Taylor, W. H., and G. D. Novelli	Enzymes of the pyrimidine pathway in *Escherichia coli*. I. Synthesis by cells and spheroplasts	*J. Bacteriol.* **88**, 99–104 (1964)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Upton, A. C.	Comparative aspects of carcinogenesis by ionizing radiation	Pp. 221–42 in *Control of Cell Division and the Induction of Cancer (International Symposium Held at Lima, Peru, and Cali, Colombia, July 1–6, 1963)*, ed by C. C Congdon and Pablo Mori-Chavez, National Cancer Institute Monograph No. 14, U.S. Department of Health, Education, and Welfare, Bethesda, Md., 1964
Upton, A. C., V. K. Jenkins, and J. W. Conklin	Myeloid leukemia in the mouse	*Ann. N.Y. Acad. Sci.* **114**, 189–202 (1964)
von Borstel, R. C.	Survey of cytochemistry	Pp. 1–8 in *Methods in Cell Physiology*, vol. I (ed. by D. M. Prescott), Academic, New York and London, 1964
Vu-Thi-Suu, C. C Congdon, and A. L. Kretchmar	Lysozyme activity in radiation chimeras	*Proc. Soc. Exptl. Biol. Med.* **115**, 825–29 (1964)
Wald, Niel, A. C. Upton, V. K. Jenkins, and W. H. Borges	Radiation-induced mouse leukemia: consistent occurrence of an extra and a marker chromosome	*Science* **143**, 810–13 (1964)
Wallace, R. A.	Studies on amphibian yolk. VI. A protein kinase from the ovary of *Rana pipiens*	*Biochim. Biophys. Acta* **86**, 286–94 (1964)
Welshons, W. J., and Benedetto Nicoletti	Pseudoallelic recombination of the mutant white-coffee (w^{cf})	*Drosophila Information Service* **38**, 80 (1963)
Wicks, W. D., and F. T. Kenney	RNA synthesis in rat seminal vesicles: stimulation by testosterone	*Science* **144**, 1346–47 (1964)
Wolff, Sheldon	Handling of root tips	Pp. 215–27 in *Methods in Cell Physiology*, vol. I (ed. by D. M. Prescott), Academic, New York and London, 1964
Wolff, Sheldon, and H. E. Luippold	Chromosome splitting as revealed by combined x-ray and labeling experiments	*Exptl. Cell Res.* **34**, 548–56 (1964)
Wust, C. J., Clara L. Gall, and G. D. Novelli	Actinomycin D: effect on the immune response	*Science* **143**, 1041–43 (1964)
(Members of the Biology Division)	Report of Conference on Lens Differentiation – abstracts of conference held March 20–21, 1964, at the Biology Division, ORNL (Tuneo Yamada, chairman)	Reprint No. 2635
	Report of Conference on Blood Platelets – abstracts of second conference, held July 10–11, 1964, at the Biology Division, ORNL (T. T. Odell, Jr., chairman)	Reprint No. 2805 (Small Conference Booklet No. 10)

LECTURES

During the period February to August 1964, members of the Biology Division presented 219 lectures and speeches compared with 182 for the corresponding period of 1963. Of these, 98 were before professional societies at home and abroad, 18 were given as part of the Traveling Lecture Program, and the remainder were presented on invitation of universities, laboratories, and scientific organizations in this country and abroad.

Details on speaking activities outside the continental United States will be found in the section "Foreign Travel." Educational activities are described under "Education."

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Adler, H. I.	Genetic control of radiation sensitivity in bacteria	1. Spring Hill College, Mobile, Ala. 2. University of Kansas Medical Center, Kansas City 3. Virginia Polytechnic Institute, Blacksburg
	Radiation microbiology (five lectures)	NSF-AEC Summer Institute in Radiation Biology, Lawrence, Kan.
Adler, H. I. (and Alice A. Hardigree)	A genetic locus affecting cell division and radiation sensitivity in *Escherichia coli* K-12 [*Bacteriol. Proc.*, p. 39 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
	Genetic control of cell division and radiation resistance in *Escherichia coli* [*Radiation Res.* **22**, 165 (1964)]	Radiation Research Society, 12th Annual Meeting, Miami Beach, Fla.
Albright, J. F.	Aspects of cytodifferentiation associated with soluble antibody response	Paterson Laboratories, Manchester, England
	Some cytokinetic aspects of antibody formation	1. University of Bern, Bern, Switzerland 2. University of Turin, Turin, Italy
	The cellular basis of antibody production	Free University of Berlin, Berlin, Germany
Albright, J. F. (and Takashi Makinodan)	Dynamics of expression of competence of antibody-producing cells	International Symposium on Molecular and Cellular Basis of Antibody Formation, Prague, Czechoslovakia
Allen, R. C.	New techniques in the study of plasma proteins and enzymes in health and disease	Fort Detrick, Frederick, Md.
Anderson, N. G.	Viruses, centrifuges, and cancer	Virginia Chapter of Sigma Xi, Charlottesville
	Virus isolation by zonal ultracentrifugation	Michigan State University, East Lansing
	Zonal centrifugation	1. DuPont Experimental Station, Wilmington, Del. 2. Massachusetts Institute of Technology, Cambridge
Anderson, N. G. (C. L. Burger and H. P. Barringer)	Virus isolation by continuous flow centrifugation combined with banding [*Federation Proc.* **23**, 140 (1964)]	Fedration Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Arnold, W. A.	An electron-hole picture of photosynthesis	International Conference on Photosensitization in Solids, Chicago
Barnett, W. E.	Interspecies aminoacyl-sRNA formation	Indiana University School of Medicine, Indianapolis
	Role of RNA in protein synthesis	Auburn University, Auburn, Ala.
Barnett, W. E. (and K. B. Jacobson)	Evidence for degeneracy and ambiguity in interspecific amino acyl—sRNA formation [*Federation Proc.* **23**, 165 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Baum, R. H.	The isolation of a monosubstituted naphthoquinone from *Streptococcus faecalis*, 10C1	Syracuse University, Syracuse, N.Y.
Bell, Sandra (and Sheldon Wolff)	Studies on the mechanism of the effect of FUdR on chromosomes [*ASB Bull.* **11**, 39 (1964)]	Assoc. SE Biol., 25th Annual Meeting, Atlanta, Ga.
Bender, M. A.	Radiation and human chromosomes	University of Michigan, Ann Arbor
Bertsch, W. F.	Recent concepts of photosynthetic energy conversion	Centro de Studi Nucleari della Casaccia, Comitato Nazionale per l'Energia Nucleare, Rome, Italy
	Solid state physical aspects of photosynthetic quantum conversion	University of Tennessee, Knoxville
	The millisecond delayed light emission from early steps of photosynthetic energy conversion	Xth International Botanical Congress, Edinburgh, Scotland
	The spectrum of delayed light emission at 1.5 milliseconds	Fourth International Photobiology Congress, Oxford, England
Bollum, F. J.	Biosynthetic polydeoxynucleotides	1. Rockefeller Institute, New York City 2. Michigan State University, East Lansing
	Chemically defined templates and initiators for deoxypolynucleotide synthesis	101st Annual Meeting of the National Academy of Sciences, Washington, D.C.
	Chemistry and biology of DNA	UT-AEC Agricultural Research Laboratory Seminar, Oak Ridge
	Initiators and templates in DNA synthesis	University of Southern California, Los Angeles
	Synthetic DNA's	1. California Institute of Technology, Pasadena 2. Syntex Institute for Molecular Biology, Palo Alto, Calif. 3. University of Illinois, Urbana
Bollum, F. J. (Eleanor Groeniger and Masahiko Yoneda)	Polydeoxyadenylic acid [*Federation Proc.* **23**, 218 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Cameron, I. L.	Autoradiographic analysis of giant cells in the mouse placenta [*Anat. Record* **148**, 268 (1964)]	Am. Assoc. Anatomists, 77th Annual Meeting, Denver, Colo.
Cammarano, Piero (Giovanni Giudice, Marialuisa Melli, and G. D. Novelli)	Stimulation of amino acid incorporation by preparations from regenerating rat liver [*Federation Proc.* **23**, 218 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Canning, R. E. (and N. G. Anderson)	Separation of subcellular fractions with a new zonal rotor	AIBS (Am. Soc. Zool.), Boulder, Colo.
Caro, L. G.	Replication of DNA during bacterial mating	1. Rockefeller Institute, New York City 2. Johns Hopkins Medical Center, Baltimore
Chu, E. H. Y.	Action of ultraviolet radiation on mammalian cells	1. University of Alabama Medical Center, Birmingham 2. University of Alabama, Tuscaloosa
	Human cytogenetics (two lectures)	University of Chicago, Chicago
Cohn, W. E (F. Pochon, A. M. Michelson, M. Grunberg-Manago, L. Dondon, and W. E Cohn)	Poly-pseudouridylic acid: synthesis and some physico-chemical and biochemical properties [*Federation Proc.* **23**, 218 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Cole, B. T.	Fatty acid constituents of lipid compounds in *Astasia longa*	Annual Meeting of the South Carolina Academy of Sciences, Aiken
Congdon, C. C	Secondary diseases in radiation chimeras	Jefferson Medical College, Philadelphia
	Wasting diseases in mice	Wistar Institute Thymus Conference, Philadelphia
Congdon, C. C (D. G. Doherty and Frances Hacker)	Bone marrow transplantation after intraperitoneal administration of DMBA	Protection and Recovery Discussion Meeting, Chicago
Congdon, C. C (M. A. Kastenbaum and D. A. Gardiner)	Mortality from secondary disease in homologous bone marrow chimeras [*Federation Proc.* **23**, 202 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago
Cosgrove, G. E.	Radiation effects in mammals	1. Health Physics Training Program, ORNL, Oak Ridge 2. Oak Ridge School of Reactor Technology (ORSORT), Oak Ridge
Cosgrove, G. E. (and L. H. Smith)	Effects of AET and shielding on induction of glomerulosclerosis in irradiated mice [*Radiation Res.* **22**, 180 (1964)]	Radiation Research Society, 12th Annual Meeting, Miami Beach, Fla.
Cudkowicz, Gustavo	Genetic interaction in the inheritance of mouse histocompatibility	Wistar Institute of Anatomy and Biology, Philadelphia

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Cudkowicz, Gustavo (and J. H. Stimpfling)	Immunological basis for deficient growth of C57BL/10 parental marrow in F_1 hybrid mice [*Federation Proc.* **23**, 202 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago
Curtis, Roy, III	An *Escherichia coli* K-12 Hfr strain with the fertility factor attached to or within the structural gene for β-galactosidase [*Bacteriol. Proc.*, p. 30 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
Darden, E. B.	Current research projects in mammalian radiation biology. II	U.S. Army Nuclear Science Seminar, Oak Ridge
de Serres, F. J.	Mutagenesis and chromosome structure	Symposium on The Molecular Action of Mutagenic and Carcinogenic Agents, Gatlinburg, Tenn. (ORNL Biology Division Symposium)
	The difference between x-ray-induced reparable and irreparable recessive lethal mutations in the *ad-3* region of *Neurospora* [*Radiation Res.* **22**, 182 (1964)]	Radiation Research Society, 12th Annual Meeting, Miami Beach, Fla.
Doherty, D. G.	Chemical protection against ionizing radiation	1. Oklahoma State University, Stillwater 2. University of Texas Medical Branch, Galveston 3. East Texas State College, Commerce 4. Florida State University, Tallahassee 5. University of Miami School of Medicine, Miami, Fla. 6. University of Florida, Gainesville 7. Texas Woman's University, Denton
	Enzymes — the biological catalysts	1. Texas Woman's University, Denton 2. Texas A & M University, College Station 3. Auburn University, Auburn, Ala.
Dolin, M. I. (and K. B. Jacobson)	Optical activity attributable to conformation in a pyridine dinucleotide	Sixth International Congress of Biochemistry, New York City
Dolin, M. I. (E. F. Phares and Mary V. Long)	Bound pyridine nucleotide of malic-latic transhydrogenase [*Federation Proc.* **23**, 427 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Duggan, D. E. (and H. I. Adler)	Genetic analysis in *Escherichia coli* K-12 of a double mutant sensitive to radiation [*Bacteriol. Proc.*, pp. 39–40 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
Ehling, U. H.	Frequency of x-ray induced presumed dominant mutations affecting the skeleton of mice [*Genetics* **50**, 246 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Finamore, F. J.	Oligonucleotides in amphibian embryonic development	Southern Illinois University, Carbondale, Ill.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Finamore F. J.	Ribonucleic acids in embryonic development	Charlottesville, Va.
	The oligonucleotide nature of acid-soluble RNA in amphibian eggs and embryos [*Federation Proc.* **23**, 526 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Fisher, W. D. (and R. E. Canning)	Isolation of macroglobulin by rate zonal centrifugation	AIBS (Am. Soc. Zool.), Boulder, Colo.
Fujimura, Robert (and Paul Kaesberg)	Viral RNA polymerization *in vivo* [*Federation Proc.* **23**, 526 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Gardiner, D. A.	A three-dimensional bioassay: more information for less experimentation	UT-AEC Agricultural Research Laboratory Seminar, Oak Ridge
Gaulden, Mary Esther (J. G. Carlson and John Jagger)	Changes induced in mitosis and nucleolar organizer activity by ultraviolet microbeam irradiation of the nucleolus	Fourth International Photobiology Congress, Oxford, England
Goodman, Joan W.	Test for parental antigens not expressed in F_1 hybrid mice [*Federation Proc.* **23**, 202 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago
Green, J. G.	Ion-exchange chromatography in biological research	Symposium on Liquid Chromatography, Earl M. Seagraves Co., Charlotte, N.C.
Grell, E. H.	Influence of the location of a chromosome duplication on crossing over in *Drosophila melanogaster* [*Genetics* **50**, 251–52 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Grell, Rhoda F.	Distributive pairing in *Drosophila melanogaster*	Division of Biology and Medicine, USAEC, Washington, D.C.
	Fourth chromosome nondisjunction and the length of free X-duplications [*Genetics* **50**, 252 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Grizzard, Tom	The accumulation and retention of cesium^{137} by fresh water fish	Tri-Beta Biological Fraternity, Atlanta, Ga.
Gude, W. D.	Current research projects in mammalian radiation biology. I	U.S. Army Nuclear Science Seminar,
Haber, A. H.	The inadequacies of the cell theory in explanations of morphogenesis	Washington University, St. Louis
Haber, A. H. (and D. E. Foard)	Use of radiation-induced mitotic inhibition in studies of differentiation	Xth International Botanical Congress, Edinburgh, Scotland
Harrison, A. P., Jr. (and L. E. Franklin)	Thymineless death of *Aerobacter aerogenes* and protection by culture filtrates [*Bacteriol. Proc.*, pp. 111–12 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
Heddle, J. A.	The kinetics of neutron- and x-ray-induced chromosomal aberrations in barley [*Radiation Res.* **22**, 194 (1964)]	Radiation Research Society, 12th Annual Meeting, Miami Beach, Fla.
Hollaender, Alexander	New developments in radiation biology	The Hebrew University, Jerusalem, Israel
	Principles and perspectives in radiobiology (two lectures)	IAEA-sponsored International Course on Cellular and Molecular Aspects of Radiobiology, Radioisotope Training Centre, Rehovoth, Israel

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Igarashi, Satomi (and R. F. Kimball)	Temperature sensitive mutations induced by x ray in *Paramecium* [*Genetics* **50**, 258 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Jacobson, K. B.	Ribonucleic acid: fact and fancy	1. Gettysburg College, Gettysburg, Pa. 2. Mt. St. Joseph College, Emmitsburg, Md. 3. Western Maryland College, Westminster, Md.
	Studies on lactic dehydrogenase	1. Gettysburg College, Gettysburg, Pa. 2. Mt. St. Joseph College, Emmitsburg, Md. 3. Western Maryland College, Westminster, Md.
Jagger, John	Indirect photoreactivation in *Escherichia coli*	Institute of Genetics, University of Cologne, Cologne, Germany
	1. Life on other worlds 2. Ultraviolet radiation — a tool for the study of life 3. Why be a scientist?	Norris High School, Norris, Tenn.
Jagger, John (and R. S. Stafford)	Relative contributions of direct and indirect photoreactivation in strains of *Escherichia coli*	Fourth International Photobiology Congress, Oxford, England
Johnson, E. J. (and H. D. Peck, Jr.)	Coupling of phosphorylation and carbon dioxide fixation in a cell-free system from *Thiobacillus thioparus* [*Bacteriol. Proc.*, p. 91 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
Karasaki, Shuichi	1. The sites of nuclear RNA synthesis during amphibian embryogenesis 2. Studies on cellular changes during Wolffian lens regeneration	Marine Biological Laboratory, Woods Hole, Mass.
	Ultrastructural study of Wolffian lens regeneration in the adult newt	Information Meeting on Lens Differentiation, Biology Division, ORNL, Oak Ridge
Kenney, F. T.	Regulation of enzyme levels in liver	Trinity College, Dublin, Ireland
Kenney, F. T. (D. L. Greenman and W. D. Wicks)	RNA synthesis in response to steroid hormones	NATO Advanced Study Institute on "Mechanisms of Hormone Action," Meersburg am Bodensee, Germany
	Stimulation of RNA synthesis by steroid hormones	Sixth International Congress of Biochemistry, New York City
Kimball, R. F.	Physiological genetics of the ciliates	Upstate Medical Center, State University of New York, Syracuse
	Studies on reparable premutational lesions with alkylating agents [*Genetics* **50**, 262 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Kimball, R. F. (and Stella W. Perdue)	Synthesis of RNA by fragments of the old macronucleus in *Paramecium aurelia* undergoing autogamy (read by title)	AIBS (Am. Soc. Protozool.), Boulder, Colo.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Krivanek, J. O.	Nucleic acids in the developing slime mold *Dictyostelium discoideum* [*ASB Bull.* 11, 49 (1964)]	Assoc. SE Biol., 25th Annual Meeting, Atlanta, Ga.
Long, T. J.	Some metabolic aspects of sporocarp development in the mushroom *Collybia velutipes*	1. University of Florida, Gainesville 2. Vanderbilt University, Nashville, Tenn. 3. North Carolina State University, Raleigh
Makinodan, Takashi	A mechanism of antibody formation	Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.
	1. Kinetics of antibody formation 2. Problems in radiation immunology	University of Chicago, Chicago
Malling, H. V.	Mutagenic action of nitrous acid	Rice University, Houston
Manney, T. R.	Properties of supersuppressible mutants of yeast	Yale University, New Haven, Conn.
Manney, T. R. (R. K. Mortimer and T. R. Manney)	Further evidence that super-suppressible mutants of yeast are nonsense mutants [*Genetics* 50, 270 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Mans, R. J.	DNA stimulated incorporation of ribonucleotides by extracts of maize seedlings [*Plant Physiol.* 39, Iii (1964)]	AIBS (Am. Soc. Plant Physiol.), Boulder, Colo.
Mazur, Peter	Physical-chemical factors underlying injury in frozen cells	Harvard University, Cambridge, Mass.
Mead, C. G.	Enzymic condensation of oligodeoxyribonucleotides [*Federation Proc.* 23, 373 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Muhammed, Amir	Purification of the photoreactivating enzyme from yeast	Sixth International Congress of Biochemistry, New York City
Oakberg, E. F.	Genetic effects of radiation	U.S. Army Nuclear Science Seminar, Oak Ridge
Odell, T. T., Jr.	Biological effects of radiation on mammals	U.S. Army Nuclear Science Seminar, Oak Ridge
	Regulation of blood platelet production and homeostasis	Wood Veterans Administration Center, Milwaukee, Wis.
Odell, T. T., Jr. (T. P. McDonald, and C. W. Jackson)	Hypertransfusion of blood platelets and platelet regulation	Conference on Blood Platelets, Biology Division, ORNL, Oak Ridge
Padilla, G. M.	Cell division synchrony	University of California, Davis
Padilla, G. M. (G. L. Whitson and G. M. Padilla)	Stomagenesis in temperature-synchronized *Tetrahymena pyriformis* and its inhibition by actinomycin D	AIBS (Am. Soc. Zool.), Boulder, Colo.
Peck, H. D., Jr.	Studies on the mechanism of adenosine-5'-phosphosulfate reductase	University of Georgia, Athens

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Peck, H. D., Jr.	The coupling of phosphorylation and CO_2-fixation in extracts of *T. thioparus*	University of Wisconsin, Madison
Perkins, E. H.	The role of peritoneal phagocytic cells in antibody response [*Federation Proc.* **23**, 344 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Assoc. Immunol.), Chicago
Phillips, Stephen (John Jagger and Stanley Person)	Near ultraviolet action spectrum for induction of division delay in *Escherichia coli* B [*Radiation Res.* **22**, 224 (1964)]	Radiation Research Society, 12th Annual Meeting, Miami Beach, Fla.
Pochon, F. (A. M. Michelson, M. Grunberg-Manago, L. Dondon, and W. E Cohn)	Poly-pseudouridylic acid: preparation with polynucleotide phosphorylase and some chemical and biochemical properties	Sixth International Congress of Biochemistry, New York City
Popp, Diana M. (and R. A. Popp)	A non-H-2 erythrocyte isoantigen in RFM mice [*Genetics* **50**, 276–77 (1964)] (read by title)	AIBS (Genetics Society), Boulder, Colo.
Popp, R. A. (and Diana M. Popp)	Competitive growth of hematopoietic cells in lethally irradiated mice	Protection and Recovery Discussion Meeting, Chicago
Randolph, M. L.	Production and decay kinetics of free radicals produced by irradiation of amino acids	Biophysics Colloquium, University of Arkansas Medical Center, Little Rock
Ritossa, F. M. (R. C. von Borstel and H. Swift)	The action of ribonuclease on salivary gland cells of *Drosophila* [*Genetics* **50**, 279 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Roberts, Paul	Spontaneous and x-ray-induced exchange in *Drosophila melanogaster* [*Genetics* **50**, 280 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Russell, Liane B.	Genetic activity of the mammalian X chromosome	Yale University, New Haven, Conn.
	Genetic and functional mosaicism in the mouse	Symposium of the Society for the Study of Development and Growth, "Role of the Chromosomes in Development," Amherst, Mass.
Russell, Liane B. (Jean W. Bangham and Clyde S. Montgomery)	The use of X-autosome translocations in the mouse for the study of properties of autosomal genes [*Genetics* **50**, 281–82 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Russell, W. L.	Effect of radiation dose fractionation on mutation frequency in mouse spermatogonia [*Genetics* **50**, 282 (1964)]	AIBS (Genetics Society), Boulder, Colo.
	The effect of dose, dose rate, and dose fractionation of gamma, x, and neutron irradiation on mutagenesis in mice	Donner Laboratory, Berkeley, Calif.
Sado, Toshihiko (I. H. B. Hoppee and Paul Nettesheim)	Further cytokinetic studies on antibody response [*Federation Proc.* **23**, 345 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Assoc. Immunol.), Chicago

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Sansing, N. G.	Deoxyribonuclease activity in the roots of germinating maize [*Plant Physiol.* **39**, Iii–Iiii (1964)]	AIBS (Am. Soc. Plant Physiol.), Boulder, Colo.
Schram, Eric	Etude analytique des ribosomes 30s et 50s d'*Escherichia coli* (in French)	Meeting of the Belgian Biochemical Society, Brussels, Belgium
Schram, Eric (and H. Roosens)	Application de la "disc"-électrophorèse et de la scintillation à l'étude des protéines ribosomiales marquées au soufre-35 (in French)	Meeting of the Belgian Biochemical Society, Brussels, Belgium
Setlow, Jane K.	The molecular basis of photoreactivation	1. Laboratorio Internazionale di Genetica e Biofisica, Naples, Italy 2. Experimental Radiopathology Unit and Microbial Genetics Unit, Hammersmith Hospital, London
Setlow, Jane K. (and M. E. Boling)	Evidence for photoreversal of an ultraviolet lesion other than the thymine dimer	Fourth International Photobiology Congress, Oxford, England
Setlow, R. B.	Physical changes and mutagenesis	Symposium on The Molecular Action of Mutagenic and Carcinogenic Agents, Gatlinburg, Tenn. (ORNL Biology Division Symposium)
	The effects of uv on DNA and their relations to error-correcting mechanisms	1. Carnegie Institution of Washington, Cold Spring Harbor, N.Y. 2. Laboratorio Internazionale di Genetica e Biofisica, Naples, Italy 3. Istituto Superiore di Sanita, Rome, Italy 4. Institute for Biological Research, Ness-Ziona, Israel
Setlow R. B. (and W. L. Carrier)	Thymine dimers and the recovery of cells from the effects of uv irradiation	Fourth International Photobiology Congress, Oxford, England
Seto, Frank	Age study of natural and immune hemagglutinin formation in chickens	AIBS (Am. Soc. Zool.), Boulder, Colo.
Smith, L. H.	Radiation protection and recovery measures	U.S. Army Nuclear Science Seminar, Oak Ridge
Stapleton, G. E.	Radiation resistance and balanced synthesis of macromolecules in *Escherichia coli* [*Bacteriol. Proc.*, p. 40 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
Stimpfling, J. H. (Gustavo Cudkowicz and J. H. Stimpfling)	Deficient growth of parental marrow grafts in F_1 hybrid mice: Association with the H-2^b allele [*Genetics* **50**, 243 (1964)]	AIBS (Genetics Society), Boulder, Colo.
Swartzendruber, Donald	Cellular changes in mouse spleen following injection of chicken erythrocytes [*Anat. Record* **148**, 343 (1964)]	Am. Assoc. Anatomists, 77th Annual Meeting, Denver, Colo.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Swenson, P. A. (and R. B. Setlow)	The wavelength dependence of the inhibition of DNA synthesis in radiation sensitive and resistant bacteria	Fourth International Photobiology Congress, Oxford, England
Takata, Chinami (and J. F. Albright)	Lens antigens in Wolffian lens regeneration	Information Meeting on Lens Differentiation, Biology Division, ORNL, Oak Ridge
Tyndall, R. L. (K. B. Jacobson and Ernestine Teeter)	Analysis of RNA from virus-infected HeLa cells [*Bacteriol. Proc.*, p. 121 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
Upton, A. C.	Cancer research 1964: thoughts on the contribution of radiation biology	Am. Assoc. Cancer Research, 55th Annual Meeting, Chicago
	Effects on mammals	Radioecology Institute, ORINS Training Building, Oak Ridge
	Neoplasia in mammals	USPHS--University of Colorado Comparative Pathobiology Conference, Aspen, Colo.
	Response of cells to radiation injury	Armed Forces Institute of Pathology, Washington, D.C.
Upton, A. C. (J. W. Conklin and M. L. Randolph)	Observations on "wasted" radiation in chronically irradiated mice [*Radiation Res.* **22**, 245 (1964)]	Radiation Research Society, 12th Annual Meeting, Miami Beach, Fla.
Uziel, Mayo	Characterization of the reaction products of pancreas RNase A and 1,5 difluoro-2,4-dinitobenzene (read by title)	Sixth International Congress of Biochemistry, New York City
Van Dreal, P. A.	Biochemical events associated with temperature synchronized cell division in *Astasia longa*	University of South Florida, Tampa
Volkin, Elliot	Nucleic acid enzymology	Union Carbide Research Institute, Tarrytown, N.Y.
Volkin, Elliot (and M. Helen Jones)	Biochemical transformations of bacteriophage T2 DNA	Sixth International Congress of Biochemistry, New York City
	Disproportionation in the transfer of parental T2 DNA deoxynucleotides to progeny DNA [*Bacteriol. Proc.*, p. 135 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
von Borstel, R. C.	Recent developments in research on radiation and chemical mutagen effect on insects	Panel on Advances in Insect Control by the Sterile Male Technique, IAEA, Vienna, Austria
	Spontaneous and acridine-induced reversions during mitosis and meiosis in yeast	1. International Laboratory of Genetics and Biophysics, Naples, Italy 2. Biophysics Institute, University of Geneva, Geneva, Switzerland
von Borstel, R. C. (Martha J. Bond and C. M. Steinberg)	Spontaneous reversion rates of a supersuppressible mutant during mitosis and meiosis [*Genetics* **50**, 293 (1964)]	AIBS (Genetics Society), Boulder, Colo.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
von Borstel, R. C. (G. E. Magni, R. C. von Borstel, and S. Sora)	Mutation induction and antimutagenesis by 5-aminoacridine in the dark in yeast [*Genetics* **50**, 266 (1964)]	AIBS (Genetics Society), Boulder, Colo.
von Borstel, R. C. (O. L. Miller, Jr., Romance F. Carrier, and R. C. von Borstel)	X-ray-induced breakage of lampbrush chromosomes *in vivo* and *in vitro*	Chromosome Research Conference, Oxford, England
Walburg, H. E., Jr. (E. I. Mynatt and D. M. Robie)	Survival of x-irradiated germfree mice [*Radiation Res.* **22**, 247—48 (1964)]	Radiation Research Society, 12th Annual Meeting, Miami Beach, Fla.
Wallace, R. A.	Vitellogenesis and protein transport	Marine Biological Laboratory, Woods Hole, Mass.
	Yolk-granule structure and formation	The Ottawa Biological and Biochemical Society, Ottawa, Canada
Whitson, G. L.	Cell differentiation in ciliated protozoa	University of South Florida, Tampa
	Evidence for RNA synthesis during regeneration in *Stentor coeruleus*	AIBS (Am. Soc. Protozool.), Boulder, Colo.
Wicks, W. D. (and F. T. Kenney)	Stimulation of RNA synthesis by testosterone [*Federation Proc.* **23**, 482 (1964)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Williams, G. R. (and G. D. Novelli)	Effect of preillumination in an amino acid incorporating system from etiolated plants [*Plant Physiol.* **39**, Ii (1964)]	AIBS (Am. Soc. Plant Physiol.), Boulder, Colo.
Williams, Joy P. (J. T. Davidson and H. D. Peck, Jr.)	Components of the formic hydrogenlyase from *Desulfovibrio desulfuricans* [*Bacteriol. Proc.*, p. 110 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
Wolff, Sheldon	Are sister chromatid exchanges sister strand crossovers or radiation induced exchanges?	Brookhaven National Laboratory, Upton, N.Y.
	1. Introduction to radiation genetics 2. Biophysical and biochemical studies on radiation mutagenesis. I 3. Biophysical and biochemical studies on radiation mutagenesis. II	Cornell University, Ithaca, N.Y.
	Radiation effects as measured by chromosome damage	18th Annual Symposium on Fundamental Cancer Research, M. D. Anderson Hospital and Tumor Institute, Houston
	Time of chromosome splitting as revealed by combined labeling and x-ray experiments	Washington University, St. Louis
Wust, C. J.	Aspects of diversities in biological disciplines	St. Mary's University, San Antonio, Tex.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Wust, C. J.	Biochemical studies of antibody synthesis	1. Our Lady of the Lake College, San Antonio, Tex. (two lectures) 2. St. Mary's University and Trinity University (jointly), San Antonio, Tex. 3. Incarnate Word College, San Antonio, Tex.
	The effect of actinomycin D on the immune response [*Bacteriol. Proc.*, p. 64 (1964)]	Am. Soc. Microbiol., 64th Annual Meeting, Washington, D.C.
Yamada, Tuneo	A cell-biological study of lens regeneration	University of Utrecht, Utrecht, Netherlands
	An autoradiographic and immunochemical study of lens-regeneration	University of Marburg, Marburg, Germany
Yamada, Tuneo (Chinami Takata and J. F. Albright)	Appearance and localization of lens antigens in transformation of the newt iris into the lens	Association for Research in Ophthalmology (Eastern Section Meeting), New York City
Yamada, Tuneo (Chinami Takata, Sara Eisenberg, and P. J. Kohonen)	The pattern of macromolecular synthesis in Wolffian lens regeneration	Information Meeting on Lens Differentiation, Biology Division, ORNL, Oak Ridge
Zimmerman, B. K.	Primary activity of cross-linked DNA for *in vitro* DNA synthesis	Sixth International Congress of Biochemistry, New York City

VISITING LECTURERS

During the six months preceding this report, 37 lectures were given on the Biology Division Seminar Program by guest speakers from scientific institutions and universities in this country and abroad. Visiting lecturers included scientists from Belgium, Brazil, England, France, Germany, Italy, Mexico, Scotland, Sweden, and Uruguay.

SPEAKER	AFFILIATION	SUBJECT
Seymour Abrahamson	Department of Zoology, University of Wisconsin, Madison	Radiation studies with Drosophila
Peter Alexander	Chester Beatty Research Institute, London, England	Variations in the radiosensitivity of primary tumours: immunological and radiobiological factors
Erik Arrhenius	The Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden	Effect of carcinogenic amines on hepatic protein synthesis
K. C. Atwood	Department of Microbiology, University of Illinois, Urbana	Some aspects of the control of gene action
Charlotte Auerbach	Institute of Animal Genetics, Edinburgh, Scotland	Studies on mutagenesis at Edinburgh
D. E. Buetow	Gerontology Branch, Baltimore City Hospitals, Baltimore	Cellular responses during carbon deprivation and repletion in *Euglena*
David Catcheside	Department of Microbiology, University of Birmingham, Birmingham, England	Some aspects of allelic recombination
Orio Ciferri	Istituto di Genetica, Università de Pavia, Pavia, Italy	Resistance to actinomycin in *Bacillus subtilis*
M. M. Cohen	Department of Human Genetics, The University of Michigan Medical School, Ann Arbor	The effects of certain antibiotica on human chromosomes
Ben-Zion Dorfman	Department of Biology, Yale University, New Haven, Conn.	Allelic complementation and recombination in yeast
J. R. Einstein	Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York City	Protein crystallography
Thomas Eisner	Department of Entomology and Limnology, New York State College of Agriculture, Cornell University, Ithaca	The chemical language of arthropods
R. L. Elder	Department of Radiological Sciences, The Johns Hopkins University, Baltimore	Dark repair of ultraviolet damages in *Micrococcus lysodeikticus*, and in *Hemophilus influenzae*-transforming DNA
John Godfrey	Oxford University, London, England	The evolution of sexual isolation in a rodent
Roland Goutier	Section of Physiological Chemistry, Centre d'Etude de l'Energie Nucléaire, Mol-Donk, Belgium	The acid desoxyribonuclease activity in normal and irradiated tissues

SPEAKER	AFFILIATION	SUBJECT
D. M. Greenberg	Division of Biochemistry, University of California Medical School, San Francisco	Studies on the use of enzymes in the treatment of cancer with special reference to glutaminase
Walter Harm	Institut für Genetik der Universität zu Köln, Köln-Lindenthal, West Germany	Suppression and repair in ultraviolet-irradiated phage systems
R. D. Holsten	Laboratory for Cell Physiology, New York State College of Agriculture, Cornell University, Ithaca	Organization and metabolism in cultured plant cells: some factors affecting growth induction
Gerhart Hotz	Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, Germany	Radioprotection of normal and base-substituted phage-DNA by compounds of the cysteine-cysteamine group
L. Ledoux	Section of Cellular Biochemistry, Centre d'Etude de l'Energie Nucléaire, Mol-Donk, Belgium	Separation and biological behavior of fractionated DNA
Walter Marshall	Radiobiological Unit, Harwell, England	Mössbauer effect in the study of proteins
Taiju Matsuzawa	The Lobund Laboratory, University of Notre Dame, Notre Dame, Ind.	Studies on radiation leukemia and acute intestinal radiation death in germfree mice
Heather D. Mayor	Department of Virology and Epidemiology, College of Medicine, Baylor University, Houston	An experimental and theoretical approach to capsid formation in icosahedral viruses
Susumu Ohno	Department of Biology, City of Hope Medical Center, Duarte, Calif.	Phylogeny of vertebrate sex chromosomes and the origin of X-linked genes
Crodowaldo Pavan	Department of General Biology, University of São Paulo, São Paulo, Brazil	Nucleic acid synthesis and differentiation in polytene chromosomes
R. M. Pearlstein	University of Maryland, College Park	Photosynthetic unit and 2-quantum hypothesis in photosynthesis
G. B. Petersen	Oxford University, Oxford, England	Studies of pyrimidine nucleotide sequences in DNA
T. H. Pittinger	Agronomy Department, Kansas State College, Manhattan	Somatic recombination in Neurospora
E. L. Powers	Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Ill.	Isotope effects and radiation sensitivity
José Ramirez	Departamento de Bioquimica, Centro de Investigacion y Estudias, Instituto Politecnico Nacional, Mexico City, Mexico	Changes in fluorescence of heart muscle during mechanical activity
H. H. Rossi	Department of Radiology, College of Physicians and Surgeons, Columbia University, New York City	Distribution of radiation energy in the cell
A. Ruhenstroth	Max-Planck Institute, Munich, Germany	Studies on the electrophoretic mobility of blood cells — prompt effects of radiation

SPEAKER	AFFILIATION	SUBJECT
F. A. Saez	Department of Cytogenetics, Instituto de Investigacion de Ciencias Biologicas, Montevideo, Uruguay	Heterochromatinization gradient in the evolution of neo-X neo-Y sex determination system
L. Szabo	Centre de Recherche sur les Celles Normales et Cancereuses, Ville-jeuf, France	Some new approaches to the synthesis and analysis of sugars and sugar phosphates of biochemical interest
Adolph Wacker	Institut für Therapeutische Biochemie, Universität Frankfurt, Frankfurt am Main, Germany	Photoinactivation and photoreactivation
Robert Wheat	Department of Biochemistry, Duke University Medical Center, Durham, N.C.	Biochemistry of Viosamine, 4-amino-4:6-dideoxy-D-glucose
A. A. Yousten	Army Chemical Corps, Ft. McClellan,	Succinic acid degradation by bacteria

SPEAKERS AT PROFESSIONAL MEETINGS, FALL–WINTER 1964

Gordon Research Conference on Cancer – O. L. Miller, Jr.

Fifteenth Annual Meeting of the American Institute of Biological Sciences – Speakers and their papers are listed by title in the Lectures section

148th National Meeting of the American Chemical Society – A. H. Warner (coauthor, F. J. Finamore) and R. B. Setlow (Symposium on Helical Molecules)

Tenth Congress of the International Society of Haematology – T. T. Odell, Jr. (coauthors, T. P. McDonald and C. W. Jackson), C. C Congdon (coauthors, M. A. Kastenbaum and D. A. Gardiner), Gustavo Cudkowicz (coauthors, Michael Bennett, G. M. Shearer, and A. C. Upton), A. C. Upton (coauthors, Niel Wald, B. K. Jenkins, E. B. Darden, Jr., and Sylvia Pan), L. H. Smith, and Joan W. Goodman

Tenth Congress of the International Society of Blood Transfusion – Joan W. Goodman and Gustavo Cudkowicz (coauthor, J. H. Stimpfling)

International Society for Cell Biology, Eleventh Congress – L. G. Caro

International Conference on Organogenesis – Tuneo Yamada (coauthors, Chinami Takata and J. F. Albright)

CNRS Meeting on Graft of Allogenic Hematopoietic Cells – Joan W. Goodman and G. Cudkowicz

Nineteenth Annual Meeting of the Society of General Physiologists – P. A. Swenson (coauthor, R. B. Setlow) and T. H. Turney, Jr. (coauthor, K. B. Jacobson)

Technicon International Symposium – Herbert Schuel (coauthor, N. G. Anderson) and S. R. Tipton (coauthors, Herbert Schuel and N. G. Anderson)

Fifteenth Annual Meeting of the Animal Care Panel – M. L. Simmons (coauthors, H. E. Williams and Everline B. Wright), D. M. Robie (coauthors, H. E. Walburg, Jr., and Edna I. Mynatt), and H. E. Walburg, Jr. (coauthors, Edna I. Mynatt, G. E. Cosgrove, R. L. Tyndall, and D. M. Robie)

American Veterinary Radiology Society – G. E. Cosgrove

Southeast Electron Microscopy Society – L. G. Caro

Third Spore Conference – J. E. Donnellan, Jr. (coauthors, E. H. Nags and H. S. Levinson)

Third International Symposium on Advances in Enzyme Regulation – F. T. Kenney (coauthors, D. L. Greenman, W. D. Wicks, and W. L. Albritton)

New York Academy of Sciences Conference on Forms of Water in Biologic Systems – Peter Mazur

Sixth Medical Symposium – A. C. Upton

Cell Synchrony Conference – P. A. Van Dreal (coauthor, N. G. Anderson), G. L. Whitson (coauthors, G. M. Padilla and W. D. Fisher), G. M. Padilla (coauthors, I. L. Cameron and O. L. Miller, Jr.), and I. L. Cameron

American Cancer Society Conference on Cryobiology – Peter Mazur

Second Symposium on Protection Against Radiations in Space – G. E. Stapleton and M. A Bender (coauthor, H. F. Smith, Jr.)

Conference on the Mechanisms of the Dose Rate Effect at the Genetic and Cellular Level – Sheldon Wolff, R. F. Kimball, E. F. Oakberg, and W. L. Russell

Southeastern Section Meeting of the American Physical Society – M. L. Randolph

Fourth Annual Meeting of the American Society for Cell Biology – R. A. McGrath, Sandra Bell (coauthor, Sheldon Wolff), G. L. Whitson (coauthors, G. M. Padilla and L. H. Elrod), Ellen Mattingly (coauthor, K. M. Jakob), I. L. Cameron (coauthor, E. E. Guile, Jr.), Tuneo Yamada (coauthor, Chinami Takata), Shuichi Karasaki, O. L. Miller, Jr., J. E. Trosko (coauthor, Sheldon Wolff), and D. C. Shepard

Third Mammalian Cytology and Somatic Cell Genetics Conference – J. G. Brewen, P. Carolyn Gooch (coauthor, M. A Bender), E. H. Y. Chu, and M. A Bender

Eighth International Congress of Internal Medicine – M. A Bender

Symposium on Genes and Chromosomes: Structure and Function – Sheldon Wolff, D. L. Lindsley, Jr., Rhoda Grell, and F. J. de Serres

Reticuloendothelial Society – C. J. Wust (coauthor, M. G. Hanna, Jr.)

American Society of Zoologists – R. A. Popp, D. C. Swartzendruber (coauthor, M. G. Hanna, Jr.), R. F. Kimball (coauthor, Stella W. Perdue), M. G. Hanna, Jr., P. W. Whiting, Judith G. Heddle (coauthors, R. A. Popp, R. E. Canning, and R. C. Allen), R. C. Allen (coauthor, D. J. Moore), J. E. Trosko (coauthors, E. H. Y. Chu and W. L. Carrier), and I. L. Cameron (coauthor, W. D. Fisher)

FOREIGN TRAVEL

Members of the Biology Division traveled to Europe, Asia, and South America during this report period. They attended and participated in meetings and conferences, presented lectures and seminars, and consulted with other scientists.

In February, Alexander Hollaender attended the 13th session of the United Nations Scientific Committee on The Effects of Atomic Radiation in Geneva, Switzerland. In April, he traveled to Tel Aviv, Rehovoth, and Jerusalem, Israel. Dr. Hollaender gave two lectures entitled "Principles and perspectives in biology" at the IAEA-sponsored International Course on Cellular and Molecular Aspects of Radiobiology at the Radioisotope Training Centre, Rehovoth, on April 20–21. He also spoke on "New developments in radiation biology" at the Hebrew University, Jerusalem. On his return trip, Dr. Hollaender attended a World Health Organization meeting in Geneva, May 1–2.

Eric Schram, who spent the latter part of 1963 in the Biology Division as a visiting investigator from abroad, presented two papers concerning his work in the Division at a local meeting of the Belgian Biochemical Society, Brussels, in May.

Also in May, F. T. Kenney went to Dublin, Ireland, to consult with members of the Biochemistry Department at Trinity College. While there, he lectured on "Regulation of enzyme levels in liver." Then he attended, as an invited participant, a symposium on Mechanisms of Hormone Action, an NATO

Advanced Study Institute, in Meersburg am Bodensee, Germany. His speech was entitled "RNA synthesis in response to steroid hormones." Kenney concluded his trip with a visit to Madrid, Spain, where he consulted with staff members of the Centro de Investigaciones Biologicas.

M. A Bender was in Rio de Janeiro, Brazil, for one month from May 13 to June 13 advising and collaborating with the Instituto de Biofisica of the University of Brazil. While in Brazil, he made side trips to Guarapari and to São Paulo.

En route to Prague, Czechoslovakia, J. F. Albright visited the Universities of Turin (Italy) and Bern (Switzerland). At each of these institutions he lectured on "Some cytokinetic aspects of antibody formation." In Prague, he presented a paper entitled "Dynamics of expression of competence of antibody-producing cells" at the International Symposium on Molecular and Cellular Basis of Antibody Formation (June 1–5). After the meeting in Prague, Dr. Albright visited Germany, France, and England. In Germany, he spoke on "The cellular basis of antibody production" at the Free University of Berlin; in France, he visited the Institut Pasteur in Paris; and in England he visited Guy's Hospital Medical School, London, and Christie Hospital and Holt Radiation Institute, Manchester. While in Manchester, he gave a talk entitled "Aspects of cytodifferentiation associated with soluble antibody response" at the Paterson Laboratories.

Tuneo Yamada was in Scotland, England, the Netherlands, France, and Germany in June and July. He discussed research programs and culture techniques with fellow investigators at laboratories and universities in each of these countries. He presented lectures on "A cell-biological study on lens regeneration" at the University of Utrecht, Utrecht, Netherlands, and on "An autoradiographic and immunochemical study of lens regeneration" at the University of Marburg, Marburg, Germany.

Six members of the Biology Division participated in the Fourth International Congress of Photobiology, Oxford, England, July 26–30. Alexander Hollaender, a past president of the Congress, and now a Free Member of the Executive Committee of the Comité Internationale de Photobiologie (sponsor of the congress), served as chairman of the session on "Photochemistry of Nucleic Acids and Its Biological Implications." John Jagger was rapporteur for this session. Attending and submitting papers were:

W. F. Bertsch	The spectrum of delayed light emission at 1.5 milliseconds
John Jagger	Relative contributions of direct and indirect photoreactivation in strains of *Escherichia coli*
Jane K. Setlow	Evidence for photoreversal of an ultraviolet lesion other than the thymine dimers
R. B. Setlow	Thymine dimers and the recovery of cells from the effects of uv irradiation

R. B. Setlow was also coauthor of a paper, "The wavelength dependence of the inhibition of DNA synthesis in radiation-sensitive and resistant bacteria," presented by P. A. Swenson, formerly with the Biology Division as a USPHS fellow. Although unable to attend in person, Mary Esther Gaulden participated through submission of an invited paper, "Changes induced in mitosis and nucleolar organizer activity by ultraviolet microbeam inactivation of the nucleolus." A. H. Haber also attended this conference. Each of the investigators attending the Photobiology Congress also visited other institutions during his sojourn abroad.

Dr. Hollaender reviewed the Italian radiobiology program in Rome and Casaccia and considered sites for the 1966 International Congress of Radiation Research at Venice and Cortina. He also consulted with colleagues at the Chester Beatty Research Institute, London, England, and continued discussions on a world health research center with the World Health Organization in Geneva, Switzerland.

W. F. Bertsch attended the Tenth International Botanical Congress in Edinburgh, Scotland, and presented a paper entitled "The millisecond delayed light emission from early steps of photosynthetic energy conversion." He also traveled to Utrecht and Leiden, Netherlands; Copenhagen, Denmark; Berlin, Germany; and Rome and Naples, Italy. While in Rome, he lectured on "Recent concepts of photosynthetic energy conversion" at the Centro de Studi Nucleari della Casaccia.

Before the Photobiology Congress, Dr. John Jagger visited laboratories in Frankfurt and Cologne, Germany, and in Paris, France. In Cologne, he spoke on "Indirect photoreactivation in *Escherichia coli*" at the Institute of Genetics of the University of Cologne. After the congress, he visited fellow scientists at the Rothamsted Experimental Station, Hertsford, England.

Jane K. Setlow traveled to Naples, Rome, and Paris, as well as to England, during this trip abroad. She spoke on "The molecular basis of photoreactivation" at the Laboratorio Internazionale di Genetica e Biofisica in Naples, and the Experimental Radiopathology Unit and Microbial Genetics Unit, Hammersmith Hospital, London, as well as holding discussions with colleagues at various institutions in each of the cities visited.

R. B. Setlow also visited Naples and Rome prior to his attendance at the Photobiology Congress. He lectured on "The effects of uv on DNA and their relations to error-correcting mechanisms" at the Laboratorio Internazionale di Genetica e Biofisica, Naples, and at the Istituto Superiore di Sanita in Rome. Then he went to Israel where he visited the Weizmann Institute in Rehovoth, the Hebrew University in Jerusalem, and the Technion in Haifa. At the Institute for Biological Research, Ness-Ziona, Israel, he spoke on the same subject as in Naples and Rome.

In July and August, R. C. von Borstel was in Italy, Austria, Switzerland, and England. On July 20–24, he attended an IAEA panel meeting on Insect Control by the Sterile Male Technique in Vienna, at which he spoke on "Recent developments in research on radiation and chemical mutagen effect on insects." Before the meeting he visited colleagues at the Laboratorio Internazionale di Genetica e Biofisica in Naples and lectured on "Spontaneous and acridine-induced reversions during mitosis and meiosis in yeast." On July 27–28 he visited the World Health Organization in Geneva and the University of Geneva. At the latter institution he lectured on the same subject as in Naples. The last three days of the month were spent in England, where Dr. von Borstel attended the Chromosome Research Conference, Oxford, and presented a paper "X-ray-induced breakage of lampbrush chromosomes *in vivo* and *in vitro*."

A. H. Haber presented a paper entitled "Use of radiation-induced mitotic inhibition in studies of differentiation" at the Tenth International Botanical Congress in Edinburgh, Scotland.

E. F. Oakberg visited McGill University, Montreal, Canada, from August 10–16, to discuss methods of identification and quantitation of cell types in the seminiferous epithelium of man.

Also in Canada, R. A. Wallace spoke to the Ottawa Biological and Biochemical Society on the subject of "Yolk-granule structure and formation," before his return to the United States from a year's leave of absence.

EIGHTEENTH ANNUAL BIOLOGY RESEARCH CONFERENCE

The 1965 Research Conference, sponsored by the Biology Division of the Oak Ridge National Laboratory, will be held April 5–8 at the Riverside Motor Lodge in Gatlinburg, Tennessee. The organizing committee, F. T. Kenney (chairman), A. H. Haber, K. B. Jacobson, D. L. Lindsley, R. C. von Borstel, and Tuneo Yamada, have announced the following tentative program:

HORMONAL CONTROL OF PROTEIN BIOSYNTHESIS

Monday, April 5

Control of Enzyme Levels

Morning

Chairman – W. Eugene Knox, Cancer Research Institute, New England Deaconess Hospital, Boston, Massachusetts

Hormonal influences on the synthesis of lactic dehydrogenase isozymes – Nathan O. Kaplan, Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts

Glucocorticoid control of glutamic-alanine transaminase synthesis – Harold L. Segal, Department of Pharmacology, St. Louis University School of Medicine, St. Louis

Afternoon

Chairman – Sidney P. Colowick, Department of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee

Insulin dependent synthesis of glucokinase – A. Sols, Instituto G. *Maranon*, Centro de Investigaciones Biologicas, Madrid, Spain

Hormonal control of enzymes participating in gluconeogenesis and lipogenesis – Henry Lardy, Institute for Enzyme Research, University of Wisconsin, Madison

Gibberellic acid controlled synthesis of α-amylase in barley endosperm – J. E. Varner, RIAS, Baltimore

Tuesday, April 6

Control of Chromosome Activity

Morning

Chairman – Crodowaldo Pavan, Department of General Biology, University of São Paulo, São Paulo, Brazil

Biochemical studies of ecdysone control of chromosomal activity – Peter Karlson, Physiologisch-chemisches Institut der Universität, München, Germany

Genetic studies of ecdysone control of chromosomal activity – Hans J. Becker, Zoologisches Institut der Universität, München, Germany

Afternoon

(Free)

Wednesday, April 7

Control of RNA Synthesis

Morning

Chairman – Elliot Volkin, Biology Division, Oak Ridge National Laboratory

Estrogen control of uterine RNA and protein synthesis – Jack Gorski, Department of Physiology, University of Illinois, Urbana

Androgen control of RNA and protein synthesis in male accessory organs – H. G. Williams-Ashman, The Ben May Laboratory for Cancer Research, University of Chicago

Afternoon

Chairman – Arthur B. Pardee, Program in Biochemical Sciences, Princeton University, Princeton, New Jersey

Hydrocortisone stimulation of RNA synthesis in induction of hepatic enzymes – F. T. Kenney, Biology Division, Oak Ridge National Laboratory

Allosteric proteins and RNA synthesis in hormonal inductions – Gordon M. Tomkins, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

Growth hormone effects on RNA and protein synthesis in liver – Asher Korner, Department of Biochemistry, University of Cambridge, Cambridge, England

Thursday, April 8

Control in Neoplastic Tissues

Morning

Chairman – Philip P. Cohen, Department of Physiological Chemistry, University of Wisconsin Medical School, Madison

Regulation of enzyme levels in rat hepatomas – Henry C. Pitot, McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison

Conference Summary – Van R. Potter, McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison

General Discussion

Final Summation – Van R. Potter

1964 BIOLOGY SYMPOSIUM

The Proceedings of the Seventeenth Annual Biology Symposium, *The Molecular Action of Mutagenic and Carcinogenic Agents*, held April 6–9, 1964, in Gatlinburg, Tennessee, are being published as an October supplement to the *Journal of Cellular and Comparative Physiology*.

BIOMEDICAL DIRECTORS MEETING

The Biomedical Directors of the United States Atomic Energy Commission held their spring meeting at the Biology Division in Oak Ridge, May 11–12. The first day was devoted to regular sessions in the large conference room. After opening remarks by S. R. Sapirie and a welcome by A. M. Weinberg, Alexander Hollaender outlined the program of the Laboratory. Several section leaders then summarized the Division's activities. On the second morning the visitors toured Division research laboratories and consulted with individual scientists; in the afternoon there was an executive session of the AEC Division of Biology and Medicine.

BONE MARROW CONFERENCES

A Radiation Protection and Recovery discussion meeting was held in Chicago on April 13. C. C Congdon presented a paper coauthored by D. G. Doherty and Frances Hacker. R. A. Popp served as chairman of the meeting and gave a paper coauthored by Diana M. Popp.

Additional meetings on bone marrow transplantation are to be held in Paris, France, September 7–9, and in Houston, December 4–5.

The Paris meeting is being organized by Georges Mathé. Joan W. Goodman and Gustavo Cudkowicz will present papers at this conference, while C. C Congdon, who coauthored a paper to be given by A. L. Kretchmar, will serve as cochairman of the session on *Tolerance of the Host with Regard to the Graft.* T. T. Odell, Jr., will also attend.

Daniel Billen and John Trentin are organizing the December meeting to be held at the M. D. Anderson Hospital and Tumor Institute, Houston.

SYMPOSIUM ON THE NUCLEOLUS, ITS STRUCTURE AND FUNCTION

On May 24–26, 1964, Alexander Hollaender (Committee President), Mary Esther Gaulden, O. L. Miller, Jr., and R. C. von Borstel met with W. S. Vincent, Department of Anatomy, University of Pittsburgh, Pittsburgh, Pa., R. P. Perry, The Institute for Cancer Research, Philadelphia, and Committee Vice President F. A. Saez, Institute of Biological Sciences, Montevideo, Uruguay, to prepare the program for a meeting on *The Structure and Function of the Nucleolus* to be held in Montevideo, December 6–10, 1965.

Committee members in addition to those named above are Honorary President Clemente Estable, Instituto de Investigacion de Ciencias Biologicas, Montevideo; M. E. Drets, Instituto de Investigacion de Ciencias Biologicas, Montevideo; G. Gerard, Facultad de Humanidades y Ciencias, University of Montevideo; Dr. Polletti, Faculty of Medicine, University of Montevideo, and J. I. Valencia, University of Buenos Aires, Argentina.

A tentative program has been prepared and will be announced in the spring of 1965.

SMALL CONFERENCES

LENS DIFFERENTIATION

A conference on Lens Differentiation, organized by Tuneo Yamada, was held at the Biology Division on March 20–21. The program included papers by three Division members – Shuichi Karasaki, Chinami Takata (coauthor, J. F. Albright) and Tuneo Yamada (coauthors, C. Takata, S. Eisenberg, and J. Kohonen). Eighteen papers were presented by investigators from several other states. Subjects of discussion included experimental analysis of lens development and regeneration, the ultrastructure of normal and regenerating lenses, cell biology of lens differentiation, and biochemical aspects.

BLOOD PLATELETS

The Second Conference on Blood Platelets was held July 10–11 at the Biology Division, Oak Ridge National Laboratory. Approximately 80 scientific investigators from the United States and Canada attended the meeting which was organized by T. T. Odell, Jr. Abstracts of the conference were submitted for publication in *Blood* and also appeared in a booklet published by the Biology Division.

CELL SYNCHRONY

A committee comprised of G. M. Padilla (chairman), I. L. Cameron, and G. L. Whitson has planned a program of 20 papers on Cell Synchrony to be held October 9–10 at the Biology Division. The abstracts of the papers presented will be published in a Biology Division booklet and will cover different methods of cell synchrony and the physiological, biochemical, structural, and mathematical aspects of synchronization.

EDUCATION

Student Trainee Program. – During the summer months sixteen students between their junior and senior years at undergraduate colleges, principally in the Southeast, worked under the direct supervision of a Biology Division senior investigator. Each student was required to present a final oral and written report of his summer's work.

The 1964 student trainees, their affiliations, the groups to which they were assigned, and their supervisors were:

STUDENT TRAINEE	INSTITUTION	ASSIGNED TO
Ballard, Anne H.	Randolph-Macon Woman's College, Lynchburg, Va.	C. J. Wust, Enzymology
Davis, Glenda A.	Alabama College, Montevallo	A. H. Haber, Plant Physiology
Gaskins, Patricia I.	Columbia College, Columbia, S.C.	D. E. Foard, Plant Physiology
Grant, W. C.	Livingston College, Salisbury, N.C.	C. C Congdon, Mammalian Recovery
Guile, E. E., Jr.	Morehouse College, Atlanta, Ga.	I. L. Cameron, Cytochemistry and Cell Reproduction
Heath, Connie J.	Centenary College, Shreveport, La.	E. F. Oakberg, Mammalian Genetics
Huffman, G. H.	Texas Lutheran College, Sequin	F. J. Bollum, Molecular Biology
Matheson, Rebecca J.	Mount Mercy College, Pittsburgh, Pa.	Peter Mazur, Cell Biophysics
Mills, Jane B.	Randolph-Macon Woman's College, Lynchburg, Va.	M. P. Stulberg, Enzymology
Phillips, Myra D.	University of Chattanooga, Chattanooga, Tenn.	L. H. Smith, Mammalian Recovery
Randall, G. R.	Oklahoma City University, Oklahoma City	W. D. Fisher, Cell Physiology
Renshaw, Janet	University of Richmond, Richmond, Va.	Roy Curtiss III, Radiation Microbiology
Shaffer, Carolyn R.	San Diego College for Women, San Diego, Calif.	R. A. McGrath, Biophysics
Stewart, Diane M.	Hood College, Frederick, Md.	P. A. Roberts, Drosophila Cytology and Genetics
Walker, Susan C.	Trinity College, Washington, D.C.	W. D. Gude, Pathology and Physiology
Willett, Margaret L.	Brescia College, Owensboro, Ky.	E. H. Perkins, Radiation Immunology

Four other students who were not under the Student Trainee Program were selected to train in the same manner. They were:

NAME	INSTITUTION	ASSIGNED TO
Albritton, W. L.	Medical College of Alabama, Birmingham	F. T. Kenney, Enzymology
Groeniger, Eleanor	Johns Hopkins University, Baltimore	F. J. Bollum, Molecular Biology
Kendrick, Joseph	University of Tennessee, Knoxville	C. C Congdon, Mammalian Recovery
Leininger, S. R.	School of Veterinary Science and Medicine, Purdue University, Lafayette, Ind.	M. L. Simmons, Experimental Animal Facility

Small College Program. – Two Biology Division members participated in the Small College Program during this report period. H. I. Adler presented a lecture entitled "Genetic control of radiation sensitivity in bacteria" at Spring Hill College, Mobile, Alabama, and at Virginia Polytechnic Institute, Blacksburg. K. B. Jacobson spoke on "Ribonucleic acid: fact and fancy" and "Studies on lactic dehydrogenase" at Western Maryland College, Westminster; at Mt. St. Joseph College, Emmitsburg, Maryland; and at Gettysburg College, Gettysburg, Pennsylvania.

Science Fair. – S. F. Carson served as judge for the AEC special awards at the 15th National Science Fair-International, Baltimore, May 6.

Courses. – Five members of the Biology Division participated in the U.S. Army Nuclear Science Seminar held in the ORINS Special Training Building in August. Speakers and titles were:

W. D. Gude	Current research projects in mammalian radiation biology I
E. B. Darden	Current research projects in mammalian radiation biology II
T. T. Odell, Jr.	Biological effects of radiation on mammals
E. F. Oakberg	Genetic effects of radiation
L. H. Smith	Radiation protective and recovery measures

Those who attended the seminar were given a tour of the Biology Division on August 18.

H. I. Adler presented a series of lectures on "The effects of radiation on microorganisms" at the National Science Foundation–Atomic Energy Commission–sponsored Summer Institute in Radiation Biology at the University of Kansas, Kansas City and Lawrence, Kansas, in July.

W. D. Gude taught a University of Tennessee histology course, at the graduate level, in the spring quarter. The course was given at the Biology Division.

Alexander Hollaender gave two lectures on "Principles and perspectives in radiobiology" as part of an IAEA-sponsored international course on Cellular and Molecular Aspects of Radiobiology at the Radioisotopes Training Centre, Rehovoth, Israel, on April 20–21.

In March, John Jagger presented talks on "Life on other worlds," "Ultraviolet radiation – a tool for the study of life," and "Why be a scientist?" to students at Norris High School under the Visiting Scientist Program of the Tennessee Academy of Sciences.

C. M. Steinberg presented a three-week series of lectures in the Bacteriophage Genetics course given at the Cold Spring Harbor Laboratory for Quantitative Biology, Cold Spring Harbor, New York, in July and August.

In July, A. C. Upton spoke on "Neoplasia in mammals" in the Comparative Pathobiology course, sponsored by the U. S. Public Health Service and the University of Colorado, at Aspen, Colorado. Dr. Upton also lectured on "Effects on mammals" to graduate students attending the Radioecology Institute at the ORINS Special Training Building, Oak Ridge, in June.

Sheldon Wolff presented three lectures on radiation genetics at Cornell University, Ithaca, New York, in May. He was a visiting lecturer on genetics under a training grant. Titles of his speeches were: "Introduction to radiation genetics" and "Biophysical and biochemical studies on radiation mutagenesis I and II."

Courses Attended. – D. E. Duggan attended the Cold Spring Harbor Summer Course in Bacterial Viruses, Cold Spring Harbor, New York, July 13–August 4.

W. D. Gude attended a course on Introduction to General Pathology at the Harvard Medical School, Boston, Massachusetts, June 22–July 11.

L. J. Serrano attended an NAS-NRC Institute of Laboratory Animal Resources Workshop on Graduate Education in Laboratory Animal Medicine in Washington, D. C., March 25–27.

DIVISION MEMBERS ON LEAVE

D. R. Krieg is on leave of absence from the Division during which time he is with the Department of Chemistry, Stanford University, Palo Alto, California, working under the sponsorship of a special United States Public Health Service fellowship and the Oak Ridge National Laboratory.

R. A. Wallace, who has been on assignment with the National Research Council, Ottawa, Canada, carrying out collaborative work between the Biology Division and the Council's Division of Applied Biology, has returned to the Laboratory.

1. Cytology and Genetics

R. F. Kimball

Effects of Radiation on Paramecium (1.1–1.5)

R. F. Kimball
Satomi Igarashi[a]
G. L. Whitson[b]
E. G. Bailiff
Barbara R. Bussell
H. Marine Payne
Stella W. Perdue
Carrie L. Wells

Phage Genetics and Molecular Photobiology of DNA (1.6–1.9)

D. R. Krieg[c]
Jane K. Setlow
C. M. Steinberg
Amir Muhammed[a]
M. E. Boling
Ursula B. Steinberg[d]

Chromosome Cytology (1.10–1.16)

Sheldon Wolff
M. Ellen Mattingly
Sandra L. Bell[d]
K. M. Jakob[a]
Te Hsiu Ma[e]
J. E. Trosko[f]
H. E. Luippold
Agnes R. Patterson
J. A. Heddle[a,g]

Fungal Genetics (1.17–1.21)

F. J. de Serres[h]
H. V. Malling[h]
B. B. Webber[h]
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1.1 STUDIES ON CHEMICAL MUTAGENESIS IN *PARAMECIUM*[1]

R. F. Kimball H. Marine Payne
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Introduction. – The studies reported in the previous semiannual report[2] on chemical mutagenesis in *Paramecium aurelia* have been continued. Most of the work has been done with the alkylating agent, triethylenemelamine (TEM), which has very little effect on the cell cycle at mutagenic doses. The procedures used are those in our previous work on mutagenesis. Paramecia in known stages during the cell cycle were treated with the mutagen for a brief period of time and allowed to undergo a

number of cell divisions. Autogamy was then induced to produce homozygosis, and the amount of mutation estimated from the frequency of inviable and slowly growing autogamous isolates.

Results. – The dose curve has been worked out for TEM. If dose is expressed as concentration times time of exposure, then there is a linear relation between our measure of mutation and dose over a fourfold range of doses. The relation is the same whether the concentration or the time is varied.

The maximum amount of mutation is induced by treatment with TEM shortly before DNA synthesis begins, and less mutation is induced the earlier in the presynthesis interphase (G1) the treatment is given. Treatment of stationary phase cells yields less mutation than treatment of log phase cells, and the amount of mutation decreases the longer the stationary phase conditions are maintained after treatment. These results are similar to those obtained with x rays and can be interpreted the same way; that is, the mutagen produces premutational lesions, which are subject to repair until DNA synthesis, when the remaining lesions are fixed as mutation.

TEM produces about a third as much mutation in G2 as in G1. X rays also produce less mutation in G2, but no more than a tenth as much instead of a third as much. The meaning of this quantitative difference between the two mutagens is not clear.

Conclusions. – The general pattern of stage sensitivity with TEM is much the same as with x rays, although there are quantitative differences. The x-ray pattern can be interpreted in terms of reparable premutational damage that is converted to fixed mutation by DNA synthesis.[3] The same interpretation can be applied to the mutation production by TEM and is supported by the earlier evidence[2] that streptomycin posttreatment decreases the amount of TEM-induced mutation. These findings support the view that the cell is able to repair a wide variety of different initial lesions.

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1.2 EFFECT OF MUTATION MODIFYING TREATMENTS ON MACROMOLECULAR SYNTHESES IN *PARAMECIUM*

R. F. Kimball Stella W. Perdue

Introduction. – In earlier work, we[1] developed the view that various postirradiation treatments decrease mutation by increasing the time to chromosome doubling, thus allowing more time for repair of premutational lesions. There was also evidence that the same treatments decreased the rate of repair, presumably through their effects on cellular metabolism. The decrease in mutation is thus the consequence of the relative effectiveness of two opposing actions of the treatments. One of the most used posttreatments in our work has been exposure for 3 hr to 3 mg/ml of streptomycin. Another and somewhat less effective procedure is exposure to 0.5 mg/ml of chloramphenicol for 4 hr. Indirect evidence was available that both these treatments delayed chromosome doubling in the micronucleus, the nucleus in which mutations are detected. It seemed desirable to get more direct evidence for this and at the same time to get more information on the effect of these treatments on other macromolecular syntheses.

Methods. – Dividing paramecia were collected, and half an hour after division they were put into 3 mg/ml of streptomycin for 3 hr or into 0.5 mg/ml of chloramphenicol for 4 hr. Untreated controls were run at the same time. At various times during and after the exposure to the antibiotics, some of the paramecia were put into tritiated cytidine and uridine to label RNA or into tritiated leucine to label protein. The antibiotic was included in the mixture for the groups labeled during exposure. The paramecia were dried on slides immediately after a brief labeling period, autoradiographs were prepared, and the number of grains per unit area was determined. The time of chromosome doubling in the micronucleus was determined by measurements of cross-sectional area in the same preparations. This procedure has been found to give results in good agreement with DNA determinations by Feulgen microspectrophotometry and can be used in autoradiographs where microspectrophotometry is difficult.

Results. – Transfer to medium containing the antibiotics produces a rapid inhibition of the incorporation of both leucine and the ribonucleosides. No marked difference in this respect was

found between the two antibiotics, although streptomycin produced a somewhat greater inhibition of the incorporation of leucine than did chloramphenicol. Recovery of the normal rate of incorporation was delayed at least 3 hr after removal from streptomycin; a lesser time for chloramphenicol. Chloramphenicol delayed micronuclear chromosome doubling by between 3 to 4 hr on the average, but there seemed to be a greatly increased spread of times as compared with the controls. Streptomycin delayed doubling on the average by more than 4 hr, but the exact time was not determined because of too short a time series.

Discussion. – There are several conclusions from the present study that must be considered in any quantitative interpretation of the effect of these two treatments on mutation. (1) Both streptomycin and chloramphenicol inhibit RNA as well as protein synthesis in this organism, at least at concentrations that can be used to decrease mutation. (2) The rate of repair of premutational damage, if it is influenced by the rate of RNA and protein synthesis, probably does not return to normal before the time of chromosome doubling, when all repair ceases. (3) Micronuclear chromosome duplication is delayed, as required by our theory, and, also as expected, to a greater extent by the treatment that produces the greater effect on mutation. (4) The amount of delay in chromosome doubling may vary markedly from specimen to specimen even though they have received ostensibly the same treatment.

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1.3 INHERITANCE OF TEMPERATURE SENSITIVITY IN PARAMECIA

Satomi Igarashi

Introduction. – As described in the previous report,[1] temperature-sensitive clones have been kept in stocks, and they are still temperature-sensitive after several successive autogamies. However, it is essential to confirm that some, if not all, temperature-sensitive animals are true mutations. Thus the cross breeding test was performed.

Method and Results. – Temperature-sensitive animals were newly induced in *Paramecium aurelia*, syngen 4, stock 51, both mating type 7 and 8, with the same method as that used in the previous experiment and kept in stocks. As a control, temperature-insensitive animals, derived from the same originally irradiated animal, were also kept in stocks.

In the cross test, the killer character was adopted as a genetic marker. Both temperature-sensitive and -insensitive animals were crossed with wild-type animals of opposite mating type marked with killer trait. For instance, if the temperature-sensitive animal of mating type 7 was a KK-killer, the mate animal should be mating type 8 of kk-sensitive.

In the F1 generation, both exconjugant animals were temperature-insensitive, while killer trait was dependent on the cytoplasmic parent as expected.

In the F2 generation, the kk-sensitivity was apparently segregated from Kk-killer animals of F1. This indicates that a true cross occurred. The temperature sensitivity segregated from both exconjugant (F1) parents when the original parent was temperature sensitive, but no temperature sensitivity was found in the control.

These facts suggest that temperature sensitivity is the inheritable trait and it is recessive. The segregation ratios have not yet been analyzed statistically, but they were a little lower than the theoretical ratio of 1:1 for both traits, killer and temperature sensitivity. The technique of segregation test should be refined, and the improvement of it is now under way.

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1.4 DNA CONTENT OF ULTRAVIOLET-IRRADIATED *TETRAHYMENA*[1]

D. C. Shepard

Introduction. – In a previous report,[2] it was shown that the macronuclear DNA content of

Tetrahymena was increased considerably during the irradiated cell generation. The amount of DNA had returned to normal by the third postirradiation division. The data have been expanded considerably, and the method by which the cells return to normal has been established.

Methods. – *Tetrahymena pyriformis* W was cultured axenically in 2% proteose peptone at about 25°C. Dividing cells were isolated. After separation, some cells were air dried on microscope slides; $2\frac{1}{2}$ hr after division, others were irradiated with 2000 ergs/mm^2 incident exposure from a germicidal lamp (wavelength 254 mμ). Following each of the next three divisions, some of the cells were air dried on slides. These cells were stained by the Feulgen procedure, and two-wavelength microspectrophotometry was used to measure the relative DNA concent. In other experiments, the cells were transferred at various times to medium containing tritiated thymidine to label DNA. The cells were washed, air dried, and the resulting slides were made into autoradiographs.

Results. – At the time of division of the macronucleus, a small piece is often left behind. This piece, the so-called extrusion body, persists for some time after division but eventually degenerates. The studies with tritiated thymidine show that this body does not synthesize DNA. Consequently, the amount of DNA synthesized between divisions is the difference between the macronuclear DNA content just after the first division and twice the total DNA, including both the macronucleus and the extrusion body, after the second division. These quantities were measured by Feulgen microspectrophotometry, and it was found that about 300 microspectrophotometric units were synthesized during the irradiated cell generation, but in the next generation and thereafter the normal amount, about 100 microspectrophotometric units, was synthesized. Thus only during the irradiated cell generation is there excess synthesis.

The extrusion bodies are unusually large after the first one or two postirradiation divisions. Thus the return to the normal amount of DNA occurs by the elimination of the excess in the extrusion bodies in conjunction with a return to the normal amount of synthesis.

The autoradiographic studies show that the label from tritiated thymidine appeared in the extrusion bodies whether the labeling was done prior to irradiation, between irradiation and the first division, or at later times. There is no evidence that the excess DNA formed as a result of irradiation has special properties that make it more likely than DNA formed at other times to be eliminated in the extrusion bodies.

Studies on the uptake of tritiated thymidine by unirradiated sister cells were used to establish the stage in the cell cycle that was irradiated. In this way, it was shown that the same excess DNA synthesis occurred whether the cells were irradiated during or after the normal DNA synthesis period.

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1.5 THE ROLE OF RNA DURING REGENERATION IN *STENTOR COERULEUS*

G. L. Whitson

Introduction. – Studies concerned with alterations in nucleocytoplasmic ratios in regenerating *Stentor* point out the lack of any direct evidence indicating that renewed RNA synthesis or the presence of RNA is necessary in order for these cells to regenerate.[1] Recent findings, however, indicate that renewed RNA synthesis is necessary for oral regeneration to occur in isolated tail pieces from cut, starved cells.[2] This report presents additional evidence suggesting that preexisting RNA is also essential for the formation of new oral structures during regeneration.

Methods. – Whole *Stentor* were fed for several hours on *Tetrahymena* prelabeled with ^{3}H-cytidine and then washed free of the labeled *Tetrahymena*. Several of these cells were then fixed, sectioned, and autoradiographed for controls. The remaining cells were placed in ribonuclease (1 μg/ml) and then removed at different intervals, washed several times in fresh culture fluid, and cut for regeneration. For comparison, whole cells as well as cut cells were autoradiographed.

Results and Discussion. – Sixty percent of the isolated tail pieces of whole cells exposed for 30 min to the ribonuclease regenerated,

whereas only 20 percent of the tail pieces regenerated when exposed for 1 hr to ribonuclease. Although the whole cells remained viable for several hours after being exposed for 2 hr to ribonuclease, no isolated tail pieces regenerated after this treatment. Cells, which were exposed to the ribonuclease and then fixed and sectioned, showed an increase vacuolation of their cytoplasm and a marked decrease in radioactivity in autoradiographs. These results, although not conclusive, support the view that the presence of RNA as well as the resynthesis of RNA is necessary for regeneration in *Stentor*.

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1.6 THE RANGE OF PHOTOREACTIVABLE LESIONS IN DNA

Jane K. Setlow M. E. Boling

Introduction. – It is possible to detect the presence of photoreactivable lesions in biologically inactive polynucleotides by observing their competition with ultraviolet-irradiated transforming DNA for photoreactivating enzyme from yeast. The rate of increase of transforming ability of ultraviolet-inactivated DNA illuminated with photoreactivating enzyme may be depressed by the presence of various irradiated polynucleotides. By combining such competition experiments with chemical assay of ultraviolet lesions in synthetic and natural polynucleotides, a series of dimers have been found to be the specific sites in irradiated DNA which are concerned with photoreactivation.

Results and Discussion. – The synthetic compound poly dA:dT, with a single type of base in each chain, competes readily in photoreactivation after ultraviolet irradiation. Thymine dimers are formed in this compound by ultraviolet irradiation, and they are eliminated by illumination with the yeast enzyme, along with the ability to compete. At large doses of short wavelength ultraviolet, the number of thymine dimers reaches a constant level, and so does the competing ability. The alternating polymers poly d(AT) and poly dA do not compete. Thus it is concluded that lesions in A and in a single T cannot be involved in photoreactivation and that the thymine dimer is the only photoreactivable lesion in poly dA:dT.

The competing ability of DNA irradiated with large doses of short wavelength ultraviolet does *not* saturate along with the thymine dimers, but there appears to be another lesion which competes for the thymine dimer in photoreactivation. Irradiated poly dI:dC exhibits similar behavior, and two types of dimers have been shown to be formed in this compound, both of which are eliminated in photoreactivation: a cytosine and a uracil dimer. The cytosine dimer is converted to uracil dimer on heating (there is some conversion even at room temperature). Although the number of cytosine dimers saturates at a low dose of short wavelength ultraviolet, if there is some heating during irradiation and thus some formation of uracil dimer, which comes to equilibrium at a much larger dose, there is enough increase in dimer formation to account for the increase in competing ability with increasing dose. If poly dI:dC is irradiated at the same doses at 5°C, or for a very short time, the competing ability and the number of dimers *do* reach a constant level. Likewise the competing ability of DNA irradiated cold reaches saturation.

It is concluded that various pyrimidine dimers are the photoreactivable lesions in DNA.

1.7 IN VITRO ACTION SPECTRUM FOR *ESCHERICHIA COLI* PHOTOREACTIVATION

Jane K. Setlow M. E. Boling

Introduction. – For an understanding of the mechanism of photoreactivation in cells it is necessary to distinguish between direct repair by photoreactivating light (which apparently acts on a complex of photoreactivating enzyme and DNA containing ultraviolet lesions) and the indirect effect,[1] in which the photoreactivating light causes higher survival of ultraviolet-damaged cells by producing a growth delay, which in turn somehow makes more cellular repair possible. A crude extract of *E. coli* B can be used for photoreactivation of ultraviolet-inactivated transforming DNA from *Hemophilus influenzae*.[2] In this system all indirect photoreactivation is absent. The

wavelength dependence of this direct photoreactivation has been determined for the purpose of comparison with in vivo photoreactivation of whole bacteria, which shows both indirect and direct types together.

Results and Discussion. – Because extracts of *E. coli* contain a high level of nucleases which can destroy the transforming ability of DNA, it was found necessary to add unirradiated DNA to the photoreactivating mixture of transforming DNA and extract. The calf thymus DNA used depressed the level of transformation but did not alter the rate of increase of transforming ability. At different wavelengths of photoreactivating illumination the maximum increase in transforming ability was apparently the same, but the rate of increase at a given intensity differed. The action spectrum is very similar to that of the yeast photoreactivating enzyme,[3] with peaks at 3550 and 3850 A, the latter somewhat higher. The similarity of the two spectra suggests a similarity in the chromophores of the yeast and coli photoreactivating enzymes.

Comparison with the in vivo action spectrum of log-phase *E. coli* B/r (ref. 4) suggests that indirect photoreactivation is effective only at the shorter wavelengths, in accordance with Jagger and Stafford's prediction from dose rate and temperature dependence of photoreactivation on whole cells[5] and with their action spectrum for photoprotection in *E. coli* B,[6] which apparently has a similar indirect mechanism. However, they have concluded from their experiments that direct photoreactivation at 3341 A is considerably less effective than at 4047 A, whereas these two wavelengths are about equally efficient in the cell-free system. This suggests that there may be another as yet unknown complication in photoreactivation of whole cells.

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1.8 STUDIES ON THE YEAST PHOTOREACTIVATING ENZYME

Amir Muhammed

Introduction. – In order to clarify the mechanism of photoreactivation in cells, it is essential to purify the photoreactivating enzyme and study the phenomenon in vitro under controlled conditions. The in vitro photoreactivation system is almost unique in that the enzyme-substrate complex is stable in the absence of light, and the formation and dissociation of the complex (enzyme and transforming DNA containing ultraviolet lesions) can be studied separately. One important question with respect to the mechanism of photoreactivation is the nature of the substance which absorbs the photons. The purification of the yeast photoreactivating enzyme has been undertaken partly to answer this question.

Results. – A purification procedure has been developed so that the enzyme can be routinely purified approximately 2000-fold over the crude yeast extract. There are five principal steps: (1) ammonium sulfate precipitation, with the 35–50% fraction being retained, yielding about a tenfold increase in specific activity; (2) filtration through Sephadex G-100 gel; (3) fractionation on DEAE cellulose columns, using a phosphate buffer for stepwise elution; the enzyme is eluted at a phosphate concentration of 0.12–0.14 *M*, with a 150- to 200-fold increase in specific activity; (4) fractionation on hydroxyapatite columns; the enzyme is eluted with 0.2 *M* K_2HPO_4 at pH 8.5, and the increase in specific activity over the crude extract is 2000- to 2500-fold; and (5) concentration by dialysis against saturated ammonium sulfate at pH 8.0.

The purified enzyme shows the characteristic fluorescence spectrum of flavin nucleotides after boiling. The fluorescence is quenched on addition of sodium hydrosulfite, which is also characteristic of these compounds. These results indicate that the enzyme is probably a flavoprotein.

1.9 THE THEORY OF FORMAL PHAGE GENETICS FOR CIRCULAR MAPS

C. M. Steinberg F. Stahl[1]

Streisinger, Edgar, and Harrar-Denhardt[2] have shown that the genetic map of T4 is topologically equivalent to a circle. That is, any small region of the genome yields a conventional linear map, but the map as a whole has no ends. We have extended the theory of formal phage genetics[3] to cover the case of circular maps. Unfortunately, the results depend somewhat upon how the circularity comes about.

Now the most straightforward interpretation of the circular genetic map is that the phage chromosome is a circle, at least at the time of mating. This interpretation of the circular map is also easy to treat within the above ground rules. We need only specify the probability of exchange as a function of distance for a single mating and then combine this with our previous results to obtain a mapping function. A mating between two circular chromosomes is limited to an even number of exchanges if only intact genomes are to emerge.

Further reflection shows that the requirement for an even number of exchanges is formally more important than the circular structure. In fact, if we assume that the chromosome is structurally a rod but nevertheless requires an even number of exchanges, we obtain formally identical results. We will refer to the above models as the "closed circle" and "rod" respectively.

While we do not explicitly account for heterozygosis in our model, recent experiments with heterozygotes (Streisinger, personal communication) have led to another interpretation of the circular genetic map. This interpretation, which we call the "open circle" model, postulates that the phage chromosome is structurally a rod, but the sequence of genes is not the same in all members of a population of chromosomes. One arrives at a circular map by requiring that the gene sequences within a population of chromosomes are all circular permutations of one another. One can construct such a population of permuted rods by making one randomly placed break in each member of a population of circles. The open-circle model leads to results which are formally distinct from the closed-circle–rod model.

After deriving mapping functions for the above models, we estimate numerical values for the parameters which appear in these functions. The models are then tested against previously published data with the conclusion that each of the two models provides a fairly adequate description of the basic observations of a phage cross.

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1.10 ARE SISTER CHROMATID EXCHANGES SISTER-STRAND CROSSOVERS OR RADIATION-INDUCED EXCHANGES?

Sheldon Wolff

When chromosomes are labeled with tritiated thymidine, it is found that at the second metaphase following labeling, sister chromatid exchanges are visible. Taylor, who first observed this phenomenon, reasoned from the ratio of such exchanges that affect both homologous chromosomes (twin) to those affecting only one member of a homologous pair (single) that the strands within a chromatid are polarized. If the strands were not polarized, a ratio of at least ten singles to one twin would be observed. The actual ratio found was one single to two twins.

The above ratios are based on the hypothesis that the exchanges are caused by spontaneous sister-strand crossing-over that is equally probable in either of the two cell cycles that occur between labeling and the metaphase at which the observations are made. The expected ratio of singles to twins when the strands are polarized is 2 to 1, and not the 1 to 2 observed.

Because (1) there is this discrepancy in the ratios, (2) sister-strand crossing-over has been very difficult (or impossible) to observe genetically, and (3) incorporated tritiated thymidine can induce chromosome aberrations, we decided to analyze the possible results if the sister-strand exchanges were radiation-induced and not spontaneous sister-strand crossovers.

If the strands within the chromatids are polarized, then any exchange that occurred in the first cell cycle would appear as a twin at the second division, whereas an exchange that occurred in the second cycle would be a single. At the first cell cycle both chromatids are labeled. The dose of radiation from incorporated isotope then will be proportional to 2 and the yield of radiation-induced exchanges, which increase as the square of the dose, will be proportional to 4. At the second cell cycle, only one chromatid per chromosome is labeled. The dose of radiation will thus be proportional to 1 as will the yield per diploid genome. There are, however, twice as many chromosomes present, so the yield of singles will be proportional to 2. The expected ratio of two twins to one single is exactly the ratio observed in the experiments.

We conclude that sister-strand exchanges are more likely radiation-induced exchanges than spontaneous sister-strand crossovers.

1.11 EFFECTS OF 5-FLUORODEOXYURIDINE ON DNA SYNTHESIS IN *VICIA FABA*

Sandra L. Bell Sheldon Wolff

Introduction. – 5-Fluorodeoxyuridine (FUdR) has been shown to inhibit DNA synthesis in *E. coli* by irreversibly inactivating the enzyme thymidylate synthetase. Chromosomes of lateral roots of *Vicia faba* have gaps and are fragmented after FUdR treatment. It has been shown that these gaps and fragments can occur in G_2, the post-DNA-synthetic period. Both the effect of FUdR on DNA synthesis in *E. coli* and the chromosome-breaking effect in *Vicia faba* can be reversed or prevented by the addition of exogenous thymidine in high concentrations.

Because the visible effect of FUdR on *Vicia* was chromosome damage rather than an inhibition of DNA synthesis, it was not known if FUdR does, in fact, inhibit DNA synthesis in *Vicia*.

Results. – Lateral roots of *Vicia* were grown in 10^{-5} *M* FUdR and 10^{-4} *M* uridine for 60 min and then transferred for 30 min to ^{3}H-deoxycytidine (rather than thymidine that can reverse the FUdR effect) to label the DNA in the cells in S. Following fixation the roots were hydrolyzed in 1 *N* HCl at 60°C for 12 min and then rinsed at 60°C in TCA for 15 min to remove any label that might have been incorporated into RNA. Autoradiographs showed that there was an almost total suppression of DNA synthesis in the FUdR-treated roots. Control roots grown in 10^{-4} *M* uridine showed considerable DNA synthesis.

Since a concentration of exogenous thymidine greater than the concentration of FUdR is necessary to reverse the chromosome-breaking effect of FUdR, an experiment was undertaken to determine what concentration of thymidine will reverse the inhibitory effect on DNA synthesis. Lateral roots were grown in mixtures of 10^{-5} *M* FUdR, 10^{-4} *M* uridine, and either 10^{-3} or 10^{-5} *M* thymidine, and then transferred to ^{3}H-deoxycytidine for 30 min. Autoradiographs showed that a concentration of thymidine equal to the concentration of FUdR reversed the inhibitory effect on DNA synthesis. Autoradiographs from other experiments in which cells were treated with 10^{-6} *M* FUdR and 5×10^{-8} *M* ^{3}H-thymidine showed that even very low concentrations of thymidine can reverse the FUdR inhibition of DNA synthesis.

Conclusion and Summary. – It has been demonstrated that FUdR inhibits DNA synthesis in a higher plant, *Vicia faba*. This effect on DNA synthesis can be prevented by the addition of low concentrations of thymidine to the FUdR solution. This is in contrast to the chromosome-breaking effect of FUdR, which is prevented only when a higher concentration of thymidine than FUdR is present.

1.12 THE EFFECT OF 5-AMINOURACIL ON DNA SYNTHESIS IN *VICIA FABA* LATERAL ROOTS

K. M. Jakob Sheldon Wolff

Introduction. – The chemical 5-aminouracil (5AU) has been shown to cause partial synchronization of nuclear divisions in *Vicia faba* root meristems. It raises the mitotic index from about 10 to 40%.[1] This occurs about 12 to 14 hr after removal of the seedlings from a 24-hr 700-ppm treatment with the substance.[1] On the basis of earlier work with 5AU,[1–3] it had been assumed that DNA synthesis, possibly through interference with thymidine synthesis, is blocked. Work in this laboratory suggested that these assumptions may have been oversimplifications. Experiments

were therefore performed to see if the effect of 5AU on synchronization could be reversed by thymidine and if DNA synthesis is really affected by 5AU.

Results and Discussion. – Trosko and Jakob[4] supplied lateral roots of *Vicia faba* with thymidine either along with or after 12- and 24-hr 5AU treatments. High concentrations of thymidine similar to those of 5AU, as well as low concentrations similar to those used in an ordinary ^{3}H-thymidine labeling experiment, were used. Mitotic peaks similar to those obtained with 5AU treatment alone were observed. This suggested that the 5AU synchronization could not be altered by the deoxyriboside as might be expected, if thymidine metabolism was affected by the 5AU. Unchanged 5AU peaks were also observed when uracil, deoxyuridine, or uridine was added to 5AU.[4]

The number of cells in the DNA-synthetic portion of the cells cycle (S) was found to increase while the roots are treated with 5AU. When either ^{3}H-labeled deoxycytidine or thymidine was supplied for successive 2-hr periods during a 12-hr 5AU treatment, it was found that the proportion of labeled cells increased from about 45 to about 65%. The increase was obtained between the second and the fourth hour of 5AU treatment. Ribonuclease and deoxyribonuclease treatment of the slides showed that the label was incorporated only into DNA. When the peak of mitosis occurred following the above treatment, it was found that most of the chromosomes were labeled. This indicates that almost all of the cells that reach metaphase after a 12-hr 5AU treatment have synthesized DNA in the presence of 5AU.

Experiments in which 1-hr pulses of ^{3}H-deoxycytidine were given at various times after a 12-hr 5AU treatment was completed showed that the labeling index was near that of the controls 1 hr after 5AU treatment. It then rose at 3 hr and fell again to the control level by 6 hr. This suggests that synchronization had already occurred in midinterphase.

Preliminary counts of grains in autoradiographs of cells given a 2-hr pulse of ^{3}H-thymidine suggest that, although more cells are in S, there is less label incorporated per cell than in the water control.

The above results indicate that DNA synthesis does proceed in the presence of 5AU. The labeling patterns described here are consistent with the interpretation that, in addition to causing a partial block in G2,[5] 5AU may also slow down DNA synthesis so that the affected cells will leave S more slowly.[6] These two factors may contribute to the mitotic synchronization.

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1.13 EFFECTS OF FAR-RED AND RED LIGHT ON CHROMATID ABERRATIONS INDUCED BY X IRRADIATION OF *TRADESCANTIA* MICROSPORES

Te Hsiu Ma Sheldon Wolff

Introduction. – Far-red light has long been believed to have a synergistic effect on chromatid aberrations induced by x rays in *Tradescantia*.[1,2] In studying root tip cells of *Vicia faba*, Wolff and Luippold[3] found that a high peak of chromatid aberrations occurred around 15 hr after 150 r of x rays and at around 18 hr after 150 r of x rays plus 3 hr of far-red treatment. The so-called synergistic effect of x rays and far-red seemed to be caused by a delay of mitosis, so that after far-red light there is a shift in the time at which cells from various parts of the cell cycle reach metaphase where they can be scored. This was verified by the same authors[4] who labeled cells in S and noted that after far-red treatment it took longer for those cells to reach metaphase.

The present study shows that the same far-red induced delay of mitosis is responsible for the apparent synergism in *Tradescantia* and also shows that the red reversibility of the far-red effect on aberrations is caused by a reversal of the mitotic delay.

Results. – Inflorescences of *Tradescantia paludosa* (Sax clone 3) were excised and maintained in aerated spring water for 5–10 hr prior to treatment. Various combinations of treatment are shown in the following table.

Far-Red–X-Ray Experiment

	3 hr	3 hr	$\frac{1}{60}$ hr	3 hr	3 hr
Group 1		Far-red	X ray		
Group 2			X ray	Far-red	
Group 3			X ray (control)		

Far-Red–Red–X-Ray Experiment

Group 1	Far-red	Red	X ray		
Group 2			X ray	Far-red	Red
Group 3			X ray (control)		

A series of eight fixations were made at 1.5-hr intervals from each group starting from 10.5 hr after x irradiation up to 21 hr. Newcomer's fixative and the acetocarmine squash method were used to prepare the microslides. Chromatid aberrations were scored.

The control groups of both experiments showed a high peak at 16.5 hr after x irradiation. Far-red pre- and posttreatment groups showed two peaks of aberration. The first peak (lower magnitude) appeared at 12 hr after x irradiation, and the second peak (originally at 16.5 hr position, higher magnitude) appeared at 19.5 hr position. A 3-hr delay is apparent.

In the far-red–red–x-ray experiment, the high peaks of aberration for both pre- and posttreatment groups appeared at 16.5 hr position. This reveals the antagonistic effect of far-red and red light.

Discussion and Conclusion. – In *Tradescantia* microspores, far-red light delays the mitotic cycle and so shifts the appearance of the peak yields of aberration to later times (about 3 hr later). The peak at 12 hr position indicates that there is another sensitive stage at late G_2. This is not ordinarily seen because radiation-induced pycnosis occurs in cells reaching metaphase within 9 hr after x irradiation. Red light seems to accelerate the mitotic process so that when red light is used immediately following far-red treatment, the delaying effect of far-red is canceled.

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1.14 THE LACK OF INTERACTION OF NEUTRON- AND X-RAY-INDUCED CHROMOSOME BREAKS AND AN ESTIMATE OF THE REJOINING DISTANCE IN BARLEY

J. A. Heddle

Introduction. – In studies on *Vicia*, the lack of interaction between x-ray- and neutron-induced chromosome breaks has been attributed to the spatial relationships of the chromosomal strands and the LET values of the radiations. Equations were developed from which one could estimate a maximal value for the distance over which broken ends could interact.[1] Contrary to the results in *Vicia*, interaction has been reported in barley.[2] This would permit the calculation of both maximal and minimal values of the rejoining distance. The prediction (on the basis of the reported interaction) of two-hit kinetics for neutron-induced two-break aberrations, however, was not borne out by the experiments. Consequently the interaction experiments were repeated, and x-ray kinetic studies were performed.

Results. – Experiments with combined x-ray and neutron irradiation failed to demonstrate interaction. Although a two-hit dose response curve had previously been reported for barley,[3] the x-ray kinetic studies on four-year-old seeds from the 1959 harvest showed linear kinetics for two-break aberrations, suggesting that these seeds were not capable of a two-hit response. Accordingly, both the kinetic studies and the interaction

experiments were repeated on seeds of the 1963 harvest. These seeds did show a two-hit x-ray dose response curve for two-break aberrations, whereas with neutrons these aberrations increased linearly with dose. This conflicted with the published data of Natarajan and Narayanan, so their experiment was repeated. No interaction was found, whether or not x rays preceded neutrons.

Discussion. – The kinetic and interaction experiments support one another in the conclusion that in barley, as in *Vicia*, there is no quantitatively determinable interaction of neutron breaks with either x-ray breaks or independently induced neutron breaks. Consequently, no minimal value of the rejoining distance could be calculated. The maximal value was estimated to be 0.2 μ (in *Vicia* the estimate was 0.3 μ[1]), while the estimate that best fit the data was 0.02 μ.

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1.15 IN VIVO INDUCTION OF THYMINE DIMERS IN MAMMALIAN DNA

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W. Carrier[2]

It has been established that ultraviolet is able to induce chromosome breaks and gene mutations. It has also been shown that the action spectra for chromosome breaks and mutation are approximately parallel to the absorption spectra of nucleic acids. Since it has been demonstrated that the thymine dimers are one of the ultraviolet-induced lesions in DNA, these experiments were undertaken to see if thymine dimers might be responsible, in some way, for the ultraviolet-induced chromosome breaks.

Irradiation of monolayers of Chinese hamster cells with ultraviolet (2652 A, 5 ergs mm^{-2} sec^{-1}), showed that thymine dimers are formed as a linear function of dose within the dose range tested (100–2000 ergs/mm^2). At 100 ergs/mm^2 at 2652 A, about 0.05% of the thymine dimerized. With this same dose, 0.73 chromosome aberration per cell is induced in Chinese hamster cells. Since these cells contain about 5×10^{-12} g DNA, it was calculated that 1.5×10^6 thymine dimers are induced by the same dose that induces 0.73 chromosome aberration per cell.

Because of the demonstration of a dimer excision mechanism existing in certain bacteria,[3,4] it was thought that chromosome breaks might be the result of excision (with incomplete patching) of ultraviolet-induced dimers in chromosomal DNA. A search for an excision process indicated that no such process existed in Chinese hamster cells. Also, a test to see if these ultraviolet-induced dimers could be photoreactivated in vivo demonstrated that there is no photoreactivation. It has been shown earlier that the ultraviolet-induced chromosome breaks are nonphotoreactivable. It is not known whether the photoreactivating enzyme is absent in Chinese hamster cells or only inactive.

It was concluded that ultraviolet radiation can induce thymine dimers in mammalian chromosomal DNA. Also, it has been shown that ultraviolet-induced chromosome breaks are not the result of a mass excision process with an occasional incomplete patching of the removed thymine dimer. We have not ruled out the possibility that ultraviolet-induced thymine dimers can play a role in chromosome breakage.

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1.16 STUDIES ON MITOTIC INHIBITION AND REDUCTION OF GROWTH RATE BY 5-AMINOURACIL

Ellen Mattingly

The original paper by Duncan and Woods[1] which reported work on the cytological effects of the pyrimidine analog 5-aminouracil (5AU) contained some discussion of the inhibitory effect of this chemical on growth rate in the onion root and also

presented evidence of severe mitotic inhibition. Furthermore, it was shown that growth in equimolar concentrations of thymine and 5AU completely overcame the effect on growth rate, although mitotic inhibition was not reversed. This paper was followed by that of Martinez-Pico and Duncan[2] in which it was reported that roots of *Vicia faba* were able to recover spontaneously in the presence of 5AU, and the authors concluded that the rapid decline in mitotic index was the result of inhibition of DNA synthesis. Finally, Smith *et al.*[3] have recently reported that a relatively high degree of cell synchronization (40%) may be obtained in *Vicia* root tips by treatment with 5AU for 24 hr.

Several aspects of this work have been investigated in greater detail in this laboratory. A definite relationship of cell synchronization to root anatomy has been seen. Longitudinal sections show that 5AU affects only those cells which normally divide, that is, it does not seem to induce division in totally differentiated cells which normally do not divide. Therefore, if root cap, epidermal, and cortical cells are excluded, the synchrony induced in true meristematic cells is virtually entire. It is also seen, however, that the relatively broad peak of synchrony (about 8 hr) actually includes two rather discretely synchronized populations. The first group to pass through division are the small cells with spherical nuclei which lie in the extreme tip just anterior to the root cap. At this time, the differentiating, elongate cells of the pericycle are in interphase. Subsequently, it is seen that when the majority of the meristematic cells have passed into G_1, the cells of the pericycle then come synchronously into division. This anatomical phasing of cell synchrony is seen also in labeling studies that show that the differentiating cells of the stele incorporate ^{3}H-thymidine very vigorously for some time after the nondifferentiated meristematic nuclei have ceased to synthesize DNA.

The observations on inhibition of root elongation have been confirmed. In addition, it has been shown that while *Vicia faba* roots may recover in the presence of 5AU, this recovery applies only to mitotic inhibition. Therefore, roots which have remained in 5AU for several days show periodic cycling through division, with very little root elongation.

Finally, the relationship of mitotic inhibition to DNA synthesis has been investigated. Labeling studies with ^{3}H-thymidine show that during the first 8 hr of 5AU treatment, the progress of G_1 cells into S is normal; the number of labeled nuclei increases at the same rate as that of the controls, while during the same time, the mitotic index drops to zero.

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1.17 ANALYSIS OF THE EXTENT OF GENETIC DAMAGE IN *ad-3^{IR}* MUTATIONS IN *NEUROSPORA*

F. J. de Serres

The spectrum of forward mutations induced by x rays in the *ad-3* region includes mutants that can grow both as homokaryons on adenine-supplemented medium (*ad-3^R*) and those that cannot grow as homokaryons even on complete medium (*ad-3^{IR}*).[1] Whereas the *ad-3^R* mutations are intragenic mutations, the *ad-3^{IR}* mutations appear to result from such extragenic alterations as chromosome deletions. The *ad-3^{IR}* mutations show two-hit kinetics and appear to result from events that occur outside of the *ad-3A* or *ad-3B* locus.[2] Various tests have been developed[3,4] to analyze the *ad-3R* mutations to make it possible to determine the nature of the genetic alterations that produce this class of mutations at the molecular level. Characterizations of the mutations in the *ad-3^{IR}* class would appear to be possible by the development of a series of tester strains carrying unknown or known markers for loci in the immediately adjacent genetic regions. Homology tests could then be used to determine the extent of the functional inactivation to the left and/or right in individual *ad-3^{IR}* mutations. The ability to make tests to determine the extent and type of genetic alteration in both *ad-3^R* and *ad-3^{IR}* mutations will make it possible to investigate further the dose dependence of the spectrum of induced mutations with various mutagens.

Two different approaches are being used to develop a series of tester strains for such analyses. In the first approach known genetic markers

both to the left and to the right of the *ad-3* region are being crossed into a genetic background that is heterokaryon-compatible with 74A mutants and marked with genetic markers at the *al-2, cot, inos,* and *pan-2* loci. Dikaryons containing *ad-3*^{IR} mutations will be combined with these tester strains to form trikaryons of the following constitution:

(I) *A hist-2 ad-3A ad-3B nic-2* + ; *ad-2*; + ; *inos*; +

(II) *A* + (*ad-3*^{IR} mutation) *al-2*; + ; *cot*; + ; *pan-2*

(III) *A* (← LGIR marker →) *al-2*; + ; *cot*; *inos*; *pan-2*

Such trikaryons will be plated on pantothenate-supplemented medium and medium supplemented with pantothenate in addition to the supplement of the LGIR marker. The appearance of *cot* dikaryons only on plates of the latter type indicates that the wild-type locus of the LGIR marker has been inactivated in component (II) of the trikaryon. Since there are many closely linked markers adjacent to the *ad-3* region on both sides, such tests should be particularly rewarding. No trikaryon tests using LGIR markers have as yet been used in large scale tests of *ad-3*^{IR} mutations.

The second approach involves homology tests on *ad-3*^{IR} mutations similar to those described in a previous report[5] to select mutations with unique types of genetic damage for use as tester strains. The most recent tests of this type have demonstrated the presence of an especially interesting type of *ad-3* mutation: those that are *ad-3*^{R} mutations with closely linked, but separate, sites of recessive lethal damage (*ad-3*^{R} + *RL* mutations). These were found among a series of x-ray– or nitrous acid–induced *ad-3A* or *ad-3B* mutations, each of which contained genetic damage homologous to that found in a strain (12-1-18) carrying an extension deletion which extends both to the left and the right of the *ad-3* region. For this reason it was assumed that these mutations were *ad-3A*^{IR} and *ad-3B*^{IR}. Homology tests were made on 18 *ad-3A*^{IR} mutations and 31 *ad-3B*^{IR} mutations, along with 4 *ad-3A ad-3B*^{IR} mutations, included as "controls," by putting them together to make trikaryons in all possible pairwise combinations. Tests on the resulting trikaryons showed that $^{2}/_{18}$ of the *ad-3A*^{IR} mutations were actually *ad-3A*^{R} + *RL*, and $^{2}/_{31}$ of the *ad-3B*^{IR} mutations were actually *ad-3B*^{R} + *RL*, where *RL* indicates a separate site of recessive lethal damage closely linked to the *ad-3* region. The *RL* damage in each of these four mutants is irreparable even on complete medium, and none have as yet been mapped with reference to other known loci. It is of particular interest and significance that the site of one of the *RL* mutations is in the "X region" that was postulated to exist in between the *ad-3A* and *ad-3B* loci on the basis of previous homology tests.[5] The discovery of these four mutations has made it possible to determine the extent of genetic damage in *ad-3*^{IR} mutations to both the left and right of the *ad-3A* and/or *ad-3B* locus, as illustrated in Fig. 1.17.1. The difference between the extent of function inactivation in *ad-3*^{R} from that found in *ad-3*^{IR} mutations is quite striking, and the variation in the extent of functional inactivation in individual mutations is already quite apparent. It is to be expected that the sensitivity

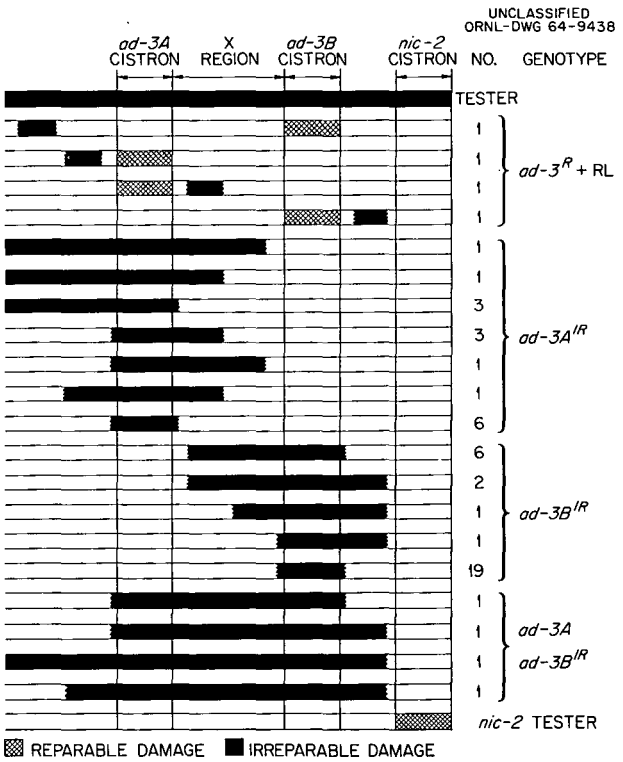

Fig. 1.17.1. Complementation Map of the *ad-3* and Adjacent Regions from Homology Tests in Various *ad-3* Mutations Containing Irreparable Genetic Damage Homologous to That Found in the Tester Strain 12-1-18.

of this distinction will be increased as other *ad-3*^{R} + *RL* mutations with closely linked *RL* damage are found for use in these tests.

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1.18 IDENTIFICATION OF THE GENETIC ALTERATIONS LEADING TO NONCOMPLEMENTING MUTANTS AND MUTANTS WITH POLARIZED OR NONPOLARIZED COMPLEMENTATION PATTERNS AMONG THE *ad-3B* MUTANTS OF *NEUROSPORA CRASSA*

H. V. Malling F. J. de Serres

Much progress has been made in the recent past in the identification of different genetic alterations at the molecular level in viruses and bacteria.[1] It is a problem of considerable importance to try to extend these findings to higher organisms where the DNA is organized in the form of chromosomes of complex structural organization. The identification of different genetic alterations in a series of allelic mutants is particularly important for obtaining some understanding of gene-enzyme relations in higher organisms. Such studies would also provide a tester set of mutants resulting from various types of base-pair substitutions, insertions, or deletions. Since such mutants revert only by means of specific types of genetic alterations, this tester set could be used to determine the spectrum of genetic alterations produced by various chemical or physical agents in quantitative terms in a series of relatively simple tests.

Recent studies[2,3] with the *ad-3* mutants of *Neurospora* have shown a high correlation between mutagenic origin and allelic complementation found among the *ad-3B* mutants. Three different classes of complementation patterns were found, and the percentages of mutants in each class varied markedly with mutagenic origin. A high percentage of mutants produced by mutagens known to produce predominantly base-pair substitutions in phage and bacteria[1] have nonpolarized complementation patterns, whereas high percentages of mutants produced by mutagens known to produce base-pair insertions and deletions have polarized complementation patterns or are noncomplementing.

The approach that is being used to attempt to identify the genetic alterations in each class is to use chemical mutagens whose mode of action is suspected or known. The use of nitrous acid (NA), ethyl methanesulfonate (EMS), and ICR-170 (a monofunctional acridine mustard) should permit one to distinguish the following three classes of genetic alteration: (1) single base-pair substitutions (revertible by NA or by NA and EMS[4]), (2) single base-pair insertions or deletions (revertible by ICR-170[5] and possibly by EMS[6]), and (3) other unidentified genetic alterations.

Reversion tests with NA-induced *ad-3B* mutants exhibiting different types of complementation patterns have been made. Thirty noncomplementing mutants, 31 mutants with nonpolarity-type complementation patterns, and 28 with polarity-type complementation patterns were chosen to determine if, indeed, some correlation exists between the mutational alteration at the molecular level and the complementation characteristics. These mutants were treated with NA, EMS, or ICR-170 under standardized conditions, and the reversion frequencies occurring spontaneously and after the treatments were determined.

Results. – The revertibility data are presented in Table 1.18.1. Whereas $^7/_{30}$ of the noncomplementing mutants and $^4/_{28}$ of the mutants with polarized patterns revert only with ICR-170, none of the mutants with nonpolarized complementation patterns are of this type. If one looks for base-pair substitution-type mutants, it is clear that $^{30}/_{31}$ of the nonpolarity-type mutants are of this type, while only $^{18}/_{28}$ of the polarity-type mutants and $^{13}/_{30}$ of the noncomplementing mutants are of this type. This is in good agreement with the observation that the majority of mutants induced by NA and by 2-aminopurine (both of which are expected to cause base-pair substitutions) have nonpolarized complementation patterns, while spontaneous and

Table 1.18.1. The Reversibility of Noncomplementing Mutants and Mutants with Polarized or Nonpolarized Complementation Patterns After Treatment with NA, EMS, or ICR-170

Mutagen				Complementation Pattern		
SP	NA	EMS	ICR-170	Nonpolarized	Polarized	Noncomplementing
0[a]	0	0	0	0	3	5
+[b]	0	0	0	1	2	0
+	0	0	+	0	4	7
+	0	+	0	0	1	1
+	0	+	+	0	0	1
+	+	0	0	2	0	0
+	+	0	+	1	0	3
+	+	+	0	18	4	4
+	+	+	+	9	14	9
+++						
+++	?	?	?	(3)	0	(1)
				31	28	30

[a]0 means below $0.05/10^6$ survivors with exception of the nonrevertible class.

[b]Assuming that when a mutant can revert with an agent it is also going to revert spontaneously.

x-ray-induced mutants are predominantly of the polarized or noncomplementing type.

The above data concern only the qualitative aspects of revertibility. The actual reversion rates are more difficult to deal with, because these will depend upon the type of DNA alteration required to restore activity and, to a lesser extent, the immediate environment of the site which must be changed. If we assume that the different chemical compounds used are affected about equally by the environment of the mutational site, then it is reasonable to use the relative reversion frequency obtained with each agent as an estimate of the effect of the DNA alteration required. The relative reversion frequency for a particular mutant with a particular mutagen (e.g., nitrous acid) is given by

$$\frac{(M/S)_{NA}}{(M/S)_{NA} + (M/S)_{EMS} + (M/S)_{ICR\text{-}170}},$$

where M/S equals the number of revertants divided by the number of surviving conidia plated after the treatment indicated in subscripts. A triangular three-parameter graph is used for plotting these relative revertibilities, and the difference between specific revertibilities of mutants of the three complementation classes can be detected readily by comparing the positions of mutants on the graphs.

The nonpolarized mutants have a relatively low reversion frequency with ICR-170 and relatively high reversion rates with NA and EMS, suggesting that their origin is by base-pair substitution. The polarized mutants include at least two very different classes, one class with high EMS reversion rates and low NA and ICR-170 reversion rates and a second class with high ICR-170 rates and moderate rates with the other two agents. Among noncomplementing mutants, one group is revertible only after ICR-170 treatment, suggesting a base-pair insertion or deletion type of origin. It appears that the three classes of mutants analyzed each consist of a different array of genetic alterations, although it is not possible at the present time to determine the nature of the genetic alteration for all of the categories of mutants. Further studies with other

mutagens and additional mutants, as well as analyses of the reversion mechanisms involved, may provide a more complete description of the genetic alterations leading to mutants in each of the nine or ten reversion categories found in the present analysis.

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1.19 INVESTIGATION ON THE MUTAGENICITY OF POTENT CARCINOGENS IN *NEUROSPORA*. 1. THE UPTAKE OF THE POLYCYCLIC HYDROCARBON 3,4-BENZPYRENE

H. V. Malling

Introduction. – Many alkylating agents which are highly mutagenic also exhibit potent carcinogenic activity. However, the polycylic hydrocarbons, a group of potent carcinogens, have shown no mutagenicity in the bacterial and fungal systems where they have been studied. Although the hydrocarbons are usually completely insoluble or only sparingly soluble in water, they can be taken up by mammalian cells. Such compounds are fluorescent and in earlier experiments,[1] in which their uptake by *Neurospora crassa* was studied by fluorescence microscopy, no change in the fluorescence of the cells was found. This may, a priori, be due either to (1) quenching of the fluorescence when the compound is bound to the cell constituents, (2) weak fluorescence of the compound, so that the amount which is taken up by the conidia cannot be detected, or (3) the fact that these compounds were not taken up by the cells.

3,4-Benzpyrene (3,4-BP) is a particularly useful polycylic hydrocarbon for studies of the questionable mutagenicity of these compounds in bacteria and fungi. It is a potent carcinogen, it can be dissolved in a protein solution, and the fluorescence emission of a colloid suspension is green, whereas that of a true solution is bright blue. Furthermore, its fluorescence is much more intense than that of most of the other polycylic hydrocarbons, so that it should be possible to detect its presence even in very dilute suspensions or solutions.

Results. – Washed suspensions of conidia which have been treated with 3,4-BP (1 mg/ml) in a protein solution exhibit a very strong blue fluorescence. Furthermore, this fluorescence is not emitted by 3,4-BP attached to the surface of the conidia but is emitted from some components within the conidia. Even *Paramecia*[2] treated with a more dilute solution (approximately 0.02 mg/liter) show a very strong blue fluorescence. Some small unidentified particles in the cell show a very strong fluorescence, but no fluorescence can be detected in the macronucleus, even when the animals have gone through divisions in the treatment solution.

The fluorescence excitation and emission spectra of 3,4-BP bound in *Neurospora* conidia have been estimated with a Zeiss spectrofluorometer (ZFM 4C). The fluorescence excitation maximum measured by the fluorescence emission at 430 mμ was found to be 385 mμ. It is nearly the same as one of the absorption maxima of 3,4-BP in cyclohexane solution,[3] and it differs from the absorption maximum in the collodial suspension.[3] Since an absorption spectrum is typically the same as the fluorescence excitation spectrum, we can conclude that 3,4-BP in the conidia is in the dissolved state.

Neurospora conidia treated 25 hr with 3,4-BP solution at 0.005–0.05 mg/ml show a linear increase in the fluorescence light intensity with concentration. The fact that the uptake of 3,4-BP by the conidia is proportional to the extracellular concentration will be useful in estimating the intracellular concentrations during the 3,4-BP treatment.

Conclusion. – The present investigations have established that dissolved 3,4-BP enters *Neurospora* conidia. When denatured DNA is treated with 3,4-BP and irradiated with near visible light (320–390 mμ), covalent linkage between 3,4-BP and DNA occurs.[4] The combined effect of near visible light

and 3,4-BP on nuclear inactivation and induction of recessive lethals and mutations is now under investigation.

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1.20 ANALYSIS OF THE GENETIC EFFECTS OF HIGH-ENERGY PROTONS AND HEAVY IONS IN *NEUROSPORA*

F. J. de Serres B. B. Webber

Experiments have been continued on the analysis of the genetic effects of various radiations to determine whether there is a systematic change in relative biological effectiveness (RBE) for cellular inactivation and mutation induction with increasing linear energy transfer (LET). A series of exposures were made at the synchrocyclotron and heavy-ion linear accelerator, Lawrence Radiation Laboratory, University of California at Berkeley, with 750-Mev protons, 39-Mev helium ions, or 101-Mev carbon ions with standardized dosimetric procedures. The direct method[1] was used with a genetically balanced heterokaryon to estimate (1) cellular inactivation of the heterokaryotic fraction of conidia and (2) induction of reparable and irreparable forward mutations in the *ad-3* region.

Cellular inactivation curves were exponential; the inactivation constants (α) in the equation $y = e^{-\alpha x}$, where y = the proportion of heterokaryotic conidia surviving the treatment and x = the dose in kilorads, are presented in Table 1.20.1. The data from the exposures with 750-Mev protons show a small unexpected difference in RBE for cellular inactivation from our 250-kv x-ray standard, but this has been attributed to differences in dosimetric techniques or exposure procedures. For the present purposes, 750-Mev protons are considered equivalent to x rays in computing RBE. The RBE's of the helium and carbon ions (with 750-Mev protons as a standard) are presented in Table 1.20.1.

Computation of the RBE's for mutation induction is more difficult since the increase of mutations with dose is exponential and the exponents are different for each series of exposures. This makes RBE dose dependent. In a study of x-ray-induced mutation in the *ad-3* region,[2] the induction curve was resolved into two different components, with one showing one-hit kinetics (the reparable mutants $ad\text{-}3^R$) and another showing two-hit kinetics (the irreparable mutants $ad\text{-}3^{IR}$). If the process of mutation induction with the present radiations is comparable to that found with x rays and the induction curves consist of one-hit and two-hit components, then these can be estimated by the equation $y = k + ax + bx^2$, where y = the proportion of heterokaryotic conidia which are mutant, and ax and bx^2 equal the incidence of one-hit and two-hit mutants respectively. The values of a and $\sqrt{b}$ will then be proportional to RBE. The estimates of RBE for $ad\text{-}3^R$ and $ad\text{-}3^{IR}$ mutant induction for these three radiations are given in Table 1.20.1.

The present data are in good agreement with those on other systems[3] where a peak of RBE is observed at an LET of about 2×10^3 Mev cm^2 g^{-1},

Table 1.20.1. Estimates of RBE for Cellular Inactivation and Induction of Forward Mutations in the *ad-3* Region of *Neurospora* with High-Energy Protons or Heavy Ions

Radiation	Linear Energy Transfer (Mev cm^2 g^{-1})	Cellular Inactivation		RBE for Mutation Induction	
		α	RBE	$ad\text{-}3^R$	$ad\text{-}3^{IR}$
750-Mev protons	2.2	0.1145	(1.00)	(1.00)	(1.00)
39-Mev helium ions	182	0.1053	0.92	1.74	1.23
101-Mev carbon ions	1850	0.3961	3.46	6.69	2.98

and the RBE values at 182 Mev cm^2 g^{-1} are the same as or only slightly higher than RBE's of radiations of much lower LET.

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1.21 EFFECTS OF STORAGE TIME, TEMPERATURE, AND ATMOSPHERE UPON SURVIVAL OF *NEUROSPORA* CONIDIA

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Since conditions proposed for the Biosatellite experiment are different from those routinely used in laboratory forward-mutation experiments, it is necessary to determine their effect on the viability of conidia used in the *Neurospora* test system. The requirement for complete sterility necessitates a sealed package that will be incubated at about 21°C for a minimum of 3 days and perhaps for as long as 6–9 days. Since the experiment will be performed on wet rather than dry (or desiccated) conidia as originally planned, the effects of different atmospheres, storage times, and possible extreme fluctuations in temperatures were tested as indicated in Table 1.21.1. Aliquots were assayed at 0, 3, 6, 9, and 22 days for survival of the heterokaryotic fraction of conidia. Aerobic and anoxic conditions were obtained by bubbling the suspensions with air or nitrogen, respectively, during storage.

Heterokaryotic survival is indicated in Table 1.21.1. Under either atmospheric condition, the conidia survive longer at 0–4°C than at higher temperatures. Above 0–4°C the loss of viability is greater under anoxic conditions than under aerobic conditions. In air at 18°C (as prescribed for the flight) survival is good through 9 days and tolerable for as long as 22 days. It would appear that satisfactory viability can be obtained under flight conditions if the package contains an adequate supply of air.

1.22 VARIATION IN THE FREQUENCY OF CHROMATID ABERRATIONS IN MAMMALIAN CELLS INDUCED BY X RAYS AND ULTRAVIOLET LIGHT DURING DIFFERENT STAGES OF THE CELL CYCLE

E. H. Y. Chu

Chinese hamster somatic cells in vitro were exposed either to 150 r of x rays or 100 ergs/mm^2 of ultraviolet radiation at 2652 A during various stages of the cell cycle, and the frequencies of

Table 1.21.1. Percentage Survival of Heterokaryotic Conidia After Incubation for Various Times and Under Different Atmospheric and Temperature Conditions

Temperature (°C)	Atmosphere	Time (days) 0	3	6	9	22
0–4	Air	(100)	96	97	90	63
18	Air	(100)	92	83	76	49
35	Air	(100)	85	40	28	1
0–4	Nitrogen	(100)	62	50	51	30
18	Nitrogen	(100)	9	10	7	2
35	Nitrogen	(100)	5	2	0.03	0.0001

resulting chromatid aberrations at metaphase were scored and compared. In the first series of experiments with randomly dividing cell populations, a 15-min pulse labeling with ^{3}H-thymidine was applied to cells prior to irradiation. Metaphase figures were accumulated with colchicine for seven successive 3-hr postirradiation periods. Both the mitotic index and the percentage of labeled cells were determined for cells collected at each period. Our earlier work[1] has shown that, with either ultraviolet or x rays at the above doses, DNA synthesis was postponed for approximately 2 hr. Similar results have been obtained in the present experiments. It was found that the frequency of the x-ray-induced chromatid aberrations reached a peak after DNA synthesis (G_2), whereas that of the ultraviolet-induced aberrations was the highest during DNA synthesis (S).

In the second series of experiments, the mitotic cycle in the hamster cells was synchronized by growing the cells in the presence of 3×10^{-3} *M* 5-aminouracil (5AU) for 24 hr. Partial mitotic synchrony has been obtained with 5AU treatment in plant cells.[2,3] When the synchronized mammalian cells were pulse labeled with ^{3}H-thymidine at various hours after the removal from 5AU, 100% of metaphases coming from the first hour were labeled. The proportion of labeled metaphases gradually decreased until it fell to 4% and 0% at the sixth and seventh hour, respectively, after removal from 5AU, indicating that practically all the cells had been in G_2 at the seventh hour. There were only a few mitoses during the first 6 hr after the removal from the chemical; mitotic figures quickly appeared thereafter, and the frequency of dividing cells reached a peak (20% in metaphase) at the eighth hour.

Successive samples of the chemically synchronized cells were pulse labeled with ^{3}H-thymidine just prior to x or ultraviolet irradiation at every hour for 7 hr. The x-irradiated and the nonirradiated cell populations were treated with colchicine for 3 hr at the seventh hour after removal from 5AU, while the ultraviolet-irradiated cells were treated with colchicine for 10 hr at the 14th hour because of a stronger mitotic inhibition by ultraviolet.

In the nonirradiated series, there was about 0.1 chromatid break/cell. The frequency remains constant throughout S and G_2. It is higher than the spontaneous rate (0.01 break/cell) and can be attributed to the action of 5AU.

In the x-irradiated series, there was certain variation of aberration frequency during different hours in S, but the most significant increase in frequency, particularly that of the terminal chromatid deletion type, occurred at G_2. Preliminary data with the synchronized cells confirm our earlier finding that chromatid aberrations can be induced by ultraviolet more frequently at S. Further analysis on the relative frequencies of various types of aberrations in relation to the cell cycle and in response to different radiations is being made.

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1.23 RADIATION SENSITIZATION BY NUCLEIC ACID BASE ANALOGS AS MEASURED BY CHROMOSOME ABERRATIONS

E. H. Y. Chu

It has been shown that there is a correlation between the base composition of the DNA of a particular bacterial strain and its radiation sensitivity. Thus, as the guanine-cytosine content increases, cells become more sensitive to killing by x rays;[1] as the adenine-thymine content increases (as G-C decreases), the cells show an increased sensitivity to ultraviolet inactivation.[1,2] Incorporation of 5-bromodeoxyuridine (BUdR) enhances the radiation sensitivity of mammalian cells both in terms of cell survival[3] and chromosome aberrations.[4] We have shown that incorporation of BUdR or 5-iododeoxyuridine (IUdR) into mammalian cells greatly increases ultraviolet-induced chromosome aberrations and that the modification of aberration frequency varies with wavelength.[5] In order to understand the molecular basis for radiation-induced chromosome breakage, the present study was undertaken to compare the relative frequencies of chromosome aberrations induced by x rays and ultraviolet when the cells are pretreated with various purine and pyrimidine analogs.

Table 1.23.1. Frequencies of Chromatid Aberrations in Irradiated Mammalian Cells in Vitro Pretreated with Purine and Pyrimidine Analogs

Series	Treatment	Total Cells	Chromatid Breaks per Cell	Sensitization Index
I	BUdR + ultraviolet	196	3.68	3.61
	IUdR + ultraviolet	200	2.31	2.43
	Ultraviolet	402	0.90	
	BUdR	300	0.12	
	IUdR	200	0.05	
	Control	500	0.01	
II	TGdR + x ray	250	1.09	1.25
	TGdR + ultraviolet	300	1.98	1.46
	TGdR	250	0.62	
	X ray	250	0.52	
	Ultraviolet	250	0.74	
	Control	100	0	

Prior to irradiation (150 r x rays or 100 ergs/mm^2 ultraviolet at 2652 A), cells were pretreated for 48 hr with 10^{-5} *M* BUdR, IUdR, or 2′-deoxythioguanosine (TGdR). The results of radiation-induced chromosome aberrations in analog-treated and untreated cells are briefly summarized in Table 1.23.1. The "sensitization index" is the ratio between radiation-induced aberration frequency in analog-treated cells and the sum of aberration frequencies in cells treated singly with either radiation or an analog. From these results, it is evident that base analog substitution greatly increases sensitivity of mammalian chromosomes to both radiations.

Since BUdR and IUdR are known to substitute for thymidine and TGdR possibly substitutes for deoxyguanosine, it appears that with ultraviolet irradiation the A-T pairs on chromosomal DNA are more radiosensitive than the G-C pairs. This observation is consistent with our present knowledge concerning the importance of thymine photoproducts in the ultraviolet inactivation of DNA. We have recently demonstrated[5] in vivo induction by ultraviolet of thymine dimers in mammalian DNA, although the direct relationship between thymine dimers and chromosome breakage has not been established.

Both BUdR and TGdR increase x-ray sensitivity of mammalian chromosomes. However, the reverse situation (increased G-C radiosensitivity to x rays) cannot be clearly demonstrated with the limited data available. Either the induction of chromosome aberrations by x rays is less dependent on the nucleic acid base composition of the chromosomes or the x-ray effect is further confounded by radiosensitization with visible light that we observed recently in BUdR-treated cells.[6] Continued experiments with x rays and chemicals are in progress to clarify this point.

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1.24 CHROMOSOME BREAKAGE AT SPECIFIC REGIONS INDUCED BY RADIATION AND 5-BROMODEOXYURIDINE

Tien-Ding Chang E. H. Y. Chu

Our earlier preliminary work[1] indicated the randomness of ultraviolet-induced mammalian chromosome breaks. Although there was an overall increase of ultraviolet-induced aberration frequency in 5-bromodeoxyuridine (BUdR)-treated cells, there was no increase of ultraviolet sensitivity at specific chromosome regions. Based on more extensive recent data, we are now able to demonstrate that ultraviolet (100 ergs/mm^2 at 2652 A) irradiation, with or without BUdR (10^{-5} *M*, 48 hr pretreatment), induced more true chromatid breaks (28.6 and 26.2% of all breaks respectively) in the secondary constriction region on the long arm of chromosome No. 1 of Chinese hamster cells. Increase of either the BUdR concentration for pretreatment or ultraviolet dose heavily damaged or killed cells and rendered analysis impossible. It must be mentioned that light conditions were rigidly controlled during the entire course of these experiments.

Further results from combined BUdR (10^{-4} *M* for 48 hr) pretreatment and x irradiation (150 r) showed that 35.1% of chromatid breaks were induced in the same secondary constriction region. X rays alone also induced breaks (37.8%) preferentially at this region. Thus, combined treatments with the analog and either type of radiation were not necessary to show the nonrandomness of break localization.

In another experiment, we discovered that BUdR alone (10^{-3} *M* and 10^{-4} *M* for 48 hr) induced breaks that were even more region-specific (95.7 and 78.6%, respectively, in the secondary constriction). Since part of the experiment was carried out in the presence of visible light, we suspected a sensitization effect by visible light similar to that shown for the inactivation of bacteriophage containing BUdR.[2] Experiments were then performed in which cells pretreated with 10^{-5} *M* BUdR for 48 hr were exposed for various lengths of time to visible light (a 15-w Sylvania "white" fluorescent lamp at 18 cm above glass culture dishes at 37°C). BUdR in the dark induced only a few breaks; prolonged illumination of cells in the absence of the analog caused no effect. However, visible light posttreatment for ½ hr induced an enormous number of breaks. With 1 hr post-BUdR illumination 20% of the breaks were found in the same region. Illumination for over 2 hr after BUdR treatment inhibited mitosis almost completely.

It appears that the induction of chromosome breaks by BUdR is largely mediated through an interaction of the substituted analog in chromosomal DNA with radiation (ultraviolet or visible light). The enhanced x-ray-induced aberration frequency in BUdR-treated cells can probably also be explained in part by this sensitization effect, as light was present in the aforementioned x-ray experiment and probably in similar experiments reported by other investigators.[3]

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1.25 PENETRATION AND RETENTION OF 3,4-BENZPYRENE IN DIVIDING MAMMALIAN CELLS[1]

E. H. Y. Chu H. V. Malling[2]

Mammalian cells offer a unique opportunity to study, within the same system, the phenomena and possible relations of carcinogenesis and mutagenesis. We propose, using chromosome aberrations and gene mutations as criteria, to test the somatic mutation theory of carcinogenesis by initially investigating whether certain carcinogens are also mutagens.

The first chemical chosen for study was 3,4-benzpyrene, which is one of many polycyclic hydrocarbons with a potent carcinogenic effect in mammals. It possibly binds physically with cellular DNA[3] and is intensely fluorescent so that its entry and accumulation inside the cell can be demonstrated with fluorescence microscopy.

One of us (H.V.M.) has recently succeeded in dissolving this chemical in a water solution of bovine serum protein, thus enabling it to be diluted appropriately and incorporated into the growth medium for culturing mammalian cells. Preliminary

experiments with Chinese hamster somatic cells in vitro indicate that the chemical readily penetrated the cell membrane and was distributed first (within 1 hr) in the juxtanuclear region and then (within 3 hr) more uniformly throughout the cytoplasm, with the exception of the cytoplasmic lipid granules where the chemical quickly and heavily concentrated. At 23 hr after addition of the chemical to medium, the chemical was clearly inside the cell nucleus. Although 0.1 mg per ml of medium caused severe damage to the cell, lower concentrations (0.01–0.001 mg/ml) did not seem to interfere with cell growth and division, nor did they cause gross cellular abnormalities.

Strong blue fluorescence was observed on or around mitotic chromosomes accumulated at metaphase by colchicine. Even at 3 to 5 days after removal of the chemical from the growth medium and the cells underwent several divisions, appreciable fluorescence was seen within both the nucleus and cytoplasm. More detailed analysis will be made on the possible effects of this chemical on chromosome structure and cell metabolism, especially in combination with near visible light (3200–3900 A).

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1.26 INDEPENDENCE OF DEFICIENT EARLY GROWTH AND LATER REGRESSION OF (C57BL × 101)F_2 MARROW GRAFTS IN (C57BL × 101)F_1 HYBRID MICE

R. A. Popp Gustavo Cudkowicz

Introduction. – Marrow cells from strain C57BL (H-2^b/H-2^b) donors do not proliferate on transplantation into irradiated F_1 hybrid recipients as well as marrow cells from several other strains of mice, or as well as in isologous recipients.[1,2,3] Moreover, marrow grafts from strain C57BL donors often regress after repopulating irradiated F_1 recipients.[4,5] Data in the above reports suggested that the initial growth and the subsequent persistence of C57BL marrow cell grafts in F_1 hybrid mice are controlled by different factors. This experiment was designed to establish whether or not the deficient early growth and later regression of marrow cell grafts are independent traits in (C57BL × 101)F_2 mice. In addition, we also studied whether or not prolonged residence of F_2 donor marrow in primary F_1 recipients affects its short-term growth pattern on retransplantation into secondary F_1 mice.

Methods and Results. – Marrow donors were (C57BL × 101)F_2 mice that were classified according to sex, coat color, hemoglobin, and red cell serotype markers. An aliquot of marrow from individual donors was injected intravenously into parallel groups of irradiated (C57BL × 101)F_1 recipients; one group was used to assay early proliferation of the marrow in the spleen of recipients, and the other group was used to determine the status of the grafted marrow at later periods. An aliquot of 5×10^5 marrow cells from F_2 donors of H-2^b/H-2^b phenotype grew deficiently (11 donors; mean ^{131}IUdR uptake of 0.02%) in 750-r-irradiated F_1 recipients, whereas cells of H-2^b/H-2^k and H-2^k/H-2^k phenotypes grew well (32 and 19 donors respectively; mean ^{131}IUdR uptake for both was 0.68%) in similar recipients. An aliquot of 10^7 marrow cells from the same F_2 donors repopulated the hemopoietic system of 900-r-irradiated F_1 recipients, and the F_2 marrow grafts that regressed (12.5%) within 180 days after transplantation were distributed among the three segregating H-2 phenotypes. Significantly, eight of nine transplanted F_2 marrows of H-2^b/H-2^b serotype did not regress. F_2 marrow cells of H-2^b/H-2^b serotype, which had been growing in 900-r-irradiated primary F_1 recipients for approximately a year, grew deficiently (21 donors; mean ^{131}IUdR uptake of 0.03%) in 750-r-irradiated secondary F_1 recipients, whereas F_2 marrow cells from 22 donors of H-2^b/H-2^k and from 2 donors of H-2^k/H-2^k serotypes grew well (mean ^{131}IUdR uptake of 0.87% and 0.75% respectively) on retransplantation into irradiated secondary F_1 recipients.

Discussion. – The results of this study demonstrate that the phenomena of deficient early growth, which is H-2 associated, and regression, which is not H-2 associated, are not governed by the same genetic factors. Furthermore, the genetic trait of deficient growth of marrow cells of H-2^b/H-2^b serotype in a H-2^b/H-2^k recipient is not altered

during the growth of such cells in a primary F_1 recipient.

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1.27 AN ANALYSIS OF THE H-2 PHENOTYPE OF STRAIN RFM/U MICE

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Much has been elucidated about the immunological role of the histocompatibility-2 system.[2] Recently the biochemical and physiological aspects of this system have attracted the attention of investigators. In view of the inclusive biological importance of this genetic determinant it becomes pertinent to characterize the H-2 phenotype of unclassified strains of mice that are used as experimental subjects. The following is an analysis of the H-2 phenotype of strain RFM/U mice.

Methods. – The RFM/U mice studied were descendants from A. C. Upton's colony, generation 53 and two subsequent generations. Red cell agglutinations were performed according to the procedures of Gorer and Mikulska.[3] In vivo and in vitro absorptions were used to remove specific antibodies. All absorption experiments included controls for nonspecific absorption and for the presence of nonspecific antibodies.

Results and Discussion. – A test of RFM red cells against a battery of stock antisera showed that RFM mice were not $H\text{-}2^a$, $H\text{-}2^b$, $H\text{-}2^k$, $H\text{-}2^q$, or $H\text{-}2^s$, but that RFM shared antigens with mice of $H\text{-}2^d$, $H\text{-}2^f$, $H\text{-}2^l$, $H\text{-}2^n$, and $H\text{-}2^s$ phenotypes. Absorption studies showed that RFM tissues could remove antibody against antigens G, F, I, and H, but not against antigens S, N, and C. RFM red cells were agglutinated by residual A′ antibody, but not by residual V, B′, or C′ antibody. RFM red cells also failed to agglutinate in various antisera containing antibodies against H-2 antigens A, D, D^b, E, K, M, V, S, C, N, Y, B′, C′, R, and Q. Action of anti-Z and anti-P on RFM red cells was not tested. A consistent similarity in the agglutination and absorption reactions was noted between strains RFM/U and A.CA. It is concluded that the H-2 phenotypes of these strains are identical and that the phenotype of strain RFM/U mice is $H\text{-}2^f$.

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1.28 SOME PROPERTIES OF SERUM ALBUMIN AND ESTERASE IN MICE

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Introduction. – This report describes some properties of purified albumin and esterase from C57BL male mouse serum.

Methods and Results. – Serum albumin and an esterase enzyme with an electrophoretic mobility similar to albumin were isolated by a method described previously.[1] The specific activity[2] of the esterase was increased, and acrylamide gel preparations[3] revealed both samples to be quite pure.

The albumin and esterase were hydrolyzed with 6 *N* HCl under vacuum at 110°C for 24 hr.[4] Amino acid analyses were then made with a Spinco automated amino acid analyzer. The amino acid composition of albumin expressed to the nearest whole number of residues per molecule of 70,000 molecular weight was: Lys_{44}, His_{14}, Arg_{18}, Cys_{28}, Asp_{60}, Thr_{44}, Ser_{35}, Glu_{83}, Pro_{33}, Gly_{22}, Ala_{65}, Val_{34}, Met_7, Iso_{14}, Leu_{64}, Tyr_{24}, Phe_{28}. The composition of esterase was: Lys_{40}, His_{13}, Arg_{19}, Cys_9, Asp_{62}, Thr_{37}, Ser_{49}, Glu_{82}, Pro_{29}, Gly_{40}, Ala_{44}, Val_{38}, Met_{13}, Iso_{32}, Leu_{67}, Tyr_{17}, Phe_{35}. The estimated number of residues in the albumin and esterase molecules was calculated to be 617 and 626 respectively.

The albumin and esterase were treated with performic acid at –7°C to –10°C for $2\frac{1}{2}$ hr[5] to convert –SH or –S–S– groups to $-SO_3H$. Unfortunately, the performic acid treatment made the esterase completely insoluble, and ultracentrifugal analysis was impossible. The performic acid–treated albumin and the native albumin were analyzed on a Spinco model E ultracentrifuge. The sedimentation value of the performic acid–treated albumin was approximately 1.66, which suggests that native albumin was split into four components of approximately equal weight, that is, 17,500. The breakdown of native albumin during performic acid treatment was verified by acrylamide gel preparations.[3]

Discussion. – The analyses suggest that, although the albumin and esterase have similar molecular weights, they differ considerably in their quantities of cysteine, glycine, alanine, methionine, and isoleucine. Although it seems most likely that performic acid split the disulfide bonds that join polypeptide chains of albumin, it is possible that three tryptophan molecules were evenly distributed over the length of the molecule and were destroyed by performic acid. This would also produce four components of similar weight.

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2. Drosophila Cytology and Genetics

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Radiation Genetics (2.4–2.7)

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2.1 DETECTION AND LOCALIZATION OF RECESSIVE LETHALS AT A SPECIFIC LOCUS IN DROSOPHILA

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In previously published work[1] it has been shown that the Notch locus on the X chromosome is composed of a pseudoallelic series of recessive lethal mutants superimposed upon another of recessive visibles. The lethal series is composed of seven separable Notch (*N*) mutants. Each individual *N* is lethal in a homozygous or hemizygous condition, but when heterozygous with a wild-type allele, females display a notched-wing phenotype from which the Notch mutants derive their name. Briefly stated, the Notches are dominant mutants with recessive lethal effects.

Reasoning by analogy with presumably similar pseudoallelic systems in microorganisms, we concluded that at the Notch locus recessive lethal mutants should be found that are not characterized by a notched-wing phenotype in heterozygotes with a normal allele. That such mutants did not already exist was probably due to selection of lethals on the basis of their dominant phenotype. Hence, a recessive lethal at the Notch locus which did not display a more or less typical phenotype would not be singled out for study.

In order to find such mutants, we decided to obtain an unselected sample of sex-linked recessive lethals and test each one to see if it was localized in the Notch region. From a sample obtained from Dr. Seymour Abrahamson (University of Wisconsin), we discovered three lethals that were either at the Notch locus or closely linked to it. A fourth lethal was inadvertently discovered in the course of routine experiments in our own laboratory. Two of these mutants, l^1 and l^2, have a normal phenotype when heterozygous with a wild-type allele of Notch. That they are mutant

sites at the Notch locus is indicated by the appearance of a mutant phenotype when heterozygous with some or all of the pseudoallelic recessive visible members, and they are lethal in combination with the recessive lethal *N*'s. A third, l^3, has an abnormal phenotype when heterozygous with the wild-type allele, but the heterozygote does not display the notched wings typical of *N* mutants; the dominant effect is confined to the removal of bristles from the thorax. As in the previous two cases, l^3 interacts with recessive visibles and is lethal with *N*'s. In the case of l^4, it is lethal when combined with Notches, but there are no other phenotypic indications that it is a real member of this locus.

Recombination experiments now in progress indicate that all four can be localized and positioned within the present limits of the Notch cistron. Three lethals, in the proximal end of the cistron, must be closely linked, but the fourth is a relatively great distance away in a more distal site.

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2.2 THE TARGET FOR IRRADIATION-INDUCED CHROMOSOME LOSS IN *DROSOPHILA MELANOGASTER* FEMALES

Rhoda F. Grell

The ability of x irradiation to induce chromosome loss is well recognized, but the radiation target, whose damage is responsible for the loss, has not been unequivocally identified. Muller[1] and Pontecorvo[2] have suggested that chromosome loss originates from a single break followed by lateral fusion of sister chromatid fragments, leading to a dicentric. If this is so, the frequency of loss should depend on the target size and a large chromosome should be more vulnerable than a small one. Other workers have assumed that unpaired chromosomes or those lacking chiasmata are more frequently lost than exchange bivalents. Most studies in Drosophila have utilized the large X chromosomes which are paired, crossover bivalents about 95% of the time. Females may be constructed, however, which carry two small nonhomologous chromosomes that pair and segregate from one another as regularly as the X's but whose pairing is initiated sometime after exchange, at the distributive phase of meiosis, and which, of course, lack chiasmata. When both kinds of chromosomes are simultaneously present in a female, the opportunity exists for comparing the frequency of radiation-induced loss of the large X chromosome exchange bivalents and the small, nonexchange heterologs.

In the present experiment, females were constructed which carried two nonhomologs of about equal size, one an extra free fourth chromosome and the other a small, free, X duplication, as well as two normal X's. The nonhomologs, whose length at mitotic metaphase is approximately one-tenth that of the X, segregate regularly from one another 99.98%, and when nondisjunction occurs (0.02%) both products are viable. Females were irradiated within 6 hr after eclosion with 4000 r of x ray given over 13 min, then held for 2 days before mating, and brooded daily thereafter for 11 days.

Table 2.2.1 gives the frequencies of radiation-induced loss of either of the X homologs (column 3) and of either of the two heterologs (column 4). Day 1 represents oocytes irradiated at stage 7, and succeeding days represent irradiation of progressively earlier oocyte stages and of oogonia. With both kinds of chromosomes, sensitivity to loss is greatest with the most mature oocytes tested and falls off rapidly in younger oocytes and particularly in oogonia (days 8–11).

Table 2.2.1. Chromosome Loss with 4000 r in *Drosophila melanogaster* Females

Day	Number	X Loss (%)	Dp or 4 Loss (%)
1	135	8.9	8.1
2 + 3	1002	3.8	4.3
4 + 5	1061	4.1	2.9
6 + 7	842	1.2	1.3
8 + 9	904	0.7	0.7
10 + 11	459	0	0.4
Totals	4403	2.5	2.3

The close similarity in loss frequencies for the two sets (columns 3 and 4) suggests that the size of the chromosome, whether large as with the X's or small as with the four and duplication, does not influence the event. Similarly, whether chromosomes are homologously paired with chiasmata or either nonhomologously paired without chiasmata or unpaired, appears to be inconsequential. These results point to an identical, single target on each chromosome which is responsible for irradiation loss.

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2.3 DISTRIBUTIVE PAIRING IN TRANSLOCATION HETEROZYGOTES

Ann C. Chandley

The importance of chromosome size in distributive pairing at meiosis in the female of *Drosophila melanogaster* has been demonstrated by Grell.[1,2] In situations where more than two nonrecombinant chromosomes in the distributive pool are in mutual competition for a pairing partner, frequency of association between any two of them is determined by similarity in length, rather than by any homology which may exist between them.

This finding forms the basis for a series of experiments which are now in progress. A study is being made into the importance of chromosome size in pairing and segregation in females heterozygous for translocations involving the X chromosome and the right arm of chromosome 2. Three translocations are being used in which reciprocal exchange has produced elements differing in length from their normal untranslocated homologs. The positions of breakpoints, which have been confirmed cytologically, are as follows, with respect to the centromere: (1) translocation No. 69 – distal in X, proximal in 2R; (2) translocation No. 10 – median in X, median in 2R; and (3) translocation No. 43 – proximal in X, distal in 2R.

The introduction of the multiply inverted chromosomes FM-6 and SM-1,[3] either singly or in combination, virtually eliminates crossing-over in the first and second chromosomes respectively. Various elements are thereby liberated into the distributive pool and made available for distributive pairing.

Females heterozygous for each translocation and carrying one, both, or neither inversion have been crossed to normal males. In this way, all regular progeny and exceptional progeny due to nondisjunction of the X chromosomes can be recovered. Nondisjunctional offspring will be produced when both parts of the translocation and the normal X chromosome pass together to one pole in the oocyte and the normal second chromosome goes alone to the opposite pole.

Table 2.3.1 gives the frequency of X-chromosome nondisjunction, expressed as a percentage, for each translocation, together with its standard error.

The significant increase in X-chromosome nondisjunction when FM-6 is introduced into translocations No. 69 and No. 10 indicates that this element, being the only noncrossover in the distributive pool, is segregating randomly as a univalent and is being recovered with the translocation with a high frequency. In translocation No. 43 there are two noncrossover elements in the distributive pool – the inverted X chromosome and the small translocated element consisting of the centromere region of the X and the tip of 2R. It is assumed that these two will pair distributively and pass to opposite poles with a high frequency. This would account for the low frequency of nondisjunction observed.

When SM-1 is introduced (Table 2.3.1, column 4), crossing-over is increased between the X chromosomes and the frequency of X-chromosome nondisjunction is thereby significantly reduced.

When both FM-6 and SM-1 are introduced, there will be four noncrossover elements available for distributive pairing. The results show a significant increase in nondisjunction in translocation

Table 2.3.1. Frequency of X Chromosome Nondisjunction for Each of the Translocations

Translocation	+; +	FM-6; +	+; SM-1	FM-6; SM-1
No. 69	9.7 ± 0.5	27.5 ± 0.7	3.3 ± 0.4	5.9 ± 0.4
No. 10	5.6 ± 0.3	23.9 ± 0.7	3.9 ± 0.3	7.8 ± 0.6
No. 43	10.7 ± 0.5	5.5 ± 0.4	4.4 ± 0.4	17.0 ± 0.8

No. 43. This may indicate that trivalent formation is occurring between the three elements of closely similar size[1] and the median-sized SM-1 chromosome is directing the large translocated element and the small FM-6 chromosome to the same pole. If this occurs and if the small translocated element is segregating randomly, there will be an expectation of increased X-chromosome nondisjunction.

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2.4 EXCHANGE IN ADJACENT TANDEM DUPLICATIONS

P. A. Roberts

Since chromosome interference in Drosophila females is usually complete over intervals less than ten crossover units in length, it is not possible to decide, under ordinary circumstances, whether homologs pair throughout their length and exchange at a limited number of points or whether pairing is discontinuous over most of the chromosome, with exchange occurring at only a few sites of effective pairing.

One approach to this problem is to study the behavior of adjacent tandem duplications, each of which regularly undergoes asymmetrical pairing and exchange. Two such duplications are Bar eye (B – 57.0) and Bx^{r49k} (Bx – 59.4). If pairing is continuous throughout the chromosome, it is conceivable that one duplication, B for example, will undergo a higher frequency of reversion to B^+ (round eye) in the presence of Bx^{r49k} than in its absence as a result of exchange in a B duplication which is asymmetrically paired owing to simultaneous asymmetrical pairing of Bx^{r49k}. Because of the likelihood that reversion frequencies will be influenced by the amount of exchange within a region, total exchange was measured between *f* (56.7) and *fu* (59.5).

The following results were obtained:

Genotype of ♀♀	Number and Percent Revertants of B to B^+	Percent Exchange *f-fu*
1. *f B/B fu*	7/9063 (0.075%)	2.49
2. *f B* Bx^{r49k}/*B* Bx^{r49k} *fu*	19/17,000 (0.11%)	3.44
3. *f B/B fu; Cy/+ Ubx/+*	16/9802 (0.16%)	4.30

A comparison of cross 3 with cross 1 reveals that the presence of autosomal inversions in females roughly doubles exchange in the *f-fu* region and that the percentage of revertants is also doubled. When cross 2 is compared with cross 1, it is apparent that the Bx duplication increases exchange in the *f-fu* region by approximately one-third and the number of B revertants is increased by about one-third. The increase in reversion of B to B^+ in the presence of Bx^{r49k} can be attributed to the increase in exchange in this region caused by addition of the duplication. Although there are other possible explanations for the failure of Bx^{r49k} to significantly increase B reversions, the results are consistent with the hypothesis that chromosome pairing at the time of exchange is discontinuous and that the effective pairing segment is less than 2.4 map units in length.

2.5 CYTOLOGY OF X-RAY-INDUCED TRANSLOCATIONS

B. J. Williams

Cytological analysis of 60 X-autosomal translocations induced by x rays in four different X chromosomes [In(1)sc^4; In(1)sc^{4L},sc^{8R}; In(1)sc^{S1L},sc^{4R}; and In(1)sc^{S1L},sc^{8R}] of Drosophila is being carried out. The chromosomes differ in amount and distribution of heterochromatin and thus might be expected to differ in their frequency of involvement in translocations. If breakability is equivalent to metaphase length, then In(1)sc^{S1L};sc^{4R} should exhibit a higher

frequency of translocations than the other three. However, it has been shown that the observed frequency of translocations is the same regardless of the X chromosome irradiated. By analyzing these translocations cytologically it was determined whether the breakpoints were heterochromatic or euchromatic. Analysis showed no correlation between the proportions of breakpoints that were heterochromatic and the amount of heterochromatin present. Thus, although heterochromatic breakability is high, it seems to be uncorrelated with heterochromatic length.

2.6 CHEMICALLY INDUCED MOSAICISM IN DROSOPHILA

J. L. Epler

A study of radiation- and chemically-induced fractional or mosaic mutations in Drosophila is in progress. The purpose of the study is to define the mechanisms of action of mutagens in a higher organism. The utilization of special screening techniques allows the detection of individuals that are gonadally mosaic for recessive lethal mutations. The relative frequency of induced mosaic and whole-body mutations can be determined, along with the size and distribution of the mutant fraction(s) in mosaic mutations. The observations can then be compared with expectations based on various models for the origin and embryonic distribution of mutations in Drosophila.

2.7 THE MUTANTS OF *DROSOPHILA MELANOGASTER*

D. L. Lindsley E. H. Grell
I. Lucille Norton

We have continued to devote full time to our revision of *The Mutants of* Drosophila melanogaster by Bridges and Brehme. The aim of this book is to present a description of every adequately described mutant and chromosome rearrangement known in *Drosophila melanogaster*. We anticipate that the finished volume will contain between two and three thousand entries. The book will be divided into seven sections as follows: (1) mutations, (2) chromosome aberrations, (3) special chromosomes, (4) departures from diploidy, (5) wild strains, (6) nonchromosomally inherited effects, and (7) cytological markers. The first category occupied approximately 80% of the original volume and will probably do so in the revised edition as well. We are still working on section 1, where the entries beginning in letters A through S are in some stage of revision. Final manuscript exists for the letters A through D.

At the same time, each mutant is being classified with respect to fifteen categories of information, and this material will be put on magnetic tape. Tables of information for the appendixes will be constructed from this tape; it will also be used to check all cross-references between entries, as well as the completeness of our listings.

3. Cytochemistry and Cell Reproduction

R. C. von Borstel[a]	
Yeast Genetics (3.1, 3.2) R. C. von Borstel[a] Martha J. Bond T. R. Manney Margaret D. Schellinger	**Insect Cytology and Genetics (3.3)** J. G. Carlson[b] Gladys S. Van Pelt[d] Mary Esther Gaulden[b] F. M. Ritossa[c] Anna R. Whiting[a,b] P. W. Whiting[b]
[a]Dual assignments. [b]Consultant. [c]Visiting investigator from abroad. [d]USPHS Fellow. [e]Student trainee.	**Cell Biochemistry (3.4)** I. L. Cameron Betty M. Wysinger E. E. Guile, Jr.[e]

3.1 MUTAGENIC ACTION DURING MEIOSIS AND ANTIMUTAGENIC ACTION DURING MITOSIS BY 5-AMINOACRIDINE IN YEAST

G. E. Magni[1] R. C. von Borstel
S. Sora[1]

Introduction. – Some acridine dyes induce mutations in T2 phage and in the *r*II region of the bacteriophage T4 under conditions where photodynamic effects have been eliminated. The mutations are attributable to either base losses or base insertions,[2] and their origin has been interpreted as being related to acridine intercalations between the bases and the DNA.[3] This type of mutation is often called a "reading-frame mutation."[2] The acridines are not mutagenic in the dark in bacteria, but an antimutagenic effect has been reported.[4]

Results. – Three haploid strains showing quite different frequencies of spontaneous mutation to canavanine resistance were studied. For the histidine reversion experiments, one haploid and one diploid strain homozygous for the same mutation were employed. The results are presented in Table 3.1.1. Each value is the average of 15 independent estimates run concurrently. Frequencies of mutation were calculated from colony counts on selective and nonselective media. 5-Aminoacridine at a concentration of 2 μg/ml appears to reduce the mutation frequencies slightly. This decrease is consistent but not significant. When the acridine was introduced at a concentration of 10 μg/ml, however, a clear-cut decrease in mutation frequency was observed. With the $CAN^s \rightarrow can^r$ system, this amounted to a 30-fold reduction, and for the $hi^- \rightarrow hi^+$ system there was a 6-fold reduction.

The meiotic experiments were performed with two different diploid strains homozygous for canavanine sensitivity and one diploid strain homozygous for histidine dependence. Mutation frequencies were again calculated from 15 independent measurements. The results, presented in Table 3.1.2, clearly show that an increase of mutation frequencies takes place in both systems.

Discussion. – Until now, mutagenic action of acridines in the dark has been observed only in the T-even bacteriophages. The lack of acridine-induced mutations in other organisms has necessarily restricted any generalizations from phage of the reading-frame hypothesis,[2] because only the acridines have been shown to be specific inducers of mutations of this type. The evidence from our experiments that forward and reverse mutations can be induced by acridines during meiosis will aid in permitting generalization of this basic hypothesis.

The differences in the responses of bacteria and bacteriophages to acridine have led to the idea that acridines might be mutagenic only in systems where active chromosomal exchange is taking place.[3] This was actually the reasoning behind our experiments. Our results on mitosis

Table 3.1.1. Effect of 5-Aminoacridine on Mutation Frequencies During Vegetative (Mitotic) Reproduction in Yeast

Mutation	Strain	Spontaneous Mutation Frequency	5-Aminoacridine Concentration (μg/ml)	5-Aminoacridine Mutation Frequency
$CAN^s \rightarrow can^r$	1060 haploid	4.3×10^{-9}	2	2.4×10^{-9}
	10284 haploid	3.7×10^{-8}	2	1.8×10^{-8}
			10	1.2×10^{-9}
	10118 haploid	2.2×10^{-7}	2	1.9×10^{-7}
$hi_{1-1} \rightarrow hi^+$	10284 haploid	2.9×10^{-9}	2	1.0×10^{-9}
			10	5.2×10^{-10}
	5243 diploid	3.8×10^{-9}	2	3.4×10^{-9}

Table 3.1.2. Effect of 5-Aminoacridine on Mutation Frequencies During Meiotic Division in Yeast

Mutation	Strain	5-Aminoacridine Concentration (μg/ml)	5-Aminoacridine Mutation Frequency
$CAN^s \rightarrow can^r$	5247	0	1.5×10^{-7}
		2	13.0×10^{-7}
	5300	0	4.5×10^{-7}
		1	9.0×10^{-7}
		2	37.0×10^{-7}
		4	110.0×10^{-7}
$hi_{1-1} \rightarrow hi^+$	5244	0	3.8×10^{-9}
		2	18.0×10^{-9}

and meiosis in the same organism support the contention that acridine mutagenesis is related to chromosomal pairing and exchange.

The antimutagenic effect may be a common feature of the action of acridines in the dark in vegetatively reproducing cells, since it has been observed in bacteria,[4] as well as in yeast.

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3.2 AN APPROACH TO A GENE-ENZYME RELATIONSHIP IN YEAST

T. R. Manney Margaret Schellinger

Introduction. – Supersuppressible mutants are believed to make incomplete proteins owing to a noncoding ("nonsense") sequence in the messenger RNA. These incomplete protein fragments provide a unique approach to the study of the enzyme tryptophan synthetase (TSase) in yeast. This enzyme has two distinguishable catalytic sites, which can be assayed independently, and mutants (nonsupersuppressible) retaining one or the other of the two activities have been isolated. By mapping these mutants we have been able to correlate their active sites with different regions of the genetic map.[1] The physiologically important activity of the enzyme depends on interaction between the two sites.

From mapping and complementation studies it has been possible to relate the supersuppressible mutants at this locus to these two regions. Four types of protein fragments are predicted: (1) some that are small polypeptides in which neither active site is present, (2) some that contain only one of the sites, (3) some that contain one site and probably part of the other, and (4) some that contain both sites. Among the mutants we have studied, only those of types (2) and (4) show allelic complementation with any testers.

If it is true that these supersuppressible mutants are nonsense mutants, then they provide a tool for genetically dissecting this enzyme to study its structure and the enzymatic and antigenic properties of its fragments. The object of the current studies is to characterize the enzymatic and antigenic properties of the proteins produced by these supersuppressible mutants, first, to test the hypothesis that they are nonsense mutants and, second, to study the structure and function of the enzyme.

Results and Discussion. – The first steps of a procedure for purifying *Neurospora* TSase were applied, with slight modifications, to crude extracts of a wild-type haploid yeast.[2] A 20-fold increase in the specific activity has been obtained. Dr. C. J. Wust generously prepared rabbit antiserum against this preparation. The serum will neutralize at least one of the catalytic activities of the wild-type enzyme (In → Tryp) and can therefore be used to detect immunological cross-reacting material (CRM) in mutant extracts.

We have studied extracts from five mutant strains. Different volumes of the mutant extracts were incubated at 37°C with a constant volume of serum – either the immune serum or a nonimmune control serum. The incubated mixtures were stored in the cold overnight, centrifuged, and the supernatants assayed for remaining neutralizing activity with homologous enzyme. With two of the mutant extracts the amount of antibody in the supernatant was less following incubation; these evidently contain a CRM. With the other three extracts the amount of antibody remained undiminished.

These results agree with predictions from genetic studies. Both of the CRM positive mutants are of the complementing type, and two of the CRM negative ones are noncomplementing. The other CRM negative mutant complements weakly with one other mutant; it is a supersuppressible, complementing mutant that evidently makes only one of the two catalytic sites. The CRM tests described above, however, utilized the activity of the other site (In → Tryp). There remains the possibility, therefore, that this mutant makes a CRM, but only with respect to the enzymatic activity of the site that it contains (In → InGP). This would mean that the two active regions of the enzyme could be distinguished antigenically, providing still another tool for studying their interaction.

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3.3 ELECTRON MICROSCOPY OF RIBONUCLEASE-INDUCED PUFFS

F. M. Ritossa H. Swift[1]

It was found that if ribonuclease is added to living salivary glands of *Drosophila buskii* a large number of puffs in the chromosomes are induced after 5 hr.[2] Since ribonuclease is able to break down RNA in these cells, we wondered if these induced puffs, in spite of their abnormally large dimensions, could represent regions that do not contain RNA. Our general approach was to understand more about the structural aspect of puffed structures and to observe the alterations produced by ribonuclease in the living cell.

After 2 hr of treatment with ribonuclease (5 mg/ml), the cells were unable to sustain protein synthesis,[3] yet at this time no apparent modification in their structure was found. In particular the microsomes showed no modification. We concluded that this blockage of protein synthesis does not involve apparent destruction of the ribosomal structure.

After 5–7 hr of treatment, the ribosomes appeared almost completely destroyed and large vacuoles appeared in the cytoplasm. The chromosomes were condensed but contained large puffs. Contrary to our expectation, these puffs were filled with dense granules that were much larger than the grains normally found in puffed sites. This is consistent with the finding that these structures are much more methachromatically stainable with azure B than normal puffs. We concluded that these grains are RNA. No polarity in the size of these granules was found along the axis of the puffed structure.

The nucleolar structure appeared completely distorted after 5–7 hr of ribonuclease treatment. We observed long rows of grains in the nucleoli similar to those observable in the puffs but these were surrounded by large vacuoles.

References

[1]Witman Laboratory, University of Chicago.
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3.4 RELATION BETWEEN STRUCTURAL CHANGES IN THE MACRONUCLEUS OF *TETRAHYMENA* AND THE CELL'S RNA SYNTHETIC ACTIVITY

I. L. Cameron E. E. Guile, Jr.

Phase and electron microscopy of the logarithmically growing ciliate *Tetrahymena pyriformis* reveal the presence of nuclear bodies of about 0.5 μ in diameter which are closely associated with and are randomly distributed over the nuclear membrane (Figs. 3.4.1 and 3.4.2). These bodies are believed to be nucleoli. In addition, one can discern smaller nuclear bodies with the use of the phase optics. These smaller bodies apparently correspond to the chromatin bodies which are distributed randomly throughout the nucleus (Fig. 3.4.2). As the cell culture reaches the growth deceleration phase (2–3 days after inoculation), the nucleoli begin to aggregate and fuse,

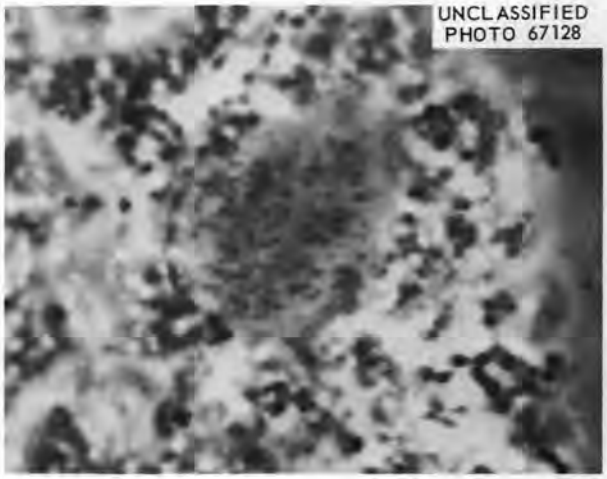

Fig. 3.4.1. Phase Contrast Picture of Nucleoli in the Nucleus from a Logarithmically Growing Cell.

thus becoming larger and fewer in number. In maximum stationary phase (4- to 6-day-old cultures) the fusion process has continued until one or very few large vacuolated bodies persist. When these stationary phase cells are placed into fresh medium, they show a lag phase of 2.5 hr before cell division begins. During this lag phase the large spherical vacuolated nuclear bodies flatten out against the nuclear membrane and begin to disaggregate into numerous bodies of about 0.5 μ in diameter (Fig. 3.4.3 is an early stage in this process). At first the nuclear bodies are nonrandomly distributed on the nuclear membrane, but by 3 to 4 hr after inoculation they return to the random distribution characteristic of logarithmic cells. This reversible nucleolar phenomenon presumably reflects the adaptive changes that these cells undergo under different environmental conditions. In view of the tentative role of nucleoli in ribosome synthesis, an attempt was

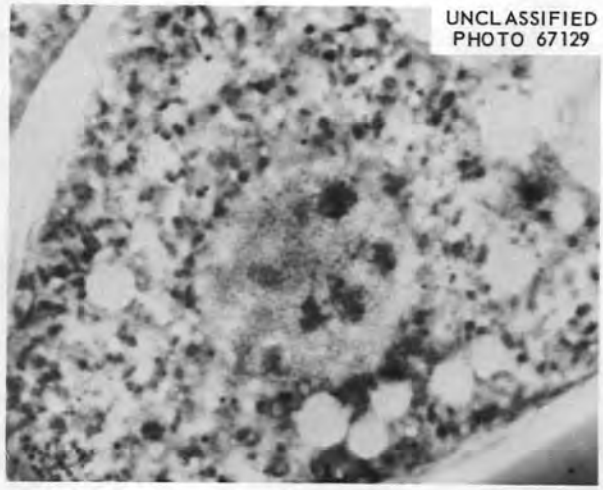

Fig. 3.4.3. Phase Contrast Picture of Cell from Early Lag Phase.

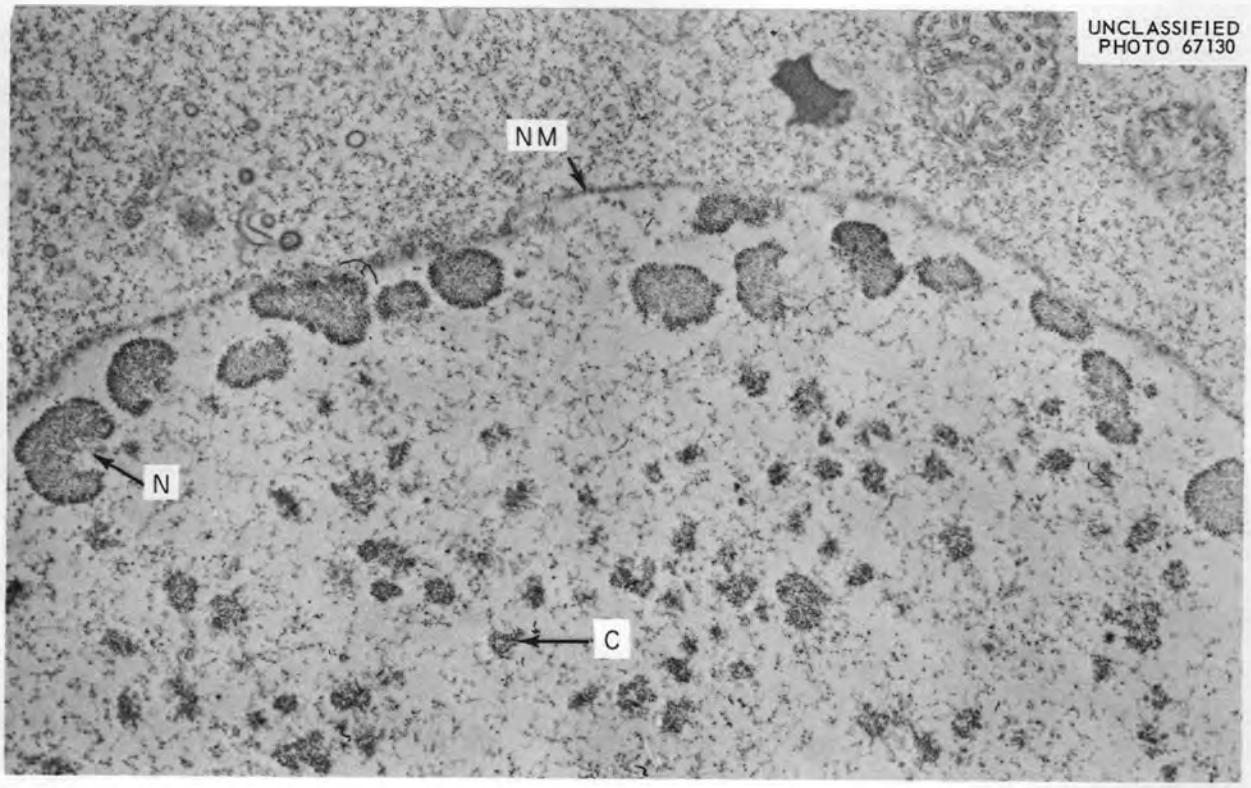

Fig. 3.4.2. Electron Micrograph of Logarithmically Growing Cell. N = nucleoli, C = chromatin bodies, and NM = nuclear membrane.

made to correlate the morphological changes with changes in RNA metabolism as determined with autoradiography after pulse labeling cells with ^{3}H-uridine for 15 min at intervals. The autoradiographic data show a general increase in ^{3}H-uridine incorporation (per unit area over nucleus and cytoplasm) throughout the lag phase. The increase is more pronounced in the cytoplasm than in the nucleus. The ratio of nuclear cytoplasmic incorporation shows a drop throughout the lag phase.

The morphological and autoradiographic data can be interpreted as follows. Nucleoli, which are involved in the synthesis of ribosomal RNA, aggregate, and the production of ribosomes slows down. In stationary phase cells the nonnucleolar chromatin continues to synthesize nonstable RNA (perhaps of the messenger type) and does not contribute appreciable stable macromolecular RNA to the cytoplasm. The recovery of cell growth and cell division that occurs during lag phase sees the reversal of this process.

4. Cell Growth and Differentiation

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4.1 EXTRACHROMOSOMAL NUCLEOLAR DNA IN AMPHIBIAN OOCYTES

O. L. Miller, Jr. Bessie G. Lukes

Introduction. – In thin sections, the peripheral nucleoli of amphibian oocytes exhibit the bipartite morphology of the typical nucleolus, that is, a fibrous core component surrounded by a granular cortical layer. Unlike the typical nucleolus, however, the peripheral nucleoli of these oocytes show no structural association with the chromosomes of the cell. A recent report[1] has suggested that such extrachromosomal nucleoli may contain DNA, an implication which, if substantiated, would rule out a constancy of DNA per chromosome complement within the nucleus of this cell. This report describes a direct demonstration of the presence of DNA in the peripheral nucleoli of an amphibian oocyte.

Results and Discussion. – The nucleoli and chromosomes of nearly mature oocytes of *Triturus pyrrhogaster* were isolated in 0.025 *M* KCl. At this molarity, the cortices of the nucleoli disperse, and the cores float free in circular configurations resembling loosely beaded necklaces. The beads are irregularly spaced, usually spherical, and vary in diameter from 0.5 to 3 μ. The strand connecting the spheres generally is submicroscopic as viewed by use of phase optics.

The circular cores were treated with two proteases and two nucleases with the following results: (1) Trypsin (0.1 mg/ml, pH 7): the beads become less refractile and decrease greatly in size. The diameter of the circles decreases about one-half, but no breaks occur in the necklaces. (2) Pepsin (0.1 mg/ml, pH 2): the beads (which swell at low pH) decrease in size and become refractile. The necklaces decrease somewhat in diameter, but again no breaks occur. (3) Ribonuclease (0.1 mg/ml, pH 7): the beads become quite refractile, and the circles decrease in diameter by fusion of adjacent spheres. However, no breaks in the continuity of the circles occur. (4) Deoxyribonuclease (0.01 mg/ml plus 0.0003 *M* Mg, pH 7): the beads exhibit little or no change in morphology. Breakage of the necklaces is observed, occurring slowly at first, then with increasing rapidity until most of the spheres show independent movement. Electron microscopy of similarly isolated cortical necklaces shows the interbead fibers to be similar in diameter (50 A)

and morphology to the DNA-containing axes of the lateral loops of lampbrush chromosomes. Measurements of collapsed circles indicate that the circular cores may fall into a geometrical progression of 11.25, 22.5, 45, 90, and 180 μ in circumference.

The presence of DNA in the 1000 to 1200 peripheral nucleoli present in the maturing amphibian oocyte suggests the possibility of a single chromosomal locus endoreplicating independently of the rest of the genome. The similarity between the circular configuration of the DNA component of the nucleolar cores and the circular chromosomes demonstrated in certain microorganisms is noted.

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4.2 COMPARATIVE STUDIES ON AVIAN AND AMPHIBIAN LIPOVITELLIN

R. A. Wallace

Granules from hen's egg yolk and yolk platelets from the frog's egg are both exclusively comprised of an insoluble lipovitellin-phosvitin complex. The lipovitellin (Lv) from avian yolk granules, however, has been resolved by a recently developed chromatographic procedure into two distinct populations (α and β), whereas the amphibian Lv seems to be a single, homogeneous entity.[1] Further comparative studies have been undertaken on the three Lv's in order to define the parameters needed for future studies on protein transport and alteration during vitellogenesis.

Molecular weight estimations by hydrodynamic and light-scattering methods provided values of 3.8 to 4.3 $\times$ 10^5 for the three proteins in neutral solvents. Lipid determinations gave values for all samples ranging from 16 to 20%. Composite and terminal amino acid analyses also failed to demonstrate significant differences among the Lv's (with the possible exception of arginine content, highest in amphibian Lv).

Protein-phosphorus content, however, was found to differ significantly among the Lv's (Table 4.2.1) and is thus the only unequivocal chemical difference found to date. Assuming that the molecular weight for the three Lv's is 400,000 and that their average lipid content is 18%, the number of protein-phosphate groups per protein molecule has been calculated to be 29, 40, and 50 for avian β-Lv, amphibian Lv, and avian α-Lv respectively.

The same relative progression was also noted for the "elution position" (in terms of the volume fraction of total gradient through the column) of the Lv's when they were eluted with a continuous gradient from TEAE-cellulose columns[1] (Table 4.2.1). Avian α-Lv, with the greatest number of phosphate groups, was also the most tightly adsorbed to TEAE-cellulose. The isolated Lv's were then dialyzed against distilled water, after which a very marked difference in solubility properties was noted: β-Lv was the least and α-Lv was the most soluble of the three.

Finally, when the Lv's are placed in alkaline solvent systems, a second, slower-sedimenting (7S) component is present in addition to the main (11S) component during ultracentrifugation. The amount of slower component observed is inversely proportional to the hydrogen ion concentration.

Table 4.2.1. Comparative Analytical Data for Avian and Amphibian Lipovitellins

Component	Protein Phosphorus (%)	Protein Nitrogen (%)	Elution Position (chromatography)	Percent Dissociated at pH 10.5
Avian α-Lv	0.48	16.13	0.53	35
Amphibian Lv	0.38	16.23	0.40	57
Avian β-Lv	0.27	16.46	0.32	83

Light-scattering studies on β-Lv solutions subjected to rapid pH changes indicated that the protein rapidly underwent a dissociation from dimer to monomer under alkaline conditions but that the back or reassociation reaction was very slow, probably due to limited reacting sites. The two peaks observed by ultracentrifugation, therefore, directly represent dissociated (molecular weight = 200,000) and undissociated (molecular weight = 400,000) forms of dimer Lv since, according to Gilbert's theory,[2] such a pattern typically would be observed when the associated form was either in slow equilibrium with two subunits or in rapid equilibrium with *three* or more subunits. The extent of monomer formation from dimer Lv at pH 10.5 was then measured for the three Lv's by ultracentrifugation, employing the proper corrections (Table 4.2.1). Again, a direct relationship between the protein-phosphorus content and the extent of dissociation was observed.

These results suggest that the causative factor for the chromatographic and physical behavior of the various Lv's is their extent of phosphorylation. Further experiments on the enzymatic alteration of Lv phosphate content are now needed to verify this suggestion and to indicate the possible mechanism whereby the Lv's change from soluble to insoluble proteins during passage from the serum into the developing oocyte.

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4.3 STUDY OF DNA SYNTHETIC ACTIVITY AND CELL CYCLE DURING LENS REGENERATION

Sara Eisenberg-Wieder

Introduction. – Earlier data have demonstrated that in the lens regenerating system there is an intense activation of DNA synthetic activity in the iris cell population after lens removal. Highest values of percentage of cells in DNA synthesis were obtained at early stages, in the dorsal as well as in the ventral iris. These values then decrease very sharply in the ventral iris and gradually in the dorsal iris to reach the control values (Fig. 4.3.1). Data in regard to mitotic activity indicate the appearance of a low number of mitotic figures at 7 days that reach a peak at 15 days after lens removal.[1] Therefore, this stage was chosen to determine the duration of the DNA synthetic period (S), G_1, and G_2 in the cell cycle. Cell generation time has also been calculated. In these studies it was hoped to obtain some understanding of the relationship between DNA synthesis, cell division, and cell differentiation.

Material and Methods. – Lentectomized animals at 15 days after lens removal were injected with ^{3}H-thymidine (1 μc per gram of animal weight) and killed at 15 min, 1, 2, 4, 6, 7, 8, 12, 14, 20, 24, and 48 hr, and 4, 7, and 10 days after injection. Duration of DNA-S, G_1, and G_2, and cell generation time were calculated according to Quastler and Sherman.[2]

Results. – Labeled cells appeared in the regenerate as early as 15 min after injection of the

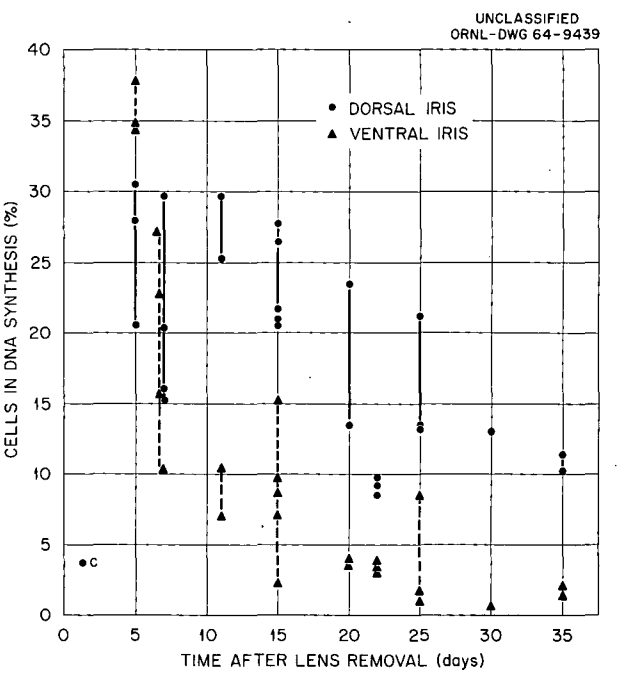

Fig. 4.3.1. DNA Synthesis at Different Stages of Lens Regeneration.

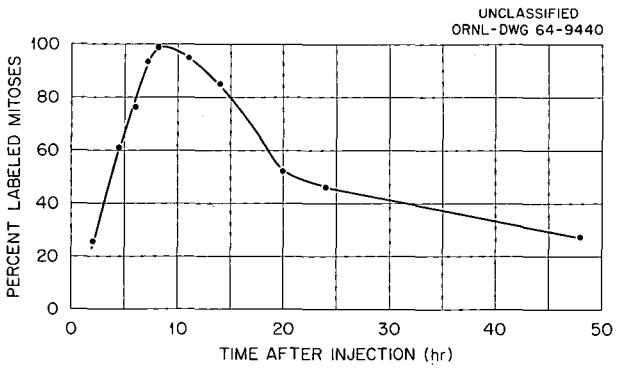

Fig. 4.3.2. Percent Labeled Mitoses in the 15-day Lens Regenerate as a Function of Time.

isotope. Labeled mitoses appeared 2 hr after injection and increased rapidly with time to reach 94% at 6 hr and 99% at 8 hr. The value for DNA-S was calculated from the interval between attainment of 50% labeling in mitotic figures after injection of the nucleoside and the time when the percentage of labeling is reduced to 50% in a later phase. In the regenerating lens it was calculated to be of the order of 15.5 hr (Fig. 4.3.2). Values for G_1 and G_2 have been calculated from our own data and for those of Hughes and Preston[3] for duration of mitosis in the newt. Duration of G_2 is 3 hr; G_1 lasts for 2.5 days. The synthetic index at this stage is 25%, and the approximate generation time is 3 days.

Discussion. – From these data and the data reported elsewhere,[1] we have been able to conclude that cells of the dorsal iris divide at least two times before they start differentiation into primary lens fibers. Studies on the synthetic indices and cell generation times at different stages of lens differentiation are presently being carried out.

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4.4 IMMUNOHISTOCHEMICAL STUDY ON APPEARANCE AND LOCALIZATION OF THREE MAIN COMPONENTS OF LENS PROTEINS IN THE REGENERATING NEWT LENS

Chinami Takata J. F. Albright[1]
Tuneo Yamada

Introduction. – It has been shown by using the immunofluorescent technique that adult *Triturus viridescens* lens proteins first appear in the cytoplasm of some inner layer cells of the regenerating lens vesicle at stage IV and increase in amount with the elongation of lens fiber cells.[2] The present study has been made to examine the distribution of three main components of lens proteins in the same system by means of an immunohistochemical method.

Materials and Methods. – Rabbit antiserum was prepared against newt α-, β-, and γ-crystallin as previously described.[3] The "specific immunofluorescent reagent" conjugated with isothiocyanate was prepared for each serum and absorbed with mouse powder.[2] A test of specificity indicated that the reagent for γ-crystallin was specific, while those for α- and β-crystallin faintly cross-reacted with one of other crystallins. The cross-reactivity was removed by keeping the reagents in the presence of corresponding crystallins for one week.

Results and Discussion. – The normal pigmented iris and regenerates of stages II and III were completely negative by the direct staining or by the indirect staining using the goat antirabbit serum conjugated with isothiocyanate.

At stage IV, α and β crystallins were first detected in the prospective primary lens fiber cells. Later they were always detected in the primary lens fiber cells. The reaction for γ crystallin became positive in the primary lens fiber cells from stage V. In stages IV to V, reactions for all crystallins were stronger in the cytoplasm and weaker or absent in the nuclei. These reactions increased their intensity in the nuclei and in the cytoplasm of primary lens fiber cells during the further course of their differentiation.

The secondary lens fiber cells which appear in the subsequent stages also indicated the presence of three crystallins. The sequence of events seems to be similar to that described above for the primary lens fiber cells.

Before stage VIII most of the lens epithelial cells did not contain a detectable amount of any crystallin. After stage IX, some lens epithelial cells became positive for α- or β-crystallin in their cytoplasm, but γ-crystallin was not found in those cells. It seems therefore that γ-crystallin is specific for lens fiber differentiation.

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4.5 THE EFFECTS OF X RAY ON LENS REGENERATION IN *TRITURUS VIRIDESCENS*

P. J. Kohonen Tuneo Yamada

Introduction. – Within the framework of our cell biological analysis of lens regeneration in the newt, x irradiation is applied to approach the control mechanism of the differentiation process occurring during tissue transformation. To study the relative resistance of various cells of the regenerating tissue at various stages of regeneration, a dose of 1000 r was found to be adequate. Irradiation at 200 r does not affect the progress of regeneration, while that at 5000 r or 10,000 r indiscriminately and immediately stops the regeneration.

Methods and Results. – The unilaterally lentectomized adults of *Triturus viridescens* were irradiated with 1000 r at various time intervals after lens removal. Lens regeneration is completely inhibited when a dose of 1000 r is given before the 10th day after removal of the lens. This dose, when given during the period from the 10th to the 20th day after lens removal, allows a certain extent of further development. However, the development results in formation of highly abnormal lens tissue. First, the lens epithelium is formed, which soon disintegrates into individual cells and, within several days, completely disappears. The lens fibers show certain progress of differentiation and maintain their differentiated condition for several days after irradiation. Later they show vacuolization and may degenerate completely. Observations show that differentiation processes occurring in the fiber area are more resistant than those occurring in the lens epithelium. When the abnormal regenerate finally disappears, the dorsal iris is able to develop a second regenerate which subsequently develops into a normal lens. Such recovery was not observed when irradiation was made in the early stages. When a dose of 1000 r is given later than the 20th day after lens removal, the regeneration process is temporarily retarded but results in a normal lens.

Discussion. – The distribution pattern of cell damage observed in this study coincides in a general way with that of mitotic activity in the same system as reported elsewhere.[1,2] It should also be emphasized that the results obtained have much in common with the results obtained with actinomycin treatment.[3,4]

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4.6 LENS REGENERATION FROM THE CORNEA IN THE ADULT SALAMANDER, *AMBLYSTOMA OPACUM*

Tuneo Yamada Marion Roesel

It is well established that in the family Salamandridae the adult can regenerate lens from the iris after complete lens removal. During the course of experiments carried out for a different purpose, it has been found that in the young adult of *Amblystoma opacum* the lens can be regenerated from the cornea but not from the iris. The cell population for regeneration is derived from the inner layer of the corneal epithelium. The cells invade the underlying substantia propria and form

a cell aggregate which later differentiates into the lens. The regenerated lens retains a connection with the cornea through its lens epithelium. The larvae of some amphibians (*Xenopus laevis* and *Hynobius unnanso*) are known to regenerate lens from the cornea. No report so far has been published, however, of regenerative transformation of the cornea into the lens in an adult amphibian. According to Freeman[1] the cornea of *Xenopus laevis* regenerates lens only before metamorphosis. He relates the loss of capacity for tissue transformation after metamorphosis to differentiation of the corneal epithelium. Our finding indicates the capacity for tissue transformation even in the definitive corneal epithelium. A comparison of cellular events occurring in transformation of cornea into lens in *Amblystoma opacum* with those occurring in transformation of iris into lens in *Triturus viridescens* may provide us some useful information for understanding the control mechanism of tissue specificity.

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4.7 ELECTRON MICROSCOPIC EXAMINATIONS OF THE SITES OF NUCLEAR RNA SYNTHESIS DURING AMPHIBIAN EMBRYOGENESIS

Shuichi Karasaki

Introduction. – The previous autoradiographic study using the electron microscope suggested that tritiated uridine is first incorporated into the fibrous region of the nucleolus and the chromatin fibrils.[1] During the early stages of amphibian development, uridine can be incorporated into DNA.[2] Water-soluble embedding media and enzyme extraction procedures[3] have been employed in this electron microscopic study to estimate the extent of incorporation of uridine into DNA and RNA. Some attempts were also made to study the cytochemical nature of nuclear ultrastructure with thin sections.

Methods. – Isolated tissues from the embryos of *Triturus pyrrhogaster* at various stages were treated in ^{3}H-uridine (50 μc/ml) for 3 hr and were transferred into nonradioactive solutions for different time intervals (0–45 hr). The explants were then fixed with glycidaldehyde and embedded in glycol methacrylate. For the cytochemical examination and autoradiography, some enzyme digestions have been carried out on ultrathin and thick sections with 0.01% ribonuclease (RNase), 0.01% deoxyribonuclease (DNase), and 0.05% pepsin.

Results and Discussion. – Glycidaldehyde fixation followed by glycol methacrylate proved to be more satisfactory than other techniques studied. According to the uranyl staining at various pH's and RNase digestion, the distribution of RNA seems to be uniform in the nucleolar body. The primary nucleus at the gastrula stage and the large nucleolus at the tail-bud stage are similar, except for their size. DNase has an immediate and strong effect on all chromatin structure. DNase treatment decreases the density of the nucleolus and severely disrupts the interchromatin materials. After 15 min treatment with pepsin, two central and peripheral regions of the nucleolus become clearly distinguishable. These may correspond to the fibrous and granular area as seen in osmium-fixed and Epon-embedded material. By enzyme digestion and uranyl staining, the presence of chromatin fibrils is clearly demonstrated in the central portion of the nucleolus which significantly incorporates ^{3}H-uridine. This may suggest that the intranucleolar chromatin participates in the synthesis of nucleolar RNA.

Experiments were performed to test whether labeling was detectable after treatment with DNase and RNase. During gastrulation, labeling over the chromatin is remarkably reduced by DNase treatment but is not significantly changed by RNase.

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4.8 A FURTHER STUDY OF THE NUCLEAR RNA SYNTHESIS DURING EMBRYONIC INDUCTION

S. K. Brahma Tuneo Yamada

Introduction. – Our earlier data[1] indicated that when the isolated newt ectoderm is experimentally induced to differentiate the neural tissue under the influence of the normal organizer (dorsal mesoderm), RNA synthesis in the nucleus is enhanced compared with that in the isolated ectoderm, which was not influenced by the organizer and only differentiates epidermal cells. In the present report a further study along this line will be discussed which supports the suggestion stated above.

Methods. – From the early gastrula of *Triturus pyrrhogaster*, a piece of the prospective ectoderm was removed and cut into two pieces of approximately equal size. One of the pieces (experimental) was combined to an explant of the dorsal mesoderm (organizer). The other piece (control) was not combined with any other tissue. Both pieces were cultured in sterile Holtfreter solution (pH 7.3) at 18°C. Three, six, and twenty two hours after the beginning of culture, the explants were exposed to 50 μc/ml of ^{3}H-uridine in Holtfreter solution for 3 hr, washed, fixed, and processed for autoradiography. Relative radioactivity of the nucleus of the ectoderm cells was estimated by grain count per unit area of the nucleus. The grain count data were compared between the experimental and control belonging to each pair. RNase and DNase were used to determine the extent of incorporation of ^{3}H-uridine into RNA and DNA in each series. To check the possible difference in the nuclear volume between experimental and control, the size of the nucleus sections in the autoradiographs used was estimated.

Results. – The data given in Table 4.8.1 concern the autoradiographic slides which were not treated with enzymes. They indicate higher incorporation of ^{3}H-uridine into the nuclei of the experimental series compared with that of the control series, except for some pairs in series 1 hr, 3 hr, and 48 hr. The ratio between experimental and control grain counts becomes larger in the later stage of embryonic induction. Data of RNase treatment and DNase treatment show that ^{3}H-uridine is incorporated into both RNA and DNA in the series 1–22 hr, but in series 48 hr it is incorporated almost exclusively into RNA under our experimental conditions. The percentage of RNA incorporation as estimated by RNase treatment is reported in Table 4.8.2. No indication was obtained for a difference in the RNA/DNA

Table 4.8.1. Comparison of Mean Grain Counts per Unit Nuclear Area Between Each Pair of Ectoderm Pieces at Various Time Intervals from the Contact with the Organizer

1 hr Series	3 hr Series	6 hr Series	22 hr Series	48 hr Series
Experimental Control	Experimental Control	Experimental Control	Experimental Control	Experimental Control
8.04 > 5.30	7.34 > 5.28	10.85 > 8.74	13.97 > 10.80	12.62 > 8.12
5.54 > 4.74	11.00 > 5.95	13.35 > 9.54	14.33 > 11.06	12.00 > 7.18
12.68 > 10.32	7.48 > 4.64	4.82 > 2.72	9.25 > 6.8	13.64 > 5.88
17.02 > 14.24	6.22 – 5.49	11.67 > 6.97	7.56 > 3.22	19.32 > 10.04
11.42 – 10.84	7.16 – 7.68	6.94 > 3.04	22.61 > 11.91	17.98 > 8.32
12.18 – 13.38	(1.38)	(1.67)	(1.63)	19.04 > 11.30
12.38 < 14.54				23.02 > 7.16
(1.13)				21.76 > 13.96
				11.06 – 10.66
				(1.89)

The statistical difference at 95% confidence limit was tested for each pair of data and the result indicated with signs. In parentheses are the average ratios between grain counts of experimental data and those of control data.

Table 4.8.2. Average Percent of Grains per Unit Area over the Nucleus Removed by the RNase Treatment Both from the Experimental and the Control Sets of Explants Fixed at Different Time Intervals

Hour of Culture	Experimental	Control
1	72.9	69.6
3	82.5	79.2
6	79.2	64.9
22	87.7	77.7
48	100	100

ratio of incorporation between one pair of explants in all series. The measurement of the area of nuclear sections of the autoradiographs used for the data of Table 4.8.1 demonstrated that no significant difference exists between experimental and control in all series.

Discussion. – The data obtained indicate that when the ectoderm comes into contact with the organizer and is neuralized, the RNA synthesis in the nucleus of the ectoderm cells is enhanced. This enhancement precedes any reported changes in cytological, ultrastructural, cytochemical, or biochemical parameters, as they occur in the corresponding ectodermal areas in the normal development under the influence of the organizer.[2]

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4.9 THE EFFECT OF HETEROGENOUS HISTONES ON THE DIFFERENTIATION OF ISOLATED AMPHIBIAN ECTODERM

P. J. Kohonen Tuneo Yamada

Introduction. – The *Triturus* ectoderm isolated at the early gastrula stage and cultivated in vitro develops into ciliated embryonic epidermis. This ectoderm, however, will differentiate into neural and other tissues in response to a wide variety of influences, among them adult tissues and their protein fractions. It has been postulated that histones play an important role in differentiation, acting as gene suppressors. A survey of the literature shows that a role of histones in the normal course of embryonic induction is not excluded. In the present experiment, the inductive effect on the isolated ectoderm of two relatively pure histone preparations was studied.

Methods. – *Triturus pyrrhogaster* or *T. alpestris* ectoderm was isolated at the early gastrula stage and treated for 24 hr in sterile Holtfreter-Ringer solution containing 50, 100, or 300 μg/ml of lysine-rich or arginine-rich histone from calf thymus. The solutions were buffered to pH 7.1–7.3. The samples of histones were prepared at the laboratory of Dr. Mirsky. After the treatment the ectoderm explants were transferred to Holtfreter-Ringer solution of pH 7.3 and cultured at 18° for 6 to 12 days. They were fixed in Bouin's solution and studied in routine histological sections. In the control series similarly isolated ectoderm of both species was treated with Holtfreter-Ringer solution having the pH value of experimental solution for 24 hr and then cultured with Holtfreter-Ringer solution of pH 7.3.

Results and Discussion. – Treatment of *T. pyrrhogaster* ectoderm with lysine-rich histone at concentrations of 50 or 100 μg/ml caused neural, mainly archencephalic differentiation in 78% of explants which were alive at the time of fixation. In several cases there was also some evidence for mesenchyme differentiation. A larger percentage of *T. pyrrhogaster* explants died before the end of the experiment when treated at a concentration of 300 μg/ml of the same histone preparation. Explants of this group that remained viable were partially cytolyzed and did not differentiate. When treated with arginine-rich histone, only a small number of *T. pyrrhogaster* explants died, but the highest concentration of this protein caused severe cytolysis. The inductive effect of arginine-rich histone was weaker, only 10% of the explants being differentiated into unspecific neural tissue. None of the *T. alpestris* explants was neutralized, and the death rate in this species was very low. In control series of both species, all explants formed aggregates of ciliated epidermal cells.

In view of the data obtained, it appears worthwhile to study the effect of histones of the same species on differentiation of the ectoderm.

5. Radiation Microbiology

Alexander Hollaender[a]	
Mutagen Mechanisms – Bacterial Metabolism (5.1)	**Bacterial Genetics (5.2–5.6)**
G. E. Stapleton[a] Annie S. Angel	H. I. Adler[a] Roy Curtiss III D. E. Duggan[b] L.J. Charamella Alice A. Hardigree D. E. Axelrod[c] Claire M. Berg[d] Janet Renshaw[e] W. S. Reznikoff[c]
[a]Dual assignments. [b]Visiting investigator from abroad. [c]Loanee. [d]ORINS Fellow. [e]Student trainee.	

5.1 THE ROLE OF EXCESS MACROMOLECULES AND RADIORESISTANCE IN *ESCHERICHIA COLI*

G. E. Stapleton Annie S. Angel

Introduction. – Several growth conditions increase the radioresistance of *Escherichia coli.*[1–3] These conditions, in general, yield cell populations with larger amounts of DNA, RNA, and/or protein per cell than are found in more sensitive cell populations. It is not clear from any published data whether excess macromolecules per se are responsible for greater radioresistance or indicative of a general or specific synthetic capacity, which may suggest a greater ability to repair or cope with radiation damage. We are investigating possible roles of excess DNA and RNA in radioresistance of this species.

Results and Discussion. – We have attempted to demonstrate genetically any unusual property of the DNA of resistant cells by use of the conjugation system in K-12 strains. Radioresistant and sensitive F^- cells from stationary phase were mated with logarithmic-phase Hfr cells for 120 min at 37°C. The frequency of transfer of Hfr chromosome was measured by selecting for transfer of markers close to the origin of the Hfr chromosome. Incorporation of markers was tested by selection among recombinants for a distal marker and observing among these selected recombinants the frequency of occurrence of more proximal unselected markers. We found that the glucose-grown (radioresistant) F^- cells show a much higher frequency of transfer of markers, but among recombinants selected for distal markers, the frequency of incorporation of more proximal unselected markers is not different in irradiated or unirradiated resistant or sensitive cells.

No explanation is yet available for the increased frequency of transfer of markers obtained with glucose-grown recipient cells; however, the frequency is no higher than that obtained for logarithmic-phase cells.

Resistant cells, obtained from glucose–amino acid cultures in stationary phase, have a high RNA/DNA ratio as compared with sensitive cells. We are investigating, at present, the possibility that the excess RNA resides in a particular class of RNA molecules in resistant cells.

The resistance shown for glucose-grown cells has been found to depend almost entirely on the presence of amino acids in the postirradiation plating medium. This finding is suggestive that

this type of resistance involves a postirradiation synthetic activity concerned, perhaps, with repair of radiation damage. Preliminary experiments show that these cells require the supplementation for only a short period of time, about 30 min at 37°C, for expression of resistance. Survival after this treatment is similar on minimal and complete media. The reciprocal experiment shows that ability to express resistance is also rapidly lost in the absence of amino acids in the post-irradiation incubation medium. The nature of some of the processes occurring immediately after irradiation is the subject of our current research.

It is not clear how the excess macromolecules are involved in physiologically induced radioresistance. We have found that the time sequence of synthesis of excess DNA per cell and development of radioresistance are different. Moreover, some mutant strains of this species synthesize excess DNA and do not become resistant. We can conclude that the excess DNA per se does not explain this type of radioresistance.

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5.2 THE CLASSIFICATION OF RECOVERY CONDITIONS BASED ON EARLY POST-IRRADIATION DIVISION PHENOMENA

H. I. Adler Alice A. Hardigree

We have continued our studies on the formation of filaments by "lon" mutants[1] and have observed that a short postirradiation exposure to a temperature of 42°C before incubation at 37°C enhances survival by stimulating the division of filaments that would otherwise fail to produce macrocolonies. We have also observed that exposure to a minimal synthetic medium has a similar but less pronounced effect. These microscopic studies of "lon" mutants and many other bacteria suggested to us that ionizing radiation inactivates bacteria either by interfering with cell growth or cell division. Strains in which division is the radiation-sensitive process are still capable of growth after radiation exposure and form long filaments having the characteristics previously described.[1] The bacteria observed to date fall into two distinct categories as indicated in Table 5.2.1.

Based on our observation with the "lon" mutant and the published literature, we have postulated that conditions favoring division relative to growth should result in the recovery of class I organisms but not of class II organisms. This prediction has been borne out (Table 5.2.2). Furthermore, it seemed logical to predict that conditions favoring growth relative to division should be effective in promoting the recovery of class II organisms. Growth at low temperature or on very rich media are known to be such conditions, and the data obtained for representative class II organisms (*Aerobacter* B, *Proteus mirabilis, Salmonella typhimurium)* support this second prediction.

References

[1]H. I. Adler and A. A. Hardigree, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964,* ORNL-3601, p. 95.

Table 5.2.1. Classification of Bacteria on the Basis of Filament Formation After Exposure to Ionizing Radiation

Class I (division inhibited); more than 75% of the non-colony-forming cells produce filaments
Escherichia coli B (Witkin), B (ORNL), AB1899, 3.300 M-6
Class II (growth inhibited); less than 20% of the non-colony-forming cells produce filaments
Escherichia coli Monod, P678, AB531, B/r (Witkin) B/r (ORNL), B_{s-1} Hill, 7, 13
Salmonella typhimurium
Aerobacter A
Aerobacter B
Alcaligenes sp.
Erwinia sp.
Proteus mirabilis

Table 5.2.2. Recovery of Colony-Forming Ability[a]

Organism		Postirradiation Plating Conditions		
		Nutrient Agar at 42°C	Nutrient Agar + Pantoyl Lactone	Synthetic Medium
E. coli				
B (Witkin)	Class I	+	+	+
B (ORNL)	Class I	+	+	+
AB1899	Class I	+	+	+
3.300 M-6	Class I	+	+	+
E. coli				
Monod	Class II	–	–	–
B/r (Witkin)	Class II	–	–	–
B/r (ORNL)	Class II	–	–	–
B_{s-1} Hill	Class II	–	–	+[b]
S. typhimurium	Class II	–	–	–
Aerobacter B	Class II	–	–	–
Erwinia sp.	Class II	–	–	–
Proteus mirabilis	Class II	–	–	–

[a]A "+" indicates recovery (a greater number of colonies than an unsupplemented nutrient agar incubated at 37°C). A "–" indicates no recovery.

[b]A small but reproducible recovery was observed in this case.

5.3 GENETIC ANALYSIS OF A RADIATION-SENSITIVE *ESCHERICHIA COLI*

D. E. Duggan

Several loci in the linkage group of *E. coli* K-12 are involved in the determination of radiation sensitivity. The induction and isolation of two new mutations to enhanced radiation sensitivity have been described.[1] These mutations were induced by exposure of *E. coli* K-12 AB1886 to nitrous acid. AB1886 is itself a radiation-sensitive mutant and lacks the ability to repair ultraviolet-irradiated bacteriophage.

Detailed genetic analysis suggested that the radiation sensitivity of one of the new mutants is due to an alteration at a locus between the loci controlling the synthesis of serine/glycine and histidine. A mutation affecting radiation sensitivity has not previously been reported for this region. The action of the newly mutated gene is independent of the one present in the parental AB1886 strain.

The biochemical expression of the new locus is not known. It is not a mutation of the "lon" type,[2] nor is it similar to the type of alteration that results in impaired ability to repair radiation-damaged bacteriophage.

References

[1]D. E. Duggan and H. I. Adler, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964,* ORNL-3601, p. 96.

[2]H. I. Adler and A. A. Hardigree, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964,* ORNL-3601, p. 95.

5.4 THE ROLE OF THE F^- PARENT IN BACTERIAL CONJUGATION

Roy Curtiss III L. J. Charamella

Time-of-entry experiments with Hfr OR1 indicate that the F^- (recipient) is not, as previously suggested, a passive partner during conjugation. The

data obtained indicate that there must be homology between the proximal portion of the Hfr chromosome transferred and the comparable region of the F^- chromosome in order to obtain "normal" rates of chromosome transfer and "normal" recombination frequencies.

The relevant order of transfer and control entry times in parentheses for the three Hfr's used in this study are as follows: Hfr H – origin, leu^+ (8.5 min), pro_1^+ (15 min), pro_2^+ (17 min); Hfr OR1 – origin, pro_2^+ (4.5 min), pro_1^+ (7.5 min), leu^+ (15 min); Hfr Cav – origin, pro_2^+ (10 min), pro_1^+ (12.5), leu^+ (19 min). These entry times were obtained by using F^- strains with leu^-, pro_1^-, and pro_2^- point mutations. If, however, an F^- with a $/3,7,\lambda,pro_{1,2}^-$ mutation was used, entirely different results were obtained. The $/3,7,\lambda,pro_{1,2}^-$ mutation confers resistance to phages T3, T7, and λ and appears to be a deletion of 2 to 3 min of the bacterial chromosome including the pro_1 and pro_2 loci. Strains with $/3,7,\lambda,pro_{1,2}^-$ mutations do not revert to pro^+, and no pro^+ recombinants are obtained in crosses with any pro_1^- or pro_2^- strains. Time-of-entry experiments with an F^- leu^- $/3,7,\lambda,pro_{1,2}^-$ strain gave results as follows: Hfr H – leu^+ (8.5 min), $(/3,7,\lambda)^s pro_{1,2}^-$ (17.5 min); Hfr Cav – $(/3,7,\lambda)^s pro_{1,2}^+$ (13 min), leu^+ (19 min); Hfr OR1 – $(/3,7,\lambda)^s pro_{1,2}^+$ (27 min), leu^+ (31 min). In crosses between Hfr H or Hfr Cav and the F^- leu^- $/3,7,\lambda,pro_{1,2}^-$ strain, leu^+ and $(/3,7,\lambda)^s pro_{1,2}^+$ recombinants were formed at slightly reduced frequencies, whereas in the cross with Hfr OR1 these recombinant classes were reduced by a factor of 10 to 50. These results indicate that pairing of the most proximal region of the Hfr chromosome with the homologous region of the F^- chromosome is necessary for chromosome transfer to occur at a normal rate and with a normal frequency.

5.5 THE *PRO-LAC* REGION OF THE *ESCHERICHIA COLI* K-12 GENOME

Roy Curtiss III L. J. Charamella

Introduction. – We are interested in the genetic structure of the *pro-lac* region of the *E. coli* K-12 genome for two reasons. First, a fine-structure genetic map of the pro_2-*lac*-pro_3 region is necessary to understand certain mutations in the *lac* region concerned with regulating the synthesis of β-galactosidase and galactoside permease. Second, a comparison of the *pro-lac* region between *E. coli* and *Salmonella typhimurium* provides evidence on the evolutionary significance of clustering of genes controlling related functions.

Results. – By various genetic tests, the arrangement of loci was found to be: $leu \cdots pro_1$ pro_2 lac_y lac_z lac_i $pro_3 \cdots ade_3$. All pro_3^- mutants crossfeed pro_1^- and pro_2^- mutants and are therefore presumed to be blocked in the last step in proline biosynthesis from glutamic acid. The lac_y^- mutants lack galactoside permease, the lac_z^- mutants lack β-galactosidase, and the lac_i^- mutants do not produce repressor and are therefore constitutive in their synthesis of galactoside permease and β-galactosidase. The pro_1 and pro_2 loci are 1 to 2 min apart as determined by time-of-entry experiments with Hfr H and Hfr Cav. The $/3,7,\lambda,pro_{1,2}^-$ mutants have the entire pro_1 to pro_2 segment deleted. Hfr P4X6 transfers pro_1 as the first marker and pro_2 as the last. Hfr OR1 transfers lac_z as the first marker and lac_i as the last. An F′ isolated from Hfr P4X6 carries the markers pro_2^+ lac_y^+ lac_z^+ lac_i^+ but not pro_3^+. An F′ isolated from Hfr OR1 carries the markers lac_i^+ pro_3^+. The isolation of pro_2-lac_y^- deletion mutations also indicates the linkage given above and suggests that the pro_2 and lac_y loci may be contiguous. The pro_2-*lac* segment can be cotransduced by P1*kc*, as can the *lac*-pro_3 segment. A pro_2^- pro_3^- strain is being prepared in order to test for cotransduction of the entire pro_2 to pro_3 segment. The order lac_y lac_z lac_i pro_3 has been determined by performing three- and four-factor crosses by employing P1*kc* transducing phage. Fine-structure mapping of the pro_2 to pro_3 segment is now in progress, employing 64 independently isolated pro^- mutants and 24 independently isolated lac^- mutants. These mutations have been obtained in strains which are coisogenic for the *pro-lac* segment.

In the isolation of pro^- mutants, it has been observed that different substrains of *E. coli* K-12 give rise to different classes of pro^- mutants. Thus the ratio of independently isolated pro_1^-:pro_2^-:pro_3^- mutants for derivatives of W1485 was 21 : 2 : 2, for derivatives of W945 was 9 : 0 : 10,

for derivatives of C600 was 0:9:6, and for derivatives of W1895 was 0:5:2. Hundreds of nonindependent *pro*$^-$ mutants have been screened for the missing classes among derivatives of W945, C600, and W1895 without success. Mutations were considered to be independent if they were isolated from different penicillin enrichment cultures or if they were mutated in different *pro* cistrons but occurred in the same penicillin enrichment culture.

Discussion. – In *S. typhimurium* the *pro A* (pro_1), *pro B* (pro_2), and *pro C* (pro_3) loci are all cotransducible at high frequency and are therefore closely linked. In *E. coli* K-12 1 to 2 min separates pro_1 from pro_2, and about 1 min separates pro_2 from pro_3. (The maximum permissible distance between two mutations for cotransduction is about ½ to 1 min.) This comparison suggests that, in this instance, clustering of genes performing related functions is a more highly evolved state than absence of clustering. The clustering of the *pro A*, *pro B*, and *pro C* loci in *S. typhimurium* could have arisen by deletion of genetic material between *pro A* and *pro B* and between *pro B* and *pro C* in an ancestral type which gave rise to both *E. coli* and *S. typhimurium*. This notion is supported by the fact that *S. typhimurium* is *lac*$^-$; the entire *lac* region has been deleted in the evolution of this species, an act which would increase the linkage (decrease the distance) between *pro B* and *pro C*.

5.6 CYCLOSERINE INHIBITION AND RESISTANCE IN *ESCHERICHIA COLI*

Roy Curtiss III
L. J. Charamella
Paula E. Harris[1]
Claire M. Berg

Introduction. – D-Cycloserine inhibits the enzymes alanine racemase and D-alanyl-D-alanine synthetase in *Escherichia coli*. These two enzymes are involved in the synthesis of the mucopeptide which is a structural component of the rigid layer of the cell wall. In the presence of cycloserine, growing *E. coli* cells lyse due to the prevention of cell-wall synthesis. We first became interested in mutations to cycloserine resistance (*cyc*^{r}) as a useful genetic marker for crosses employing streptomycin-resistant (*str*^{r}) donor strains or for crosses in which the *str*^{s} marker from the Hfr was transferred at high frequency, thus negating the use of the F$^-$ *str*^{r} marker for contraselection against the Hfr. More recently, it has been found useful to employ cycloserine to kill growing *E. coli* cells in order to isolate auxotrophic mutations from strains which had become resistant to penicillin.

Results. – Strains K-12, M7, and M13 of *E. coli* have been used in these studies. The latter two strains are of mouse origin. The relevant order of genetic markers and the distance in minutes between them is as follows: *his* 26 *str*^{r} 8.5 *xyl* 8.5 met_1 3.5 *thi* 8.0 *leu* 9.0 *lac*. Cycloserine was prepared daily as a 0.2 *M* solution in 0.1 *M* phosphate buffer, pH 8.0.

Nongrowing *E. coli* were resistant to cycloserine, whereas growing cells were lysed exponentially. The exponential rate of killing in minimal medium increased over D-cycloserine concentrations of 5×10^{-6} to 5×10^{-5} *M*; remained constant over the range in concentrations of 5×10^{-5} to 1×10^{-2} *M*; and then declined at concentrations above 1×10^{-2} *M*. At concentrations above 2×10^{-2} *M*, D-cycloserine was bacteriostatic.

On minimal agar medium, D-cycloserine at 5×10^{-7} *M* was sufficient to completely inhibit growth. Mutations to D-cycloserine resistance occurred in a stepwise manner. First-step mutants were resistant to 1×10^{-5} *M* D-cycloserine and were formed at frequencies of 10^{-6} to 10^{-7}. However, D-cycloserine at 5×10^{-4} *M* was required to cause maximal exponential lysis of these first-step resistant mutants growing in liquid minimal medium. Second-step mutants were resistant to 5×10^{-5} *M*, and third-step mutants were resistant to 2×10^{-4} *M* D-cycloserine.

Recombination experiments with several F$^-$ strains resistant to 1×10^{-5} *M* D-cycloserine gave similar results. The *cyc*^{s} allele was not transferred by Hfr H or Hfr AB313. Results with Hfr Cav indicated that the *cyc*^{r} locus was between *thi* and met_1. Transduction experiments to determine whether the mutations resulting in high-level resistance to cycloserine are linked are now in progress.

The preparation of polyauxotrophic F^- strains by successive mutagen treatment followed by penicillin enrichment procedures frequently results in strains which are penicillin resistant. The isolation of more auxotrophic mutations in these penicillin-resistant strains thus requires other enrichment procedures. Experiments with cycloserine indicate that at concentrations of 5×10^{-5} to 1×10^{-2} *M* cycloserine is as effective as penicillin in enriching for auxotrophic mutants.

References

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6. Mammalian Genetics

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Genetic Effects of Radiation in Mice (6.1, 6.2) U. H. Ehling Elizabeth M. Kelly Louis Wickham J. R. Inman Josephine S. Gower[a] Jean W. Bangham Patricia A. Burd Georgia M. Guinn Patricia R. Hunsicker Martha M. Larsen Savanna C. Maddux Elizabeth L. Phipps Jackie N. Poole Mary H. Steele Kathren F. Stelzner H. M. Thompson, Jr. Carolyn M. Vaughan Sandra J. Weaver Florence N. Woodiel Barbara I. Yarn	**Mammalian Cytogenetics (6.3, 6.4)** Liane B. Russell B. M. Cattanach[b] Clyde S. Montgomery **Effects of Radiation on Mammalian Gametogenesis (6.5)** E. F. Oakberg[c] Evelyn Clark Jo K. Henderson Connie J. Heath[d] **Cooperative NIH-AEC Co-Carcinogenesis Program (6.4)**
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6.1 NEUTRON-INDUCED MUTATION IN MOUSE SPERMATOGONIA. LACK OF EFFECT OF DOSE RATE. RELATIVE BIOLOGICAL EFFECTIVENESS OF NEUTRONS

W. L. Russell Elizabeth M. Kelly

This work on specific locus mutations in mice exposed to fission neutrons from the Health Physics Research Reactor is an extension of that described in an earlier report,[1] in which the experimental procedure and the results from irradiated post-spermatogonial stages were given. The data presented here all come from irradiated spermatogonia.

The experiments include a variety of doses and dose rates. It is already clear that at high doses and high dose rates the mutation frequency drops below that obtained at lower doses. This phenomenon parallels that reported for acute x irradiation[2,3] and can be attributed to the same cause, which is believed to be the selective elimination, at high doses, of the more mutagenically sensitive cells in the spermatogonial population. This introduces a complexity into the calculation of relative biological effectiveness. The difficulty can be avoided by limiting comparisons to doses below the level at which the drop in mutation frequency is noticeable. Results from such a dose level are given in Table 6.1.1.

Table 6.1.1. Specific Locus Mutations Induced by Fission Neutrons in Mouse Spermatogonia

Dose[a] (rads)	Dose Rate (rads/min)	Number of Offspring	Number of Mutations at 7 Loci	Mean Number of Mutations per Locus per Gamete
				$\times 10^{-5}$
63	0.17	18,194	13	10.2
59	0.79	17,041	12	10.1
59	79	16,758	10	8.5

[a]Includes a gamma component equal to approximately one-seventh of the neutron component.

The data from three separate dose rates show no significant differences, in contrast to the marked dose-rate effects observed with x- and gamma-ray-induced mutations in spermatogonia. This will be discussed in another report.

For the present purpose it seems valid to combine the results from the different dose rates. The pooled 35 mutations in 51,993 offspring give a mutation frequency of 9.6×10^{-5} per locus per gamete for a weighted mean dose of 60 rads. A comparable biological effect, and, therefore, one suitable for the estimation of RBE, was obtained[3] in an experiment with 300 r of 90 r/min x rays which gave a mutation frequency of 8.7×10^{-5} per locus per gamete (40 mutations in 65,548 offspring). Calculating the RBE of fission neutrons to x rays from these two sets of results, with allowance for the gamma component in the reactor exposures and with conversion of the x-ray doses to rads, we obtain a value of 5.8. The estimate is not significantly different (5.1) if we limit the comparison to the high-dose-rate portion of the neutron data.

Since there is, so far, no evidence of a dose-rate effect for neutron irradiation of spermatogonia, it is clear that the RBE of neutrons to x or gamma rays will be much higher at low dose rates. This RBE can be estimated from the results already obtained at low gamma dose rates. The ratio of mutation frequency per rad of 90 r/min x rays (based on 151 mutations) to all gamma-ray exposures at 0.009 r/min and below (based on 61 mutations) is 3.3.[4] The RBE of fission neutrons to gamma rays for mutations induced in mouse spermatogonia at low dose rates can, therefore, be estimated as $5.8 \times 3.3 = 19.1$.

The mutation frequency per rad in the above neutron data is about one and a half times that reported by Batchelor *et al.*[5] In spite of this, these authors estimate a neutron-gamma RBE of 30 for chronic exposures. The reason that their figure is higher than ours, instead of lower (as would be expected from the lower neutron-induced mutation frequency), is that they are using a much lower estimate of gamma-induced mutation frequency. This estimate is based on only 5 mutations as compared with our gamma-ray figure based on 61 mutations plus an additional 10 in the experiment now to be described.

Our estimate of RBE for low-dose-rate irradiation is confirmed by the entirely independent set of neutron and gamma-ray data shown in Table 6.1.2. These results are chosen for comparison on the basis of equal exposure time at a low dose rate. The gamma-ray data, although limited, happen to be in exact agreement with the average mutation frequency obtained at all lower dose rates. The RBE calculated from the data in Table 6.1.2 is 18.1.

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[5]A. L. Batchelor, R. J. S. Phillips, and A. G. Searle, *Nature* **201**, 207–8 (1964).

Table 6.1.2. Specific Locus Mutations Induced in Mouse Spermatogonia

Radiation	Dose (rads)	Exposure Time (hr)	Number of Offspring	Number of Mutations at 7 Loci
Fission neutrons	101[a]	12.1	19,506	20
^{137}Cs gamma rays	576	12.6	28,059	10

[a]Includes a gamma component equal to approximately one-seventh of the neutron component.

6.2 GENETIC DIFFERENCES IN THE REPRODUCTIVE CAPACITY OF IRRADIATED FEMALE MICE

U. H. Ehling

Introduction. – A previous study[1] of the variation in reproductive performance of female mice of five strains after 50 r of x irradiation had established the relative fertility, expressed as percent of total production of the control group, to be: SEC (15%), A (18%), SEA (19%), NB (32%), and DBA (56%). For the most radiosensitive strain (SEC), the total numbers of offspring per female (mean and standard error) in the control and 50-r group were 73.4 ± 0.4 and 11.0 ± 0.9; for the most radioresistant strain (DBA), the corresponding values were 40.4 ± 3.7 and 22.5 ± 1.6 respectively. Crosses and backcrosses between the SEC and DBA strains were made to determine the heritability of the radiation response of the reproductive capacity of the females.

Methods. – Female mice from the SEC and DBA strains and females from crosses and backcrosses of both strains were irradiated, as contemporarily as possible, with 50 r of x rays (dose rate 80 r/min, 250 kvp, 15 ma; inherent filtration, 3 mm Al; HVL, 0.4 mm Cu; target-object distance, 84 cm). One day after exposure, each female was caged separately with a nonirradiated C3H male for the duration of the experiment. This interval between irradiation and mating was chosen to avoid the high incidence of dominant lethals in the first meiotic division.[2] Nonirradiated females of each genotype were mated in the same way. The age of females and males when first mated was 10 to 12 weeks. Litters are recorded and killed at birth. The observation time will cover the whole life-span of the females.

Results and Conclusion. – Preliminary results of some of the crosses over a period of 105 days are summarized in Table 6.2.1. The control females of the SEC strain and the hybrid females produced the same number of young per fertile female. The DBA controls produced 40% fewer offspring. Irradiated SEC and hybrid females have probably already reached the end of their reproductive period. The most radiosensitive females are the SEC mice. The radiation response of the hybrids is intermediate with regard to SEC and DBA females. The most radioresistant females are the DBA mice. On the basis of the earlier results, it is expected that the irradiated DBA females will produce a few more offspring per irradiated fertile female.

Table 6.2.1. Mean Number of Offspring per Fertile Female over a Period of 105 Days

Cross	Control Groups		Irradiated Groups		
	Number of Offspring	Offspring per Female	Number of Offspring	Offspring per Female	Percent of Control
SEC	631	30.0	214	10.7	36
(SEC × DBA)F_1	784	30.2	390	15.0	50
(DBA × SEC)F_1	260	31.3	113	18.8	60
DBA	401	17.4	359	17.1	98

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6.3 THE USE OF X-AUTOSOME TRANSLOCATIONS IN THE MOUSE FOR THE STUDY OF PROPERTIES OF AUTOSOMAL GENES

Liane B. Russell Jean W. Bangham
Clyde S. Montgomery

In X-autosome translocations of the mouse, autosomal genes located within a certain distance of our postulated X-chromosome inactivation center are inactivated in an average of 50% of cells. Thus, the homologous gene on the intact autosome is expressed in an effectively hemizygous state that permits studying some of the properties of such genes.

First, it can be determined whether certain alleles behave as amorphs or hypomorphs. As expected, *chinchilla*, c^{ch}, is in the latter class, effectively $0/c^{ch}$ areas being of typical c/c^{ch} color. Surprisingly, *pink-eye*, *p*, in its effect on coat color is also a hypomorph: R(*p*)/*p* ♀♀ show light mottling (0/*p*) on a *p*/*p* background. On the other hand, *brown*, *b*, appears to be an amorph, R(*b*)/*b* ♀♀ being nonvariegated and phenotypically indistinguishable from *b*/*b*.

Second, the system has been used to determine whether mutations that are homozygous lethal (possibly deficiencies) act as cell lethals. Twelve independent *brown* lethals and a *pink-eye* lethal (general symbol, *l*) have already yielded variegated R(+)/*l* ♀♀, indicating that these mutations are *not* cell lethal. However, the frequency of translocation females is much smaller than that obtained in crosses with viable alleles. We know that p^l/p^l embryos die around implantation. The survival – in reduced numbers – of R(+)/p^l ♀♀ may indicate that p^l-lethal action is confined to a short stage, overlapped by the period during which X inactivation occurs. Survival may result when the number of cells with both X's still active (i.e., the lethal not uncovered) at the stage critical for lethal action exceeds a certain threshold. Other hypotheses are possible.

6.4 TEM-INDUCED MUTATIONS IN MICE

B. M. Cattanach

The procedure and initial results of experiments to test for triethylenemelamine (TEM)-induced mutations in mouse spermatogonia have been described in earlier reports.[1,2] Further data on the spermatogonial mutation frequency have now been accumulated, and the tests have recently been extended to cover postmeiotic germ cells. The results are shown in Table 6.4.1. The number of mutations in the 4 mg/kg dose group is significantly higher than the spontaneous mutation frequency of 0.75×10^{-5} observed by Russell[3] (P = 0.00029, or 0.0036 if the one untested mutant is excluded).

A comparison of the effectiveness of TEM and x rays may be made, using the data of the highest TEM dose. The mutation frequency in the 4 mg/kg dose group (8.00 to 10.66×10^{-5}) is similar to that produced by 300 r of x rays (8.72×10^{-5}).[3]

Postmeiotic germ cells are extremely sensitive to TEM-induced chromosome breakage; consequently, in order to determine a TEM-induced mutation frequency in this type of cell, only a very low dose of the chemical could be employed. In spite of this, three mutations were recovered from 2027 animals scored (Table 6.4.1). It may be concluded, therefore, that postmeiotic germ cells are extremely sensitive to the induction by TEM of gene mutations as well as of chromosome breakage.

The comparison of the sensitivities of pre- and postmeiotic germ cells can now be made on the basis of a single criterion, mutation frequency. In the spermatogonial data there is no suggestion of a departure from a linear relationship between mutation frequency and dose within the range of doses employed. If one can assume that this permits the calculation of a mutation frequency per dose, then, ignoring the low spontaneous mutation rate, the spermatogonial mutation frequency is 2.3×10^{-5} per mg/kg per locus per gamete (upper and lower 90% confidence limits, 1.1 and 4.5×10^{-5}). When the untested mutant is excluded, it is 1.9×10^{-5} (upper and lower 90% confidence limits, 0.8 and 4.0×10^{-5}). In the postmeiotic germ cell data the frequency is 106×10^{-5} per mg/kg per locus per gamete (upper and lower confidence limits, 39 and 265×10^{-5}).

Postmeiotic germ cells are, therefore, much more sensitive to TEM than premeiotic germ cells.

The distribution of the mutations among the seven loci is shown in Table 6.4.2. One mutation recently found in a new spermatogonial experiment is included. Russell's[4] data on x- and gamma-irradiated pre- and postmeiotic germ cells are presented for comparison. It is apparent that the distribution of the TEM mutations does not show any tendency to mimic the x- and gamma-ray distributions. The fact that three of the rare *a* mutations appeared, together with an excess of *d* mutations, is striking. More significant, perhaps, is the complete absence of *s* mutations that occur with high frequency in irradiation experiments. The possibility of chemical specificity may therefore be suggested.

References

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Table 6.4.1. Mutations at Specific Loci Induced in Mouse Pre- and Postmeiotic Germ Cells by Triethylenemelamine (TEM)

Dose (mg TEM per kg body weight)	Stage Treated	Number of Offspring Scored	Number of Mutants	Mutations per Locus per Gamete
				$\times 10^{-5}$
2 + 2 (two 24-hr fractions)	Spermatogonia	5360	3 (+1 ?)[a]	8.00 (10.66)
1	Spermatogonia	6496	1	2.20
0.5	Spermatogonia	5427	0	
1	Spermatocytes	264	1	
0.2	Spermatozoa and spermatids	2027	3	21.14

[a]Untested.

Table 6.4.2. Distribution of Mutations Among the Seven Tested Loci

Treatment	Stage Treated	Locus							
		a	b	c	p	d	d se	se	s
TEM	Premeiotic	2[a]	0	0	2	3	0	0	0
TEM	Postmeiotic	1	0	0	0	2	0	0	0
X or γ ray[b]	Premeiotic	5	45	27	38	42	1	3	108
X or γ ray[b]	Postmeiotic	4	16	4	10	6	7	3	25

[a]One untested.

[b]W. L. Russell, in *Genetics Today*, ed. by S. J. Geerts, Pergamon, Oxford, in press.

6.5 RELATIVE BIOLOGICAL EFFECTIVENESS OF NEUTRONS TO X RAYS AS ESTIMATED BY KILLING OF TYPE A SPERMATOGONIA OF THE MOUSE

E. F. Oakberg Evelyn Clark
Jo K. Henderson

Earlier, we had reported a relative biological effectiveness of approximately 2 for 2.5- and 14.1-Mev neutrons as compared to ^{60}Co gamma rays.[1] On the basis of LET, it had been expected that the 2.5-Mev neutrons would be more effective than those with an energy of 14.1 Mev. Recently, study of the effectiveness of neutrons approximating a fission spectrum was made possible in conjunction with studies by Russell and Kelly[2] on mutation induction by neutrons from the ORNL Health Physics Research Reactor (HPRR).

Twelve males were removed for histological study from each of the exposures used by Russell and Kelly. Histological procedures and quantitation of spermatogonia were the same as we have used in previous experiments.[1]

Dose rates were approximately 0.15, 0.7, 7.3, and 70 rads/min; total neutron doses ranged from 18 to 258 rads. The neutron to gamma-ray dose ratio was about 7. All dose rates were not represented at each dose. However, no effect of dose rate on cell survival was observed at those doses where comparisons could be made. This is in agreement with our results with x and gamma rays, where no effect of dose rate was observed over the range of 90 to 0.8 r/min.[1]

Relative effectiveness of HPRR neutrons, after correction had been made for gamma-ray contamination, was estimated for each dose by comparison with the x-ray dose required for equal cell survival. An average RBE of 5 was indicated. This is higher than the value of 2 previously observed for 2.5- and 14.1-Mev neutrons.[1] Further experiments at different neutron energies should be undertaken in order to more fully understand the relationship between LET and RBE for spermatogonial killing.

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7. Radiation Immunology

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7.1 ENRICHMENT AND PURIFICATION OF CELLS INVOLVED IN ANTIBODY SYNTHESIS

G. C. Cozad J. F. Albright

Introduction. – Suitable methods for obtaining cell suspensions rich in antibody-synthesizing cells and their progenitors will stimulate rapid progress along two lines: (1) analysis of cytokinetics of antibody response and (2) biochemical analysis of antibody protein synthesis. Under best present conditions, progenitors of antibody-synthesizing cells are present in a proportion of only 1 cell in 10^5 total spleen cells, and at peak antibody response antibody-synthesizing cells do not exceed a proportion of 1 in 10^3 total cells. We are exploring methods for enrichment of cells involved in antibody formation.

Methods and Results. – In initial studies, exposure to hypotonic saline[1] (0.037 *M* NaCl) for 30 sec was found to rid the spleen cell suspension of virtually all the erythrocytes, as verified by microscopic observation. In addition, during the shocking and washing process much other cellular debris was removed, suggesting that shocking damages other types of cells. The survival of immunologically competent cells exposed to hypotonic solutions was studied since previous work[2] suggested that these cells are unusually resistant to injury. Antibody-synthesizing capacity of shocked spleen cells from normal mice and those primed previously with either sheep or rat erythrocytes were tested. Washed, shocked spleen cells in varying concentrations, along with optimal numbers of erythrocyte antigen, were injected intravenously into irradiated recipient mice. After 6 or 8 days, mice were bled and hemagglutinins were determined by using the appropriate antigen. Titer enrichments of three- to fourfold were obtained, employing the cells exposed to hypotonic solutions. Treatment by hypotonic shock eliminated 50 to 70% of the nucleated spleen cells. Cells surviving the shocking procedure were tested in an "all-or-none" assay to determine if the titer enrichment was due to an increase in the number of potentially competent cells or if, under these conditions, the cells which survived displayed greater antibody-forming capacity. The proportion of immunologically competent cells in a primed spleen suspension subject to one 1-min exposure to hypotonic saline was increased by a factor of about 3. This indicates that the enrichment in terms of antibody titer is a reflection

of an increased ratio of antibody-producing to non-antibody-producing cells, rather than an increase in amount of antibody synthesized per cell.

The effects of varying time of exposure to hypotonic saline and using a more hypotonic salt solution were investigated. In general, spleen cells from mice obtained shortly after a second exposure to the same antigen were more resistant to osmotic damage than those from nonimmunized animals, as judged microscopically.

We have completed testing of a variety of possible media for their toxicity to antibody-forming cells and have found that certain dextrans are quite satisfactory. Preliminary experiments with dextran gradients indicate that two distinct zones of antibody-forming cells can be separated.

Discussion. – Limited previous experience with density gradient centrifugation of spleen cells[3] showed that enrichment by a factor of 10 may be achieved. It is envisioned that, by employing cells pretreated by hypotonic shock, separation by gradient centrifugation will result in obtaining spleen cell suspensions with greater than 100-fold enrichment of progenitors of antibody-forming cells.

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7.2 HEMAGGLUTININ FORMATION IN CHICKENS AS A FUNCTION OF AGE

Frank Seto

Introduction. – Since earlier reports of the occurrence of natural antibodies and age studies of immune precipitin[1] and immune heteroagglutinin[2] production in chickens are not sufficiently quantitative,[3] it was necessary, as a preliminary phase in the analysis of the antibody formation in chickens, to determine the incidence of natural agglutinins and the primary antibody-forming potential of chick embryos and young chickens of various ages.

Methods. – Serum samples from embryonated eggs, 12 to 19 days of incubation, and from chickens, 1 day to 6 months of age, were tested for the presence of natural agglutinins against sheep, hamster, mouse, rat, and rabbit erythrocytes. Sheep and hamster erythrocytes were selected as antigens to study the primary immune response. Various amounts of erythrocytes (10^5 to 10^{10}) were injected intravenously into 18- to 19-day-old embryos and into 1-day- to 9-week-old chickens to determine the optimum antigen dose for each age group. Serum samples were collected at specified intervals and stored in a frozen condition, and hemagglutinin titers were determined in the usual way.

Results and Discussion. – Agglutinins for sheep erythrocytes were absent in embryos and in chickens up to the third week after hatching and then detectable in low amounts thereafter. Natural antibodies against the other erythrocytes were present in trace amounts in 12- to 14-day-old embryos, were absent in late embryos, but reappeared again in low concentration soon after hatching. During the third and fifth weeks the agglutinin titers for rabbit and rat cells increased 64-fold, those for mouse and hamster erythrocytes about fourfold, and all increased steadily in subsequent weeks. Peak antibody production in the primary immune response to sheep and hamster erythrocytes occurred about 6 days after antigenic stimulation, and, of the antigen doses tested, 10^8 erythrocytes was found to be optimum for most ages studied. The frequency of positive responders increases during the first week, and practically all chicks one week old or older respond to antigenic stimulation. Associated with this change is a 1000-fold increase in antibody output during the first four weeks and a further fourfold increase thereafter. The results indicate that the primary antibody-forming potential (PAFP) is present in the newly hatched chicks and grows maximally during the first four weeks after hatching.

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7.3 EFFECT OF ERYTHROPOIETIN ON THE ANTIBODY RESPONSE OF PLETHORIZED MICE

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E. Goldwasser[1]

Introduction. – Previous studies in this laboratory[2] have shown that in primary antibody response certain multipotent cells can differentiate into functional antibody-forming cells when they are adequately stimulated by an antigen and become unipotent and refractive to other stimulants. It is very probable that these multipotent, potential antibody-forming cells are also susceptible to erythropoietin because studies in red blood cell formation[3] indicate that erythropoietin also stimulates multipotent cells to differentiate into hemoglobin-synthesizing cells. Preliminary experiments were therefore carried out to test this notion.

Methods. – Twenty 3-month-old mice were plethorized by injecting intraperitoneally on two consecutive days 1 ml of 80% isologous RBC. Five days later ten mice received 5.4 units of erythropoietin intraperitoneally, and a day later all the mice were given 1 ml of 10% sheep RBC intravenously. At the end of the log phase of response or 6 days after antigen injection, the body weight, hematocrit, and blood volume were determined, in addition to the antibody titer of individual plasma samples.

Results and Discussion. – The results showed that although erythropoietin pretreatment had no effect on the body weight, hematocrit, and blood volume of these plethorized mice, it did significantly reduce the antibody-forming potential of these mice; that is, the response of plethorized mice pretreated with erythropoietin was only 35% of that of the untreated plethorized mice. These results strongly suggest that potential antibody-forming, progenitor cells can be made refractive to the test antigen by pretreatment with erythropoietin. Further studies are now being carried out in an attempt to obtain definitive evidence that there exist in young adult mice multipotent progenitor cells susceptible to antigens, erythropoietin, and possibly other stimulants.

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7.4 COMPETITION OF ANTIGENS FOR PROGENITORS OF ANTIBODY-FORMING CELLS

J. F. Albright T. W. Evans

Introduction. – Recent evidence[1,2] that progenitors of antibody-forming cells are multipotential has prompted a thorough analysis of competition between non-cross-reacting antigens for progenitor cells available at a given time in nonprimed animals. We showed previously that the degree of competition is dependent upon the interval of time separating the administration of two antigens.[3] This report considers results obtained when three distinct types of erythrocytes are injected at intervals such that maximum competition occurs.

Methods and Results. – Twelve-week-old C31F mice were injected intravenously with 2×10^9 chicken erythrocytes (Crbc). After 12 to 15 hr the mice were injected with 2×10^9 sheep erythrocytes. After a further 12- to 15-hr interval the mice received 2×10^8 rat erythrocytes. The agglutinating antibody response to Srbc and Rrbc was followed and compared with normal primary responses of mice to equivalent injections of either Srbc or Rrbc. The results are illustrated in Fig. 7.4.1. Maximum primary responses are elicited in mice of this age by 2×10^8 Rrbc or Srbc, that is, increasing the antigen beyond this optimum does not result in additional antibody production. The prior injection of Crbc clearly interferes with the response to optimum amounts of either Rrbc or Srbc. The effect of Crbc on response to Rrbc is no greater than giving Srbc alone 12 hr prior to injection of the optimum dose of 2×10^8 Rrbc. Injection of isologous mouse rbc 12 hr prior to the test injection of Rrbc has no effect on anti-rat agglutinin response. Thus there is competition among the three foreign

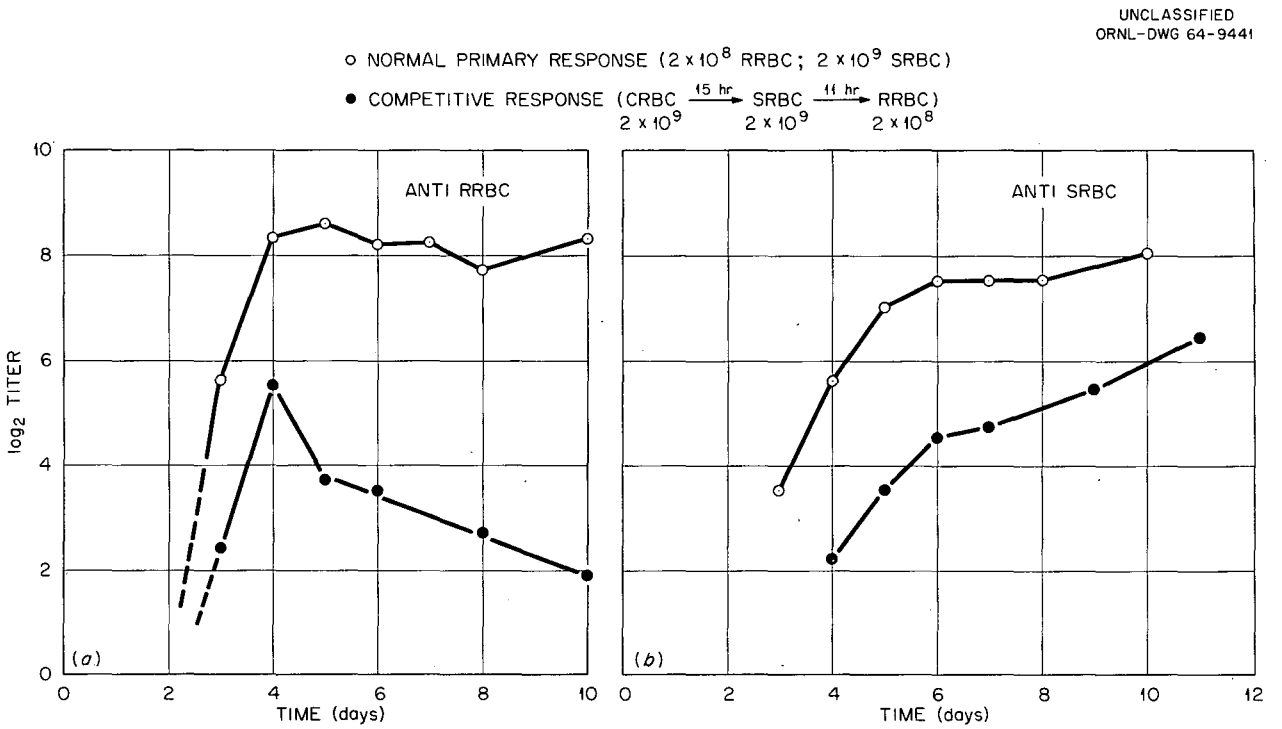

Fig. 7.4.1. (*a*) Antibody Formation as Function of Time After Injection of 2×10^8 Rrbc into (O) Normal, Untreated Mice and (●) Mice Which Had Been Injected 26 hr Previously with 2×10^9 Crbc and 11 hr Previously with 2×10^9 Srbc. (*b*) Antibody Formation on Injection of 2×10^9 Srbc into (O) Normal, Untreated Mice and (●) Mice Which Were Injected 15 hr Previously with 2×10^9 Crbc and Were Injected 11 hr Subsequently with 2×10^8 Rrbc.

erythrocyte antigens which is not merely mechanical or a matter of limiting volume.

Discussion. – These and other recent results indicate that a considerable portion of the progenitors of antibody-producing cells in nonprimed mice are pluripotential. The present results suggest that at a given time a pool of cells serves as a common source of progenitors for antibody response to a variety of different antigens and that these antigens compete for the available progenitors.

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7.5 FURTHER STUDIES ON THE SUPPRESSIVE ROLE OF PERITONEAL PHAGOCYTES IN AGGLUTININ RESPONSE

E. H. Perkins

Introduction. – Previous in vitro and diffusion chamber studies[1] have demonstrated that peritoneal phagocytes destroy red blood cell (RBC) antigens. Based on these findings, RBC antigens are expected to be phagocytized and destroyed when injected intraperitoneally into intact animals. If so, the antibody response of these animals should be lower than those receiving their RBC antigen intravenously, provided the dose of antigen is not overwhelmingly large relative to the number of peritoneal phagocytes.

Results. – Glycogen-treated or untreated mice were injected either intravenously or intraperitoneally with 10^6, 10^7, or 10^8 sheep RBC. Serum samples were collected on alternate days for 10 days and titrated (Fig. 7.5.1). It was apparent that as the dose of antigen becomes limiting the

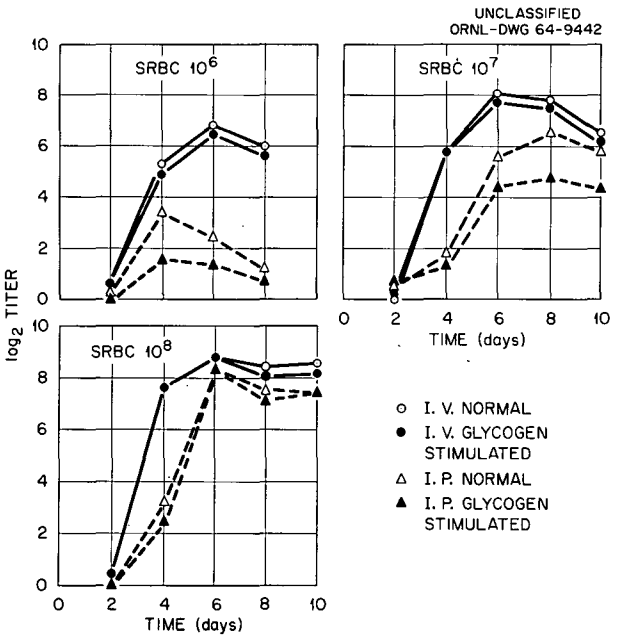

Fig. 7.5.1. Primary Ab-Response of Normal and Glycogen-Stimulated Mice as Affected by Route of Ag Injection.

intraperitoneal route is vastly inferior to the intravenous route. Additional evidence to support this contention was obtained by injecting 10^7 opsonized sheep RBC either intraperitoneally or intravenously into glycogen-treated or untreated mice. As expected, agglutinin titers were lowest when opsonized sheep RBC were injected intraperitoneally into glycogen-treated mice and highest when nonopsonized sheep RBC were injected intravenously into untreated mice. To demonstrate that these findings were not peculiar to mammals, sheep RBC ranging a million-fold in dose (10^4–10^{10}) were injected either intraperitoneally or intravenously into 3-week-old chicks, and agglutinin titers of 6-day serum samples were determined. Findings comparable to those in mice were obtained.

Discussion. – These results show that peritoneal phagocytes respond to foreign RBC as scavenger cells, act as destructive competitors rather than symbionts of potential antibody-forming cells, and are not essential for the initiation of the hemagglutinin response.

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7.6 SOME UNUSUAL FEATURES OF PHENYLALANINE ABSORPTION

R. A. Brown

Stated in its simplest form and from a molecular point of view, the problem of immunology hinges about the question of how the antigen can feed back into and regulate the synthesis of proteins or antibody. In this respect the immunological problem is similar to that of the regulation of protein synthesis. It seems reasonable to assume that the initial event in the immunological process is the formation of a specific complex between antigen or a degradation product thereof and some molecule present in the cell. If one accepts this point of view, the first question one must ask is: To which class of macromolecules does the molecule which forms the initial specific complex belong? In the original work on gene regulation, evidence was presented that the repressor was an RNA molecule. Recently the ability of RNA to form specific complexes with other molecules has been questioned,[1] and it has been concluded that the repressor must be a protein.

The author has presented evidence in previous publications that ribosomal RNA is capable of specific interactions with other molecules.[2,3] Jehle *et al.*[4] have presented a theoretical discussion of how nucleic acids may participate in specific interactions. In order to gather more data on this matter, an investigation of the interaction of amino acids with RNA is in progress. Results would suggest that the type of ions present, the ionic strength, and pH are important in these interactions. Since the pH region under study is from pH 5.5 to 7.7, the pH dependence is unusual and difficult to understand.

Spectral studies of phenylalanine in this pH region establish that the vibrational fine structure of the phenylalanine absorption spectra depends on pH in an unusual manner. There are abrupt transitions of the absorptivity of phenylalanine at wavelengths less than 255 mμ. These transitions depend in a complex way upon pH, temperature, and the ions present. In Fig. 7.6.1 the absorptivity at 247 mμ of a 1.21 m*M* phenylalanine solution

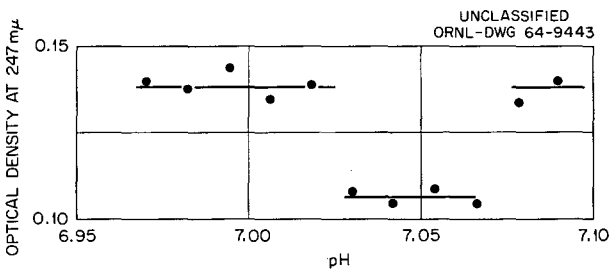

Fig. 7.6.1. Absorptivity at 247 mμ of a 1.21 m*M* Phenylalanine Solution in 0.30 *M* KCl and 0.02 Ionic Strength Potassium Phosphate Solution Plotted as a Function of pH.

in 0.30 *M* KCl and 0.02 ionic strength potassium phosphate solution is plotted as a function of pH over a very narrow range.

The complex dependence of the phenylalanine absorption spectra on several variables is difficult to rationalize in a simple manner. The very abrupt transition which takes place as a function of pH is good evidence that a large number of hydrogen ions participate in the transition. This can only reflect changes taking place in the surrounding water. Current theories dealing with hydrophobic forces assign a special structure to the water surrounding nonpolar groups. The above results would suggest that there are phase transitions in the water surrounding the nonpolar side chain phenylalanine. One would expect that the observed phenomenon plays an important part in the interaction of phenylalanine with other molecules. It may play a part in current difficulties with the code.

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8. Human Cytogenetics

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8.1 KINETICS OF X-RAY-INDUCED CHROMATID ABERRATIONS

J. G. Brewen

Recently Revell[1] has suggested that radiation-induced isochromatid-type chromosomal deletions are the consequence of interactions between breaks induced by independent ionizing events. In order to test this hypothesis, the frequencies of isochromatid deletions induced by three different doses of x rays were measured at various times during the cell cycle.

Lateral root tips of the broadbean, *Vicia faba*, were irradiated with 37.5, 75, or 150 r of x rays, after which continuous 2-hr colchicine collections of metaphase cells were made for a 20-hr period. The metaphase figures obtained at each 2-hr interval were scored for chromatid-type aberrations, and the frequencies of the various classes of aberrations were plotted against time after irradiation for each dose employed. The total number of chromatid-type aberrations induced by each dose, for the period of time studied, was calculated and a dose curve calculated for isochromatid deletions and chromatid exchanges. It was found that isochromatid deletions have primarily one-hit kinetics while chromatid exchanges have two-hit kinetics, as predicted by the classical theory of aberration production. An additional experiment was performed in which a mitotically synchronous population of cells was irradiated, and the same result was obtained. It thus appears that Revell's hypothesis is not valid.

In addition, it was found that the frequency of isochromatid deletions induced by a given dose of x rays remains constant with time after irradiation, indicating a uniformity of stage sensitivity. The frequency of chromatid exchanges is highest in those cells which presumably were in G_2 at the time of irradiation. This apparent increased G_2 sensitivity is interpreted as an increase in the number of sites at which an exchange can occur.

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8.2 NUCLEOLAR ALTERATION IN PHYTOHEMAGGLUTININ-STIMULATED HUMAN LEUKOCYTE CULTURES

Hiroshi Yamamoto

Although many reports indicate prominent nucleolar enlargement in phytohemagglutinin-stimulated

human leukocytes, details of nucleolar behavior are not known. This phenomenon has been studied by means of fluorescence microscopy and electron microscopy.

Nucleolar, nuclear, and cytoplasmic areas were determined by means of measurements of major and minor diameters in smear preparations. Nucleolar area increases greatly by the 72nd hour of culture in the presence of phytohemagglutinin, but not in cultures without this substance. During the first 96 hr, fragmentation of nucleoli can be seen; nucleolar fusion can be recognized after 96 hr.

In acridine-orange-stained preparations from stimulated 72-hr cultures, the nucleoli of lymphocytes were ring shaped and showed an orange fluorescence, while those of blastic cells were of bizarre shapes and fluoresced with a red-orange color. Only the outer shell of the ring-form nucleoli showed marked fluorescence. The nucleolar chromatin of the small lymphocytes fluoresced green, but that of the blastic cells showed yellow fluorescence.

The fine structure of the nucleoli of cultured cells is quite different from that of the nucleoli of peripheral lymphocytes. With double fixation with neutral formalin and osmium tetroxide, nucleoli in peripheral lymphocytes consist of an outer dense area which is composed of granules 150–200 A in diameter, densely packed together, and a central homogeneous body without granules. With potassium permanganate fixation the former shows a thick fibrillar structure, while the latter shows a finer fibrillar structure, about 50 A in diameter. In intermediate-type cells, the outer granular shell had changed to threadlike structures, identifiable as nucleolonema under the light microscope. The central homogeneous body remained structureless, as in peripheral lymphocytes. In blastic cells, the nucleolonema are much more developed than in the intermediate cells, but the diameter is the same. The central homogeneous body is sometimes fragmented. The average number of ribosome-like granules per unit length of nucleolonema in intermediate and in blastic-type cells is almost the same. The amounts of nucleolar RNA in blastic-type cells may, therefore, be much larger than in peripheral lymphocytes.

8.3 CHROMOSOME ABERRATION RATES INDUCED BY STEADY-STATE URANIUM FISSION NEUTRON IRRADIATION OF HUMAN LEUKOCYTES

P. Carolyn Gooch M. A Bender

A study of the RBE values for the induction of human chromosomal aberrations is being made in order to provide a basis for radiation dose estimation from peripheral leukocyte aberration frequencies in cases of accidental human radiation exposure. Rates of aberration induction by 250-kv x rays and by 14- and 2.5-Mev neutrons have already been determined.[1–3] Studies of the effects of uranium fission neutrons administered at moderate dose rates have now been completed.

Freshly drawn whole human blood samples were irradiated in nylon chambers placed at various distances from the center of the core of the ORNL Health Physics Research Reactor to give four different doses simultaneously. Exposures were started in October 1963. Dose rates ranged from 1 to 4 rads/min. Gamma contamination was less than 10% of the total dose. After irradiation the leukocytes were placed in short-term culture, fixed, stained, and mounted as described previously.[1]

If the results of three separate experiments with three different blood donors are pooled, the coefficient of deletion production is 4.5×10^{-3} deletion $cell^{-1}$ rad^{-1}. Since the value for 250-kv x rays is only 0.9×10^{-3}, the RBE value is approximately 5. The frequency of ring and dicentric chromosomes increased linearly with dose. The coefficient for these aberrations is 5.5×10^{-3}. With 250-kv x rays, on the other hand, the frequency of rings and dicentrics increases approximately as the square of the dose, with a coefficient of 6.0×10^{-6} $cell^{-1}$ rad^{-1}.[2] Because of the difference in kinetics, it is impossible to calculate an RBE value. The RBE value of 5 for deletions is of interest since a much lower value (2) has frequently been used for calculating rem doses in cases of human radiation exposure during criticality accidents. It should also be noted that what few estimates have been made from chromosome deletion data also indicate an RBE of more than 2 (ref. 2).

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9. AEC-NASA Space Biology Program

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9.1 EFFECTIVENESS OF HIGH-ENERGY PROTONS AND OTHER FAST CHARGED PARTICLES ON VARIOUS BIOLOGICAL MATERIALS

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M. A Bender
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Our previous semiannual report[1] included data on the relative biological effectiveness (RBE) of protons within the energy range of 22 to 750 Mev for (1) bacterial inactivation and mutation, (2) forward mutation and inactivation of *Neurospora crassa*, (3) lethality in spermatogonia and oocytes of mice, and (4) production of chromosome aberrations in human leukocytes. A complete report has been prepared[2] which contains data on these several systems and compares the RBE of the protons (LET ~2.5 to 25 Mev cm^2 g^{-1}) with higher LET radiations such as heavy ions and low-energy protons produced by fast-neutron irradiation.

The data can be summarized as follows. None of the systems studied respond to LET below ~100 Mev cm^2 g^{-1}. Bacterial inactivation and mutation under aerobic conditions are less efficient at very high LET (~1800 Mev cm^2 g^{-1}). The effect of oxygen is reduced at high LET. Inactivation and forward mutations in *Neurospora* show a rapid increase in RBE above 100 Mev cm^2 g^{-1}, and the RBE for inactivation or mutation ranges from 4 to 9 at 1800 Mev cm^2 g^{-1}. The RBE for lethality in mouse spermatogonia and oocytes increases to about 2.5 in the region 100–200 Mev cm^2 g^{-1}, as does production of chromosome aberrations in human leukocytes.

Although the data accumulated on RBE vs LET are far from complete, they do suggest that the range of LET in which increased RBE is detectable is nearly the same in all systems studied. Neither

the maximum RBE nor the range of LET in which it occurs was determined in these studies. It is clear, however, that the magnitude of RBE may be different for the different systems studied and perhaps for different effects in the same biological material.

Similar studies are planned on mice and other mammals regarding RBE-LET relationships for production of late somatic effects. A facility has been constructed at the ORNL Isochronous Cyclotron for irradiating groups of mice with 40- and 80-Mev protons.

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9.2 BIOSATELLITE A EXPERIMENT

F. J. de Serres R. C. von Borstel
B. B. Webber Anna R. Whiting

The Office of Space Sciences and Applications of NASA has announced the details of this program as a series of flights of duration 3 to 30 days in a modified, scaled-up Discoverer satellite to determine the biological effect on plants and animals of weightlessness, radiation, and absence of diurnal cycle. The first flight is scheduled for late in 1965.

The satellite is designed to carry its own radioactive source to supply known doses of radiation and will permit this previous variable to be controlled. Natural radiation will be controlled to less than 2 r/day by the orbital altitude selected.

The temperature, excluding reentry temperature, will be controlled to 24 ± 2°C. The experiments will be in individual containers and hermetically sealed. These very conditions require rather elaborate ground-based experiments in connection with the viability of the biological specimens and, moreover, the possible modification of radiation effects on these conditions that are somewhat unusual in comparison with routine laboratory-controlled experiments.

In some cases it is required to increase the vigor of stocks of organisms to ensure good viability under the potential rigors of the experiment.

These are the types of experiments under way at present, and we include in this report short summaries of work in progress for the Biosatellite experiments.

9.3 PREPARATION OF THE *HABROBRACON* STOCKS FOR BIOSATELLITE EXPERIMENTS

R. C. von Borstel Anna R. Whiting

In the wasp *Habrobracon*, females are diploid and heterozygous for sex alleles; normal males are haploid, developing from unfertilized eggs and containing one sex allele. Within an inbred stock, half of the fertilized eggs have the same sex alleles. The majority of these are inviable, and the few survivors are sterile diploid males. For irradiation experiments, stocks with different sex alleles are used in the crosses so that viability of controls will be high and experimental data need not be corrected for control inviability. A constant and easily identifiable character difference is also helpful in such experiments.

In order to increase the vigor of one of the sex allele stocks to be used in the biosatellite experiment, males from stock $17o^i$, an ivory eye color, were outcrossed to females for the No. 33 stock, eye color wild-type black. Daughters from these matings were backcrossed to $17o^i$ males other than their own parent. Daughters from these crosses were selectively mated to their own male parent, and each of the lines was then maintained by brother-sister matings.

It was necessary to test these lines against the No. 33 stock to find at least one which had the same sex alleles as the original No. $17o^i$ and different from those in the No. 33 stock. Hatchability of fertilized eggs from such crosses is always high, and diploid males are never produced. Females from a number of the outcrossed, backcrossed, and inbred ivory-eyed lines were crossed with the No. 33 wild-type males and the hatchability scored. One such line was found to give high hatchability and a high female-to-male ratio. No diploid males were found in 20 separate crosses.

These two stocks (the rederived $17o^i$ and the No. 33) are in excellent condition for the genetic

analyses for dominant and recessive lethal and visible mutations. Analyses of sex alleles in two other wild-type stocks are under way to aid in the tests for the chromosomal translocations that are anticipated in the radiation experiment.

9.4 PRELIMINARY DATA ON SURVIVAL OF CONIDIA STORED UNDER SATELLITE CONDITIONS OF TIME, TEMPERATURE, AND ATMOSPHERE

F. J. de Serres B. B. Webber

Since the conditions aboard the proposed Biosatellite are somewhat different from those routinely involved in laboratory experiments, it was necessary to determine the optimal conditions for survival of conidia under the restrictions imposed by the vehicle with regard to temperature and atmosphere for the proposed 3- to 30-day orbit duration.

A series of experiments involving measurement of loss of viability of wet conidia as a function of atmosphere, time, and temperature give the following results:

1. Conidia survive longer at 0–4°C under any atmospheric conditions than at higher temperatures.
2. At temperatures above 4°C the loss of viability with time is much greater under anaerobiosis than under aerobic conditions.
3. In air, conidia survive well up to 9 days or more at temperatures prescribed for the flight.

The best summary of the survival data is that satisfactory viability of *Neurospora* conidia can be obtained under flight conditions if the package permits a supply of air. Experiments are planned to determine the amount of air required for best viability, and these considerations are being given to the engineering of the flight package.

Other conditions which will increase viability are also anticipated. These, of course, will have to be tested for their possible influence on radiation sensitivity as well.

9.5 GEMINI III EXPERIMENT

M. A Bender P. Carolyn Gooch

At the request of NASA, the Biology Division has undertaken the design and development of an experiment to test for possible unpredicted effects of the radiation encountered in space flight and for possible interactions between radiation and other space-flight parameters, such as weightlessness. A number of observations of biological systems subjected to space flights have suggested the possibility of such unpredicted effects. The Biology Division experiment is to be carried on the Gemini III manned space flight scheduled for the last quarter of 1964. The experiment will measure the frequencies of various types of chromosomal aberrations in a series of samples of whole, heparinized human blood exposed to a known quantity and quality of radiation during the weightless phase of the space flight, in unirradiated "in-flight" controls, and in a similar series of control samples exposed simultaneously on the ground. Both pre- and postflight analyses will also be made of material from the astronauts selected for the Gemini III mission.

The detailed description of the package and the results of ground-based engineering and biological testing are being prepared in a separate report.

10. Mammalian Recovery

C. C Congdon[a]

Secondary Disease (10.1–10.7)

C. C Congdon[a]
D. C. Swartzendruber
Vu-Thi-Suu[b]
W. H. McArthur[c]
M. G. Hanna, Jr.[a]
Frances E. Hacker
Kathleen H. Harrington
Alberta P. Henley
W. C. Grant[d]
Joseph Kendrick[e]

Recovery in Vitro (10.8)

L. H. Smith
T. W. McKinley, Jr.
Myra D. Phillips[d]

[a]Dual assignments.
[b]Visiting investigator from abroad.
[c]Consultant.
[d]Student trainee.
[e]Temporary summer employee.

Hemopoietic and Lymphatic Stem Cells (10.9)

Joan Wright Goodman
Mysa Mathews
Gayle C. Bosma
Fay M. Martin[c]

Bone Marrow Tissue Culture (10.10)

Henrianne G. Willard

Animal Testing Program (10.11)

M. L. Simmons
D. T. Donald
J. A. Franklin
G. L. McNabb
Everline B. Wright
S. R. Leininger[e]

Cooperative NIH-AEC Co-Carcinogenesis Program (10.5, 10.7)

10.1 MORTALITY FROM SECONDARY DISEASE

C. C Congdon[1]

Introduction. – Three series of experiments were carried out to determine the major effects and the interaction of certain variables that are thought to play a role in mortality from secondary disease. The variables selected for study were bone marrow dose, day of marrow injection, age of donor marrow, and sex of donor and host. A 2×2^3 factorial design with four center points laid out in two blocks was chosen as the experimental design in the initial phase of the study. The factor levels were doses of 10×10^6 and 50×10^6 bone marrow cells with a center point of 30×10^6 cells; injection at 0 and 3 days after irradiation with center point at 1.5 days; and donor marrow ages of 7 and 91 days with center point at 49 days. The experiments were carried out on male and female mice. Each treatment combination was applied to six cages of approximately 5 mice per cage, so that about 600 mice were used in the main series, with 600 and 300 additional animals in two other series. The marrow donors were $B6D2F_1$ mice and the recipients $1C3F_1$ animals 3 months of age exposed to 950 r.

The proportions of mice dead at 30, 60, and 90 days were determined for the different experimental groups.

Results. – Analysis of the mortality among cages showed a "cage effect" to be present in the sense that the death of a mouse in a cage was not completely independent of whether his cage

mates were alive or dead. This finding was noted in the three series of experiments.

Study of the main effects demonstrated that 7-day-old marrow gave 16% less 90-day mortality than 90-day-old marrow. In one of the supplemental series this beneficial effect of young marrow was lost by 21 days of age. Interaction of factors at the two levels for each variable gave no consistent or unexplainable results except in the case of an interaction of age of donor cells with sex.

The most interesting finding in all three series was the reduced 90-day mortality at the center points. If a mean 90-day mortality of about 50% is taken as the expected finding in the donor-host combination of this study, then the lowest 90-day mortality of 22% was seen with 30×10^6 14-day-old donor marrow cells given at day 1.5 after irradiation in female mice. The findings indicate that further investigation of cell dose and time of injection of young marrow cells is needed.

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[1]In collaboration with M. A. Kastenbaum and D. A. Gardiner, Mathematics Division.

10.2 MURAMIDASE ACTIVITY IN SPLEEN AND KIDNEY OF X-IRRADIATED MICE DURING THE EARLY HOURS AFTER IRRADIATION

Vu-Thi-Suu

Introduction. – In an earlier report,[1] a decrease in muramidase activity was observed in spleens of x-irradiated mice on the 3d and 12th days after exposure. To have more complete data, a study of this enzyme in spleen and kidneys of x-irradiated mice is being carried out during the early hours after irradiation.

Methods. – Approximately 200 female hybrid mice (C57L/Cum ♀ × A/He Cum ♂)F_1 9 to 23 weeks of age were used in this experiment.

Two groups were investigated: group I was normal mice, not irradiated, used as controls; group II was mice exposed to x irradiation (950 r whole-body irradiation). Irradiated animals were weighed and killed by decapitation at different intervals: one group of six mice was killed immediately after x-ray exposure, the other exposed animals were killed at 30 min, 1 hr, 2, 3, 4, 6, 9, 12, 16, 20, 24, and 48 hr. Six to fifteen mice were killed at each interval. Muramidase activity of spleen and kidney[1,2] was determined as described previously.

Results. – An increase in muramidase activity in spleen of x-irradiated mice occurred at 6 hr after irradiation, reached a maximum of six times the normal activity at 16 hr, and decreased rapidly after 20 hr. At 24 and 48 hr, the muramidase value was only slightly increased. A study of this enzyme in kidneys of the same animals at the same intervals showed no difference between normal and x-irradiated mice.

Discussion. – This increase in muramidase activity in spleen of mice during the early hours after x-radiation exposure is of interest. Previously, we have shown a marked increase in muramidase activity in kidneys, spleen, and bone marrow of homologous and heterologous chimeras.[1–3] The meaning of those changes is not known. It has frequently been noted that the muramidase activity increased in conditions accompanied by reticuloendothelial system stimulation.[4,5] On the other hand, it has been demonstrated[6,7] that macrophages and polymorphonuclear leukocytes contain large amounts of muramidase activity. Further studies are needed to understand the possible relationship between the R.E.S. and those changes in muramidase activity.

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10.3 NITROGEN BALANCE STUDIES IN IRRADIATION CHIMERAS[1,2]

W. H. McArthur

Introduction. – Studies by McArthur *et al.*[3] have shown that mice given foreign bone marrow following lethal doses of x irradiation stayed in positive nitrogen balance for up to 90 days. Animals treated with isologous bone marrow recovered and showed only delayed effects of irradiation. In contrast, irradiated animals given foreign bone marrow temporarily regained their initial loss of body weight, then died with a secondary weight loss. Further study[4] has suggested that secondary disease, or the foreign bone marrow reaction, is an immunologic disorder in which cellular elements from grafted marrow attack host antigens. It has been suggested that secondary disease is associated with a "metabolic starvation," since food intake of mice given homologous marrow is adequate and comparable to that of normal or isologous-marrow-treated mice. Previous results confirmed the similarity of food intake among normal, isologous-, and homologous-marrow-treated irradiated mice. Mice of these three groups had comparable degrees of positive nitrogen balance despite loss of body weight among the animals given homologous marrow.

As a continuation of whole-animal metabolism studies in radiation chimeras, the present report summarizes the nitrogen balance studies in these forms through 90 days.

Materials and Methods. – A metabolic experiment was done on each of 24 mice. Eight mice were unirradiated, uninjected controls; eight were x irradiated without bone marrow treatment; eight were given 900 r total-body x irradiation and then injected intravenously with 40×10^6 isologous bone marrow cells. X-ray control animals not given bone marrow showed weight loss, negative nitrogen balance, and death before the tenth day after irradiation and therefore were not included in the analysis.

A regression line was fitted to the body-weight data, and the slope of this line, averaged for all animals in each of the three treatment groups, is entered in Table 10.3.1 as weight gain.

Results and Conclusions. – Animals treated with isologous marrow (Table 10.3.1) maintain a normal rate of gain in body weight through 90 days, but retain significantly more nitrogen than normal animals. Animals given homologous marrow fail to gain weight, but retain twice as much nitrogen as normal mice. Both marrow-treated groups show the same degree of increased positive nitrogen balance.

Our results show that correlation between nitrogen balance and food intake (Table 10.3.1) does not change significantly during the 90 days of these experiments.

Table 10.3.1. Average Weight Gain, Nitrogen Balance, and Correlation Between Nitrogen Balance and Food Intake for the Period 10–90 Days of Normal Mice and of Mice Given 900 r Total-Body Irradiation and Isologous or Homologous Bone Marrow Cells

Group	Weight Gain (g/day[a])	Nitrogen Balance[b] (mg/hr)	Correlation[c] Time After Treatment	
			10–30 Days	30–90 Days
Normal	0.06 ± 0.009	0.34 ± 0.08	0.56	0.63
Isologous	0.05 ± 0.011	0.67 ± 0.09	0.59	0.60
Homologous	0.009 ± 0.011	0.72 ± 0.09	0.66	0.68

[a] ±Standard error of the mean.

[b] Calculated on the assumption that there was 35.6 mg nitrogen per gram of food pellet (manufacturer's value). Our analyses ranged from 36.0 to 34.7 mg/g. All nitrogen-balance calculations are therefore subject to a systematic positive error which does not, however, affect the comparison among groups.

[c] Coefficient of correlation.

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10.4 LIPID COMPOSITION OF TISSUES AFTER ISOLOGOUS AND HOMOLOGOUS MARROW TRANSPLANTS IN MICE[1]

J. N. Bollinger Fred Snyder

Introduction. – These experiments were undertaken to assess the degree of lipid involvement in the metabolic consequence of bone marrow transplantation terminating in secondary disease. Previous data[2] suggested that a form of metabolic starvation (including the gross observation of adipose fat depletion) occurs in animals affected with this disease, thereby implying that stored fat might be used preferentially over other metabolites for energy requirements. Evaluation of this supposition has led to this report on the lipid content and composition of various tissues obtained from irradiated mice given homologous and isologous marrow. Fed, 3-day-fasted, and irradiated (950 r) mice served as control groups in this study.

Materials and Methods. – Total lipids,[3] the lipid classes separated by thin-layer chromatography (TLC),[3] and the total fatty-acid composition of each tissue as determined by gas-liquid chromatography[4] were measured on lipid extracts obtained from the liver, serum, bone marrow, genital fat pad, and the carcass.

Results and Discussion. – The data obtained reveal that transplantation of homologous marrow in irradiated mice had essentially no effect on the lipid class composition of liver, marrow, serum, adipose tissue, or carcass. An exception to this was the observation of long-chain fatty alcohols present in the carcass lipids of the 90-day chimeras. The finding of these alcohols in these chimeras might be related to the age or mouse strain used and not to the experimental treatment. The TLC chromatograms also revealed the triglyceride fatty liver of all mice that were starved for 3 days.

We observed that depletion of adipose fat occurred in some of the animals that received homologous transplants and not in others. The healthy-looking animals had ample fat in the genital areas, but those in a visibly physiologically poor state of health had no visible depot fat. These differences in adipose fat in homologous mice showed up in the total lipid determination and complicated the interpretation of these results. Our data, however, conclusively show that if mobilization of adipose fat to liver does occur in the homologous mice, it is readily used for energy requirements, since in no instance was there any accumulation of liver triglyceride or buildup of serum free fatty acids.

Gas-liquid chromatography analysis of adipose fat showed that alterations in the total fatty-acid composition did not occur in any of the experimental groups. The high content of linoleic acid demonstrated that there was no essential fatty-acid deficiency in the mice. The marrow lipids of the mice revealed a much more complex mixture of lipids than what we have previously observed for hematopoietic or fatty marrow from other species. Additional data on the lipid patterns of donor and recipient bone marrow before and after transplants would be valuable to determine the role that transplanted marrow cells and diet have in determining the type of lipids deposited.

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10.5 ELECTRON MICROSCOPIC OBSERVATIONS ON TRITIATED-THYMIDINE-LABELED GERMINAL CENTER CELLS

D. C. Swartzendruber

Introduction. – Although several electron microscopic studies of lymphatic tissue have been

interpreted as support for a reticular cell genesis of lymphocytes and plasma cells, autoradiographic studies[1] using tritiated thymidine have not verified a stem cell role for reticular cells. It is the purpose of this report to describe the ultrastructure of tritiated-thymidine-labeled germinal center cells utilizing a combined electron microscopic autoradiographic technique.

Materials and Methods. – Adult (101 × C3H)F_1 male mice were injected intravenously with 1 ml of Tyrode's solution containing 2×10^9 sheep erythrocytes and, 1 hr later, 100 μc of tritiated thymidine (0.9 curie/millimole specific activity). Four hours after the injection of sheep erythrocytes (3 hr after isotope injection), the animals were killed and spleens removed and fixed for autoradiographic and electron microscopic study as reported previously.[2]

Results and Discussion. – The labeled cells observed in the electron microscope were of two types: (1) primitive cells and (2) cells possessing many of the same ultrastructural characteristics as primitive cells but having additional features of more differentiated cells. Primitive cells are characterized by large nuclei and nucleoli, free ribosomes apparently unassociated with membraneous components of endoplasmic reticulum, and few cytoplasmic organelles. The more differentiated labeled cells of the second type contain a more highly organized Golgi apparatus and more mitochondria (Fig. 10.5.1). Multivesicular bodies are numerous in these rapidly proliferating cells.

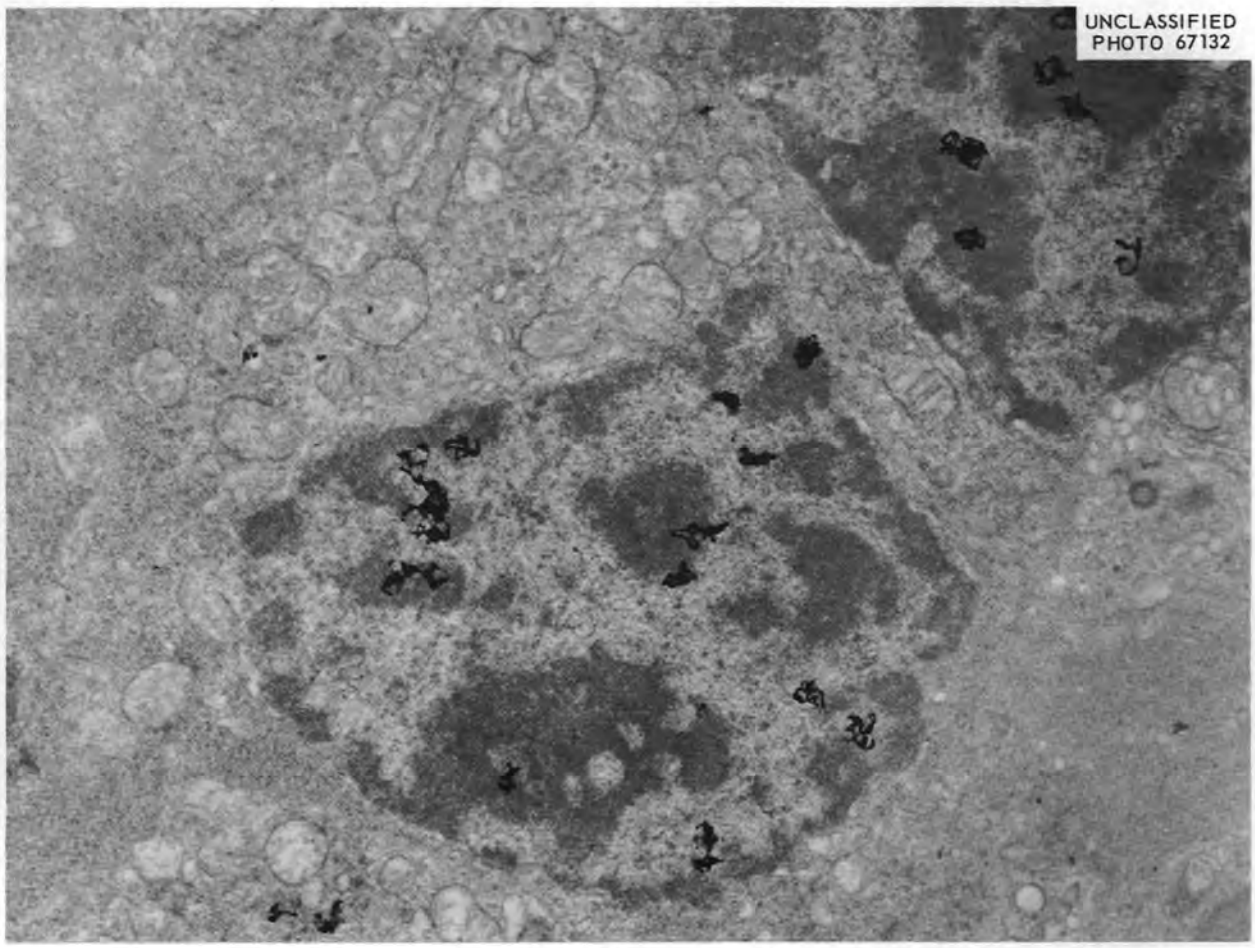

Fig. 10.5.1. Germinal Center Cell Labeled with Tritiated Thymidine. 15,000×.

It was not determined from this study whether the labeled primitive cells were transformed reticular cells, transformed small lymphocytes, or whether they represent stem cells. Reticular cells were not observed to label in this study.

Based on ultrastructural features, it seems that the primitive cells are precursors of the cells considered to be more differentiated, although the reverse may also be true. It seems important to consider that reticular cells were not observed to label – a point which must be considered in the interpretation of transitional or intermediate cells believed to arise from reticular cells.

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10.6 ACTINOMYCIN D EFFECT ON THE PRIMARY IMMUNE RESPONSE IN MICE[1]

M. G. Hanna, Jr.

Introduction. – Actinomycin D has been shown to bind to the guanosine residues of DNA. This binding results in an inhibition of in vivo and in vitro enzymic DNA-dependent RNA synthesis at low concentrations, while DNA synthesis is affected at higher concentrations. The effect of actinomycin D on dividing cells in tissue culture is to inhibit and/or block mitosis.

Convincing evidence of the direct cytotoxic action of actinomycin D on lymphoid cells, along with subsequent inhibition of the immune response in normal animals, has been presented. Wust and associates[2] showed that when the antibiotic was given simultaneously with sheep erythrocytes in rats, an increase in the latent period of the immune response was observed, but there was no effect on the rate or maximum hemagglutinin titer produced.

Histologic and autoradiographic studies during the latent period of the primary response in mice have described characteristic changes which occur in the spleen white pulp and which seem to be directly related to the production of cells capable of forming specific antibody. The purpose of these studies was to correlate the histologic changes with the appearance of hemagglutinin in serum following the injection of sheep erythrocytes and actinomycin D in mice.

Materials and Methods. – Six groups of LAF_1 mice were used for both the histologic and serologic studies. Animals in group 1 were injected intraperitoneally with actinomycin D 7 hr before sheep erythrocyte injection. Animals in group 2 were injected with 1,2-propanediol vehicle, 7 hr before sheep erythrocyte injection. Animals in group 3 were injected with sheep erythrocytes only, and animals in group 4 were injected with actinomycin D only. The animals in group 5 were nontreated controls. Group 6 was a special histologic control injected with isologous erythrocytes.

Histologic studies were performed at 2-hr intervals during the first 24 hr after injection and on days 2 through 14 after injection. Serologic studies were performed at daily intervals between days 1 through 14 after injection.

Results and Conclusion. – The results of this study showed that the optimal interval for the intraperitoneal injection of actinomycin D to give maximum delay in the appearance of circulating hemagglutinin was between 4 to 8 hr before the administration of antigen. The animals receiving actinomycin D 7 hr prior to antigen injection demonstrate a delay of circulating hemagglutinin until 5 days after antigen. A hemagglutinin titer equivalent to that achieved in the two control groups was present 6 days after antigen injection, and the slopes of the two curves were not significantly different.

The delay in the hemagglutinin production in SRBC-immunized mice treated with actinomycin D correlates with cytotoxic damage to large pyronin-staining cells in the germinal centers and in the associated lymphocyte mass. A difference was observed between the effect of actinomycin D in the lymphatic nodules of immunized and nonimmunized animals. The characteristic findings in the SRBC-stimulated, actinomycin-treated animals are large numbers of dying cells and a large amount of phagocytosis. This result was most pronounced in the germinal centers (Fig. 10.6.1). These results suggest that the cells in lymphatic nodules of animals exposed to antigen are more severely damaged by the drug than their morphologic counterparts in animals receiving actinomycin D only. These findings support the importance of

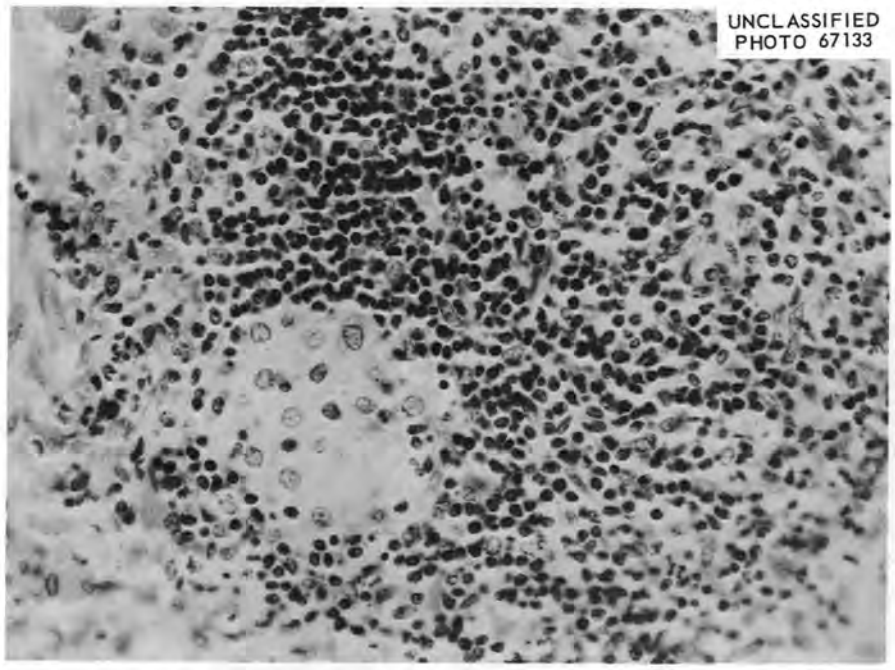

Fig. 10.6.1. Residual Germinal Center After Treatment with Actinomycin.

messenger RNA during the induction phase of the immune response and also demonstrate that unbalanced growth in antigenically stimulated cells may result in cell death and phagocytosis.

Two phases of recovery in the lymphatic nodules of immunized animals, previously treated with actinomycin D, were observed. Both phases involved a repopulation of a single morphologic cell type: the large pyronin-staining cell. The first phase results in the proliferation and repopulation of the cell type within the lymphocyte mass; the second phase constituted a repopulation of the germinal center.

Two explanations for the origin of newly formed cells responsible for the repopulation and restitution of the lymphatic nodule are transformation of reticular cells and/or replication of surviving large pyronin-staining cells.

References

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10.7 EFFECT OF AET ON ACUTE LETHALITY FROM DIMETHYLNITROSAMINE

C. C Congdon D. G. Doherty
Frances E. Hacker

Introduction. – In earlier work it was shown that some amelioration of damage to bone marrow, spleen, and thymus after administration of 9,10-dimethyl-1,2-benzanthracene was provided by bone marrow transplantation.[1] The present work is part of a program to determine the value of radiation protection and recovery agents in preventing acute lethality from representative members of the different classes of chemical carcinogens. AET administered before carcinogenic hydrocarbons and butter yellow failed to reduce acute lethality from these compounds. The present report shows that some AET protection against the acute toxicity of the highly carcinogenic substance, dimethylnitrosamine (DMN), could be achieved.

Methods and Results. – Table 10.7.1 summarizes the results in 19-week-old male and female $BC3F_1$ mice given AET orally before DMN was administered intraperitoneally. Three-tenths of a milligram of DMN killed 51% of the mice in these

Table 10.7.1. AET Protection Against Acute Lethality from Dimethylnitrosamine (DMN) in Male and Female $BC3F_1$ Mice

Group	Treatment	Number	Number Dead at Day			
			5	10	15	20
1	0.2 ml olive oil, intraperitoneally	22	0	0	0	0
2	16 mg AET, orally	30	0	0	0	0
3	16 mg AET + 0.3 mg DMN, intraperitoneally	100	11	12	14	15
4	0.3 mg DMN, intraperitoneally	100	46	47	51	51

experiments, whereas only 15% were killed when AET was given a few minutes before the DMN. At lower doses of DMN the mortality was so low that an AET protection could not be demonstrated. With 0.4 mg of DMN, all mice died regardless of the AET protection.

These preliminary results indicate that in a very restricted dose range of DMN some prevention of acute lethality with AET was obtained.

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10.8 SHORT-TERM CULTURE CONDITIONS FOR MOUSE BONE MARROW CELLS

L. H. Smith

Introduction. – This report is a continuation of work reported previously[1] and includes results of further testing of environmental conditions which support erythropoietic capacity of mouse bone marrow cells in culture for 6 hr. The effects of culture temperature and of "feeder" tissues are herein described.

Materials and Methods. – The culture procedure and assay for erythropoietic capacity has been described.[1,2] Cultures of normal mouse marrow were maintained at temperatures ranging from 2 to 45°C for 6 hr at pH 7.1 to 7.4. The medium used was TC 199 + 25% fetal calf serum. The number of cells injected into each irradiated mouse (850 r) was 0.25×10^6, which results in a 14.4 ± 1.2% uptake of ^{59}Fe by the spleen on day 8 after marrow injection. This time-zero (T_0) percentage was compared to the value obtained after 6-hr culture and the results expressed in terms of the percent of T_0.

Results and Conclusions. – Table 10.8.1 shows erythropoietic capacity of marrow cells as a function of temperature after 6 hr of incubation. Uniform maintenance of ~90% of the T_0 value is usually achieved from 0 to 30°C. Above 30°C, capacity declines to essentially 0% at 45°C. Reduction of activity in the normal physiological temperature range indicates inadequate culture conditions, differentiation of erythrocyte precursors in the direction of erythrocytes, or differentiation of precursor cells in the direction of nonerythropoietic cells. In line with the first possibility, marrow cells were cultured in the

Table 10.8.1. Effect of Culture Temperature on 6-hr Maintenance of Erythropoietic Capacity of Mouse Bone Marrow

Temperature of Culture (°C)	Erythropoietic Capacity of Spleen (% of T_0)
1–2	94
15	88
20	91
25	89
30	87
35	70
37	69
40	61
43	38
45	0.5

presence of several tissue homogenates including kidney, liver, thymus gland, and brain. It was speculated that like feeder cells employed in tissue culture, some of these tissues might condition the medium and provide a more adequate environment. However, none were effective, and the usual 20–30% reduction after 6 hr at 37°C was observed.

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Table 10.9.1. Anti-P2 Titers of Experimental P1 and Control (P1 and F_1) Mice

Mice (5 ♂♂/group)	Given F_1 Cells at Birth	Log_2 Agglutinin Titer[a] on Day		
		0[b]	7	52
P1 (C57BL/6 Cum)	$\sim 2 \times 10^7$, intraperitoneally	0	0	0
P1		0	7	14
F_1 ($B6D2F_1$)		0	0	0

[a]Each mouse received 5 to 10 × 10^7 P2 (DBA/2 Cum) bone marrow cells intraperitoneally on days 0, 7, 14, 21, 28, 35, and 42.

[b]All mice 6 months old on day 0.

10.9 SIMILARITIES AND DIFFERENCES IN SPECIFIC IMMUNOLOGICAL UNRESPONSIVENESS BETWEEN GENETICALLY TOLERANT MICE AND THOSE MADE TOLERANT AT BIRTH

Joan Wright Goodman

Introduction. – In the course of experiments designed to test for parental antigens not expressed in F_1 hybrid mice, similarities and differences were noted between genetically tolerant F_1 hybrids and parental mice rendered tolerant by cellular inocula at birth.

Methods and Results. – Inbred mice (P1) B6 or B, were given at birth an injection of spleen cells from (P1 ♀ × P2 ♂)F_1 hybrids to induce tolerance. When these mice were adults, some of them were bled for serum and then were given injections of bone marrow cells from the second parental-P-2 strain (D2 or C3). P1 and F_1 mice that had not received spleen cells at birth served as controls. Table 10.9.1 shows that primary and secondary anti-P2 titers were obtained only in control parental mice. Mice, presumably tolerant either as a result of induction or of genetic constitution, produced no hemagglutinins. Both groups were able to make antibody to A.CA or A.SW strains.

Spleen cells from P2 mice were injected into another group of presumably tolerant P1 mice. Recipients were checked for red cell chimerism and observed for evidence of wasting disease analogous to that seen in F_1 hybrids similarly injected. P1 mice did not show any evidence of erythrocyte chimerism, although F_1's ($B6D2F_1$ but not $BC3F_1$) often did. However, many (but not all) presumably tolerant mice did lose weight (20–38% of body weight at the time of spleen cell injection) and develop wasting disease. Control P1 mice never showed chimerism or developed wasting disease.

Presumably-tolerant P1 mice that underwent wasting disease were autopsied, and tissues were taken for histologic study and for comparison with those from comparable, genetically tolerant F_1's. Depletion of lymph nodes and spleen white pulp was not so severe in the P1 mice, following injection of P2 spleen cells, as it was in similarly treated wasting F_1's, and areas of lymphatic regeneration could even occasionally be found. A most striking difference between the two kinds of tolerant mice was seen in marrow sections taken from wasting animals. Whereas F_1's showed extreme hypoplasia, tolerant P1's, although also anemic, had cellular, hypergranulocytic marrow. Amyloidosis was commonly seen in spleens from tolerant P1 mice with wasting disease. This condition often occurs after chronic infections or other prolonged immunological activity. F_1 hybrid tolerant mice, on the other hand, were not often found to have amyloid spleens.

Summary. – The immunological unresponsiveness of P1 mice made tolerant by an injection of

(P1 ♀ × P2 ♂)F_1 spleen cells at birth was compared with that of genetically tolerant F_1 hybrids. Both groups were unable to make hemagglutinins to P2 bone marrow. Spleen cells from P2 mice, inoculated into either tolerant P1 or F_1, caused wasting disease. F_1 mice showed red cell chimerism after this treatment; P1's did not. Lymphoid and bone marrow pathology differed between the two groups.

10.10 GRAFT-REJECTION METHOD FOR FUNCTIONAL ASSAY OF CULTURED MOUSE HEMOPOIETIC CELLS

H. G. Willard

Introduction. – In developing a method for assay of the immunologic competency of cultured cells, a suitable end point must be selected. Analysis of morphology of hemopoietic cells cultured in vitro is difficult, and a parallel method would be advantageous whereby functional capacity can be tested. Rejection of homologous marrow by isologous immunologically competent (IC) cells may be used as an assay method whereby the 6-hr spleen ^{59}Fe incorporation is used as an end point.[1] The relative rejection efficiency of IC cells after varying periods of culture can then be compared with that of uncultured cells in terms of rejection of homologous erythropoietic tissue.

Materials and Methods. – (C3H × C57BL)F_1 female mice received 950 r of whole-body x irradiation, followed by intravenous injection of 17–20 × 10^6 (C57L × A)F_1 bone marrow cells. One day later the mice were given various doses of (C3H × C57BL)F_1 peripheral leukocytes, thymocytes, or lymph node cells. Eight days post-irradiation, ^{59}Fe uptake by the spleen was assayed to determine the extent of rejection of the homologous graft by IC cells.

Results and Conclusions. – Table 10.10.1 shows the relative effectiveness of isologous leukocytes, thymocytes, and lymph node cells in the rejection of homologous marrow grafts. The order of efficiency is leukocytes, lymph node cells, thymocytes. Approximately 10 × 10^6 lymph node cells are required to depress the ^{59}Fe uptake to the value obtained with 4 × 10^6 leukocytes. In this particular strain combination, thymocytes seem to be ineffective in rejection ability at the doses tested (up to 100 × 10^6 intravenous and 352 × 10^6 intraperitoneally). Delker,[2] using death as an end point, reported a rejection by thymocytes (5 to 17 × 10^6) in the LAF_1-A.CA strain combination. However, in Delker's experiment, a smaller number of homologous marrow cells was injected, and the time between treatment and animal death was longer than in the present study. In all three types of IC tissues studied, splenomegaly was pronounced, although there was no correlation between spleen weight and administered cell dose,

Table 10.10.1. Relative Effectiveness of Various Isologous Immunologically Competent Cells Compared to Peripheral Leukocytes in Rejection of 20 × 10^6 Homologous Marrow Cells

Number of Isologous IC Cells	Percent ^{59}Fe Uptake by Spleen After Leukocyte Treatment	Relative Effectiveness of Lymph Node Cells (%)	Relative Effectiveness of Thymocytes (%)
× 10^6			
None	16.79 (19)[a]		
0.5	15.28 (15)	81 (8)	<1 (9)
1.0	8.59 (12)	51 (5)	<1 (9)
2.0	5.12 (15)	41 (8)	
4.0	1.49 (14)	34 (10)	<1 (7)

[a]Number of mice.

or spleen weight and ^{59}Fe uptake. By means of the graded response that occurs in this rejection system, the effectiveness of cultured mouse cells in rejecting homologous marrow can be tested. This method of assay is more quantitative than 30-day survival, and, in addition, the small number of cells required to give a measurable response permits culture procedures to be kept on a small scale.

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10.11 BACTERIOLOGICAL STUDIES ON IRRADIATED MICE

M. L. Simmons

Introduction. – As there have been problems in this laboratory with the early death syndrome in lethally irradiated animals, we thought it desirable to make a bacteriological survey of organisms most likely to be involved in early deaths. Since the rooms in our animal facility are constantly being monitored and the *Pseudomonas* incidence is quite low, we did not consider this organism a major factor. On the other hand, coliforms had been cultured from heart blood and liver of mice dying within several days after irradiation.

Materials and Methods. – Adult $BC3F_1$ mice of both sexes were used. All the animals had been tested in a routine fashion when received at Receiving and Quarantine and were kept on water bottles chlorinated at 10–14 ppm. Prior to the experiment, all of the animals were randomized and were tested by composite fecal examinations (10 mice/pan) for *Salmonella sp.* and *Pseudomonas sp.* Of the 20 pans tested, 4 were found to be contaminated with nonpigmented *Pseudomonas* and 1 with *Salmonella*. These animals were sacrificed, and the remaining 150 were used in the experiment. One hundred of the mice were given 950 r of x irradiation, and fifty were used for controls. Beginning 24 hr after irradiation, ten experimental animals and five control animals were sacrificed every day for 10 days. Several animals died each day after the sixth day so that the count fell below ten thereafter. Heart blood (0.1 ml) was used as inoculum into thioglycolate liquid medium, and the medium was cultured for bacterial growth. When animals died during the experiment, heart blood clots and liver were obtained for culture.

Results and Conclusion. – Table 10.11.1 shows the *Pseudomonas* and *Streptococcus* incidence in irradiated mice. Of the irradiated mice killed and cultured, 4% were positive for pigmented *Pseudomonas*, 1% for nonpigmented *Pseudomonas*, and 20% for *Streptococcus*. Of the 50 nonirradiated control mice tested, only 1 was positive (*Streptococcus*) on day 3. This single incident may represent contamination during sterile procedure.

The early death syndrome beginning on day 5 and lasting through day 8 occurred in 14 mice. These mice were all positive for *Pseudomonas sp.* These results indicate that either the mice were very quickly recontaminated after irradiation or the infection was latent and was not picked up during initial testing. It would not seem likely that they could become so grossly recontaminated with *Pseudomonas* from drinking water since the water bottles contained 10–14 ppm of chlorine.

These results indicate that in our mouse population the involvement of *Pseudomonas* in the etiology of the early death syndrome is obscure. To further clarify the situation, we are currently investigating the possibility that nonpigmented *Pseudomonas* reverts to the pigmented form in vivo. Also to be considered is the possibility of a latent form of infection which may be in the lungs or some other locus which precludes identification by present fecal methods.

Table 10.11.1. Bacterial Culture of Heart Blood Beginning 24 hr After 950 r of X Irradiation

Day Number	Number of Animals Killed	Test Animals[a]			
		Positive *Pseudomonas*		*Streptococcus*	Negative
		Pigmented	Nonpigmented		
1	10				10
2	10				10
3	10			1	9
4	10	1			9
5	10	(4)[b]	1		8
6	10	2 (7)		1	7
7	8	1 (2)		2	5
8	7	(1)		5	2
9	6			6	0
10	5			5	0

[a]*E. coli* present in one animal of test group on day 5.
[b]Figures in parentheses indicate number of early deaths.

11. Pathology and Physiology

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Physiological and Chemical Effects of Radiation (11.2, 11.11)

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Virology (11.6, 11.7, 11.11)

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Epidemiology and Microbiology (11.8–11.11)

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Histology and Autoradiography (11.11)

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11.1 RADIOSENSITIVITY OF SNAKES AND BOX TURTLES

G. E. Cosgrove M. L. Davis

Introduction. – Exposure of snakes and box turtles to whole-body x radiation has been carried out to determine their LD_{50}. Locally captured adult snakes, *Elaphe obsoleta* (rat snake) and *Coluber constrictor* (black racer), and adult box turtles, *Terrapene carolina*, were used. They were exposed to 200–700 r of 250-kvp whole-body x rays. Exposures were determined by a Victoreen meter. In the case of turtles, this meter was

shielded in an empty turtle shell. Time of death after irradiation was recorded, and necropsies were performed on many.

Results and Discussion. – Tables 11.1.1 and 11.1.2 summarize the mortality data for the various radiation exposures. Due to the slow onset and progression of acute radiation disease in these reptiles, the usual 30-day period for study of acute radiation mortality was not suitable and observations were made to 90 days for snakes and 120 days for turtles. Exposures for turtles are those given under the shell and average 21% less than exposures in air at the same distance. The $LD_{50}/90$ for snakes was between 200 and 300 r, and the $LD_{50}/120$ for turtles was approximately 850 r (under-the-shell exposure). The mean day of death was somewhat earlier with higher exposures. The reason for the marked difference in radiosensitivity between snakes and turtles is not known. The radiosensitivity of snakes seems to be greater than that of most vertebrates.

Table 11.1.1. Mortality of Snakes Exposed to Whole-Body X Radiation

Radiation Exposure (r)	Number Exposed	Mortality in 90 Days (%)	Mean Time of Death (days)
200	7	29	33
300	3	100	38
500–800	8	100	37
1000–3500	8	100	24

Table 11.1.2. Mortality of Box Turtles Exposed to Whole-Body X Radiation

Radiation Exposure[a] (r)	Number Exposed	Mortality in 120 Days (%)	Mean Time of Death (days)
500–600	7	30	87
700–800	25	4	73
850–856	12	50	69
900	5	80	75
1000–7000	25	100	48

[a]Under-the-shell exposure.

11.2 HYPERTRANSFUSION OF BLOOD PLATELETS AND PLATELET REGULATION

T. T. Odell, Jr. T. P. McDonald
C. W. Jackson

Introduction. – It is well established that thrombocytopenia induced experimentally in rats by bleeding or specific antiserum triggers the production of new platelets after a lag period of one or two days, culminating within a few days in a transitory thrombocytosis. Current evidence appears to implicate a humoral stimulatory agent. The evanescence of the thrombocytosis produced by these and other methods raises the question whether there also exists, as part of the homeostatic mechanism, a complementary agent that is inhibitory to platelet production. Experiments were therefore carried out to explore whether the thrombocytosis induced by platelet transfusion affected the number of megakaryocytes, the source of platelets, in bone marrow or altered the rate of production of platelets.

Results and Discussion. – Platelets were injected intravenously into rats two or three times at one- or two-day intervals to maintain platelet levels well above normal. From 20 to 30 $\times 10^9$ platelets were transfused at each injection time into rats whose normal platelet level was estimated to be about 18 $\times 10^9$ platelets, thereby raising the circulating levels to two or three times normal. Counts of the number of megakaryocytes in sections of marrow taken 2, 3, and 6 days after beginning platelet transfusion were not different from control values. To measure the rate of platelet production, $Na_2{}^{35}SO_4$, a radioisotope that labels platelets by incorporation into the mucopolysaccharides of megakaryocytes (the platelet precursors in the marrow), was injected, and the radioactivity of the collected platelets was measured about 24 hr later. The radioactivities of platelets of rats killed 48 or 72 hr after beginning one to three daily platelet transfusions were as great as those of control rats not injected with platelets. (In the transfused rats, the radioactivity measured in platelet suspensions was attributed to the rat's own platelets since an earlier report indicated that platelets labeled in the circulation

lose the ^{35}S-sulfate label in less than 24 hr; the transfused platelets were therefore assumed to be unlabeled.)

The results thus far indicate that raising the circulating platelet level of rats by transfusion of large numbers of platelets does not affect the megakaryocytes nor alter the rate of platelet production. These experiments do not therefore provide any evidence for an agent that inhibits platelet production. However, since experience is still limited and the results are somewhat variable, the question whether such an agent exists remains an open one.

11.3 INDUCTION OF IMMUNITY AND OF UNRESPONSIVENESS TO PARENTAL MARROW GRAFTS IN ADULT F_1 HYBRID MICE

Gustavo Cudkowicz J. H. Stimpfling[1]
L. C. Satterfield

Introduction. – Previous work has firmly established that bone marrow cells of the C57BL/10 mouse strain (H-2^b/H-2^b) do not grow as well upon transplantation into irradiated *H-2^b* heterozygous hybrid mice as on transplantation into irradiated homozygous recipients of either of the parental strains.[2,3] The study of "hybrid resistance" to parental marrow grafts in hybrids from matings between congenic inbred parents differing from the C57BL/10 background strain by components of the H-2^b allele showed that deficient growth of C57BL/10 marrow cells in F_1 hybrids is controlled by a single noncodominant genetic factor closely linked with the K region of the complex H-2 locus.[4] The results of the experiment presented here suggest that this determinant controls an isoantigen since hemopoietic cells of C57BL/10 donor mice are isoantigenic to *H-2^b* heterozygous F_1 hybrids.

Results. – F_1 hybrids between the congenic C57BL/10 and C57BL/10.D2 (H-2^d/H-2^d) strains were exposed to 700 r of whole-body x radiation and given promptly thereafter an intravenous injection of parental strain marrow cells. Five days later, the extent of proliferation of the transplanted marrow cells was estimated by measuring the incorporation of 5-iodo-2'-deoxyuridine-^{131}I into newly synthesized DNA of the recipient spleens. C57BL/10 marrow grew deficiently in F_1 hybrids, whereas C57BL/10.D2 marrow grew as well in F_1 as in isogenic recipients (Fig. 11.3.1). Pretreatment of the F_1 mice with four weekly injections of 2×10^7 C57BL/10 viable spleen cells abolished their "resistance" toward C57BL/10 marrow grafts, as if causing immunological tolerance (Table 11.3.1), but did not affect the growth of transplanted C57BL/10.D2 or of unrelated C3H marrow. Pretreatment of the F_1 mice with three weekly injections of 15×10^6 C57BL/10 viable marrow cells enhanced their "resistance" toward C57BL/10 marrows, as if causing a second-set type of response (Table 11.3.2), without causing an effect on the growth of transplanted C57BL/10.D2 or C3H marrow. Similar pretreatment of F_1 mice with C57BL/10.D2 spleen or marrow cells or with grafting of parental skin from either strain did not affect the growth of C57BL/10 or C57BL/10.D2 marrow (Tables 11.3.1 and 11.3.2).

Conclusion. – The results strengthen the interpretation (1) that genetic interaction involving a subunit of the H-2 locus results, in F_1 heterozygotes, in modified expression of an antigenic determinant of the H-2B complex, and (2) that this

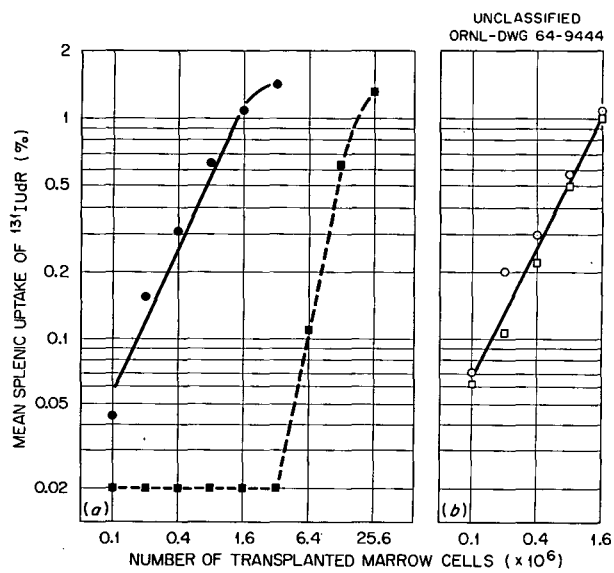

Fig. 11.3.1. Mean Splenic Uptake of ^{131}IUdR in Relation to the Number of Bone Marrow Cells Injected into X-Irradiated (700 r) Recipient Mice. (Each point represents five mice.) Section *a*: C57BL/10 cells into C57BL/10 (●—●) and into C57BL/10 × C57BL/10.D2 F_1 recipients (■—■). Section *b*: C57BL/10.D2 cells into C57BL/10.D2 (○—○) and into C57BL/10 × C57BL/10.D2 F_1 recipients (□—□).

Table 11.3.1. Proliferation of Transplanted Parental Marrow Cells in the Spleens of F_1 Hybrid Recipients Pretreated with Parental Spleen or Skin Grafts

Donor		Recipient[a]		
Strain	Number of Grafted Cells	Strain	Pretreatment	Mean Splenic Uptake of ^{131}IUdR (% ± standard error)[b]
C57BL/10	10^6	C57BL/10 × C57BL/10.D2	Tyrode's solution	0.01 ± 0.001 (29)
			C57BL/10 spleen	0.55 ± 0.04 (29)
			C57BL/10.D2 spleen	0.01 ± 0.002 (16)
			C57BL/10 skin graft	0.01 ± 0.003 (18)
			C57BL/10.D2 skin graft	0.02 ± 0.005 (10)
C57BL/10.D2	10^6	C57BL/10 × C57BL/10.D2	Tyrode's solution	0.69 ± 0.05 (10)
			C57BL/10 spleen	0.60 ± 0.06 (10)
C3H	10^6	C57BL/10 × C57BL/10.D2	Tyrode's solution	0.61 ± 0.09 (10)
			C57BL/10 spleen	0.68 ± 0.07 (10)

[a]Recipients were exposed to 700 r of x rays before marrow grafting. Five days later, they were injected with ^{131}IUdR to assess the extent of splenic repopulation by cells synthesizing DNA.

[b]Mean uptake values are given as percentage of the total ^{131}IUdR radioactivity administered ± standard error of the mean, in excess of percent retention of radioactivity in spleens of irradiated control animals not injected with marrow. The number of recipient mice is indicated in parentheses; data of several independent experiments were pooled.

Table 11.3.2. Proliferation of Transplanted Parental Marrow Cells in the Spleens of F_1 Hybrid Recipients Pretreated with Parental Marrow Skin Grafts

Donor		Recipient[a]		
Strain	Number of Grafted Cells	Strain	Pretreatment	Mean Splenic Uptake of ^{131}IUdR (% ± standard error)[b]
C57BL/10	10^7	C57BL/10 × C57BL/10.D2	Tyrode's solution	0.58 ± 0.04 (28)
			C57BL/10 marrow	0.12 ± 0.01 (25)
			C57BL/10.D2 marrow	0.60 ± 0.03 (27)
			C57BL/10 skin graft	0.73 ± 0.08 (13)
			C57BL/10.D2 skin graft	0.89 ± 0.10 (9)
C57BL/10.D2	10^6	C57BL/10 × C57BL/10.D2	Tyrode's solution	0.69 ± 0.05 (10)
			C57BL/10 marrow	0.59 ± 0.05 (10)
C3H	10^6	C57BL/10 × C57BL/10.D2	Tyrode's solution	0.61 ± 0.09 (10)
			C57BL/10 marrow	0.70 ± 0.10 (10)

[a]Recipients were exposed to 700 r of x rays before marrow grafting. Five days later, they were injected with ^{131}IUdR to assess the extent of splenic repopulation by cells synthesizing DNA.

[b]Mean uptake values are given as percentage of the total ^{131}IUdR radioactivity administered ± standard error of the mean, in excess of percent retention of radioactivity in spleens of irradiated control animals not injected with marrow. The number of recipient mice is indicated in parentheses; data of several independent experiments were pooled.

isoantigen affects the fate of hemopoietic cell grafts but not of skin grafts.

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11.4 FACTORS AFFECTING THE STEM CELL POOL IN TRANSPLANTED MOUSE BONE MARROW

Michael Bennett G. M. Shearer
Gustavo Cudkowicz

Introduction. – Serial transplantation of mouse bone marrow in x-irradiated isogenic recipients results in progressive loss (1) of the marrow's ability to promote survival and hemopoietic recovery,[1,2] (2) of its capacity to proliferate and produce undifferentiated stem cells and differentiating blood cells (i.e., depletion of its stem cell pool),[3,4] and (3) of the marrow "lymphocytes."[3,4] Although the magnitude of these effects varies with the number of successive transplantations, the impairment of proliferative capacity has also seemed to depend on the number of marrow cells transplanted.[3,4] This suggests that the hemopoietic stem cell pool can be channeled toward differentiation by the excessive pressure of transplantation of small numbers of cells into heavily irradiated recipients, differentiation occurring at the expense of proliferation. This investigation was concerned with the effects of experimental modifications that might change the need for production of differentiated hemic cells by transplanted marrow.

Results. – Femoral marrow cells from adult (BALB/c × A/He)F_1 females were injected intravenously into adult isogenic female recipients which had been exposed earlier on the same day to 850 r of whole-body x radiation ($LD_{100}/30$). Each recipient received 5×10^5, 5×10^6, or 5×10^7 nucleated cells. Fifty days later, three chimeras of each group were killed, and the competence of their marrow to proliferate on transplantation was assayed in terms of splenic repopulation in irradiated (700 r) isogenic female recipients. The splenic hyperplasia in such recipients was estimated by measuring the uptake of iododeoxyuridine-^{131}I (^{131}IUdR), a thymidine analog, into newly formed splenic DNA, according to a technique described elsewhere.[4] The marrow of chimeras (primary recipients of marrow) that had been given 5×10^5 hemopoietic cells 50 days earlier remained subnormal, but infusion of larger numbers of cells reduced or eliminated the deficiency in proliferative competence (Table 11.4.1). In fact, 5×10^7 cells produced an overshoot, suggesting that proliferation of transplanted marrow stem cells may be similar to the marrow regeneration that follows irradiation alone.[5]

As a further test, male (C3H/Anf × C57BL)F_1 mice exposed to 950 r of whole-body x rays ($LD_{100}/30$) and promptly given 5×10^5 nucleated marrow cells from isogenic female donors were hypertransfused with packed red blood cells harvested from heavily irradiated male mice of the same strain. Transfusions of 0.5–1.0 ml were initiated one day before marrow transplantation and continued for five weeks, three times a week during the first two weeks and twice a week thereafter. A second group of chimeras was hypertransfused with lymphoid cells obtained from mesenteric nodes ($\sim 5 \times 10^6$ nucleated cells per injection), beginning the day of the marrow transplantation and continuing twice weekly for five weeks. A third group received both erythrocytes and lymph node cells, but on different days. A fourth group was injected with Tyrode's solution alone. The proliferative capacity of the marrow was assayed 35 days after marrow transplantation (Table 11.4.2). Each treatment enhanced the proliferative competence of transplanted marrow compared with Tyrode's solution alone.

To explore the influence of antigenic stimulation of marrow stem cell regeneration, marrow cells were serially transplanted at 35-day intervals into both female and male, primary and secondary recipients. Male mice possess a male-specific isoantigen that elicits an antibody response adequate for rejection of male skin or spleen grafts by isogenic female recipients. This isoantigen does not, however, detectably affect the survival of irradiated males grafted with isogenic marrow from female donors, although the grafted cells are exposed to it continuously.

Reduction of the incorporation of ^{131}IUdR into spleens of test recipients occurred to a greater extent in female-male grafts than in female-female grafts (Table 11.4.3). In both types of grafts, the deficiency in marrow proliferative capacity was greater on the second than on the first transplantation, in keeping with earlier observations.[4]

Conclusion. – From the foregoing experiments, we infer that mouse marrow cells transplanted into lethally irradiated recipients may be unable to regenerate a normal hemopoietic stem cell population if the requirements for differentiated cells are great in relation to the available supply of transplanted stem cells. In the lethally irradiated host animal there is a need for rapid repopulation of all compartments of the hemopoietic system. To meet this need, stem cells may differentiate at the expense of self-perpetuation. Transplantation

Table 11.4.1. Influence of the Number of Marrow Cells Transplanted on the Regeneration of the Hemopoietic Stem Cell Pool in Isogenic Chimeras[a]

Number of Cells Transplanted into Primary Recipients	Number of Chimeras Tested[b]	Regenerative Hyperplasia in Spleens of Recipients Promoted by 10^6 Chimera Marrow Cells (% of normal, with 95% confidence limits)
5×10^5	3	56 (50–64)
5×10^6	3	80 (71–91)
5×10^7	3	116 (103–131)

[a]The stem cell concentration in marrow was estimated by transplanting 10^6 nucleated cells into irradiated recipients and determining 5 days later the extent of regenerative hyperplasia in their spleens. The latter was assessed by measuring incorporation of ^{131}IUdR into newly formed splenic DNA.

[b]Tested 50 days after marrow transplantation.

Table 11.4.2. Influence of Erythrocyte and Lymph Node Cell Transfusions on Regeneration of the Hemopoietic Stem Cell Pool in Isogenic Chimeras[a]

Treatment to Primary Marrow Recipients[b]	Number of Chimeras Tested	Regenerative Hyperplasia in Spleens of Recipients Promoted by 10^6 Chimera Marrow Cells (% of normal, with 95% confidence limits)
Tyrode's solution	7	39 (32–48)
Erythrocytes	7	84 (69–101)
Lymph node cells	7	67 (52–85)
Erythrocytes and lymph node cells	6	72 (57–92)

[a]The stem cell concentration in the marrow was estimated by transplanting 10^6 nucleated cells into irradiated recipients and determining 5 days afterward the extent of regenerative hyperplasia in their spleens. The latter has been assessed by measuring incorporation of ^{131}IUdR into newly formed splenic DNA.

[b]Treatment was initiated at the time of primary marrow transplantation and continued two or three times weekly until the transplanted marrow was tested for proliferative capacity 35–40 days after transplantation.

Table 11.4.3. Influence of the Recipient's Sex on the Ability of Serially Transplanted Marrow (from Female Donors) to Regenerate the Hemopoietic Stem Cell Pool of Isogenic Chimeras[a]

Transplant Generation	Sex of Host	Number of Chimeras Tested[b]	Regenerative Hyperplasia in Spleens of Test Recipients Promoted by 10^6 Chimera Marrow Cells (% of normal, with 95% confidence limits)
First	Female	6	59 (48–73)
	Male	7	39 (32–48)
Second	Female	10	33 (24–45)
	Male	10	17 (11–25)

[a]The stem cell concentration in the marrow was estimated by transplanting 10^6 nucleated cells into irradiated recipients and determining 5 days afterward the extent of regenerative hyperplasia in their spleens. The latter has been assessed by measuring incorporation of ^{131}IUdR into newly formed splenic DNA.

[b]Tested 35–40 days after each transplantation.

of large numbers of marrow cells or transfusion of differentiated blood cells during the critical stages of hemopoietic repopulation may allow restoration of the pool of stem cells to normal levels. Conversely, if the requirements for production of differentiated blood cells are increased, as is the case in isoantigenic recipients, a marginal marrow stem cell pool may be further depleted.

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11.5 PRELIMINARY OBSERVATIONS ON SPONTANEOUS AND RADIATION-INDUCED LEUKEMIA IN GERM-FREE MICE

H. E. Walburg, Jr. R. L. Tyndall
W. W. Harris[1] G. E. Cosgrove

Introduction. – The induction of leukemia by ionizing radiation in conventionally reared mice has been studied intensively for more than 20 years.[2] With the implication of viruses in the pathogenesis of these neoplasms,[3] it has become increasingly important to study the influence of the microbial flora of the host on the induction of leukemia and to determine the mode of transmission of any etiologic agents. Preliminary observations on x-ray-induced and spontaneous leukemia in germ-free mice associated with the appearance of "virus-like" particles in the leukemic tissues are reported here.

Methods and Results. – Non-inbred mice of the ICR strain surviving the acute effects of a single exposure to 600–900 r of x irradiation[4] have been observed for up to 9 months after exposure. The cumulative incidence of leukemia in the irradiated germ-free mice is as high as, or higher than, in the irradiated conventional mice (Fig. 11.5.1). It is higher in both irradiated groups than in the unirradiated groups, which do not differ significantly in spontaneous leukemia incidence. Germ-free RFM/U mice exposed to 300 r of x rays at 4–6 weeks of age also show a higher cumulative incidence than their conventional counterparts (Fig. 11.5.2). All leukemias examined histopathologically have been lymphocytic, and about half of them have involved the thymus, either exclusively or with generalized lymphomatosis. Electron microscopic examination of ultracentrifugates of homogenized organs of both germ-free and conventional leukemic RF mice has revealed tailed

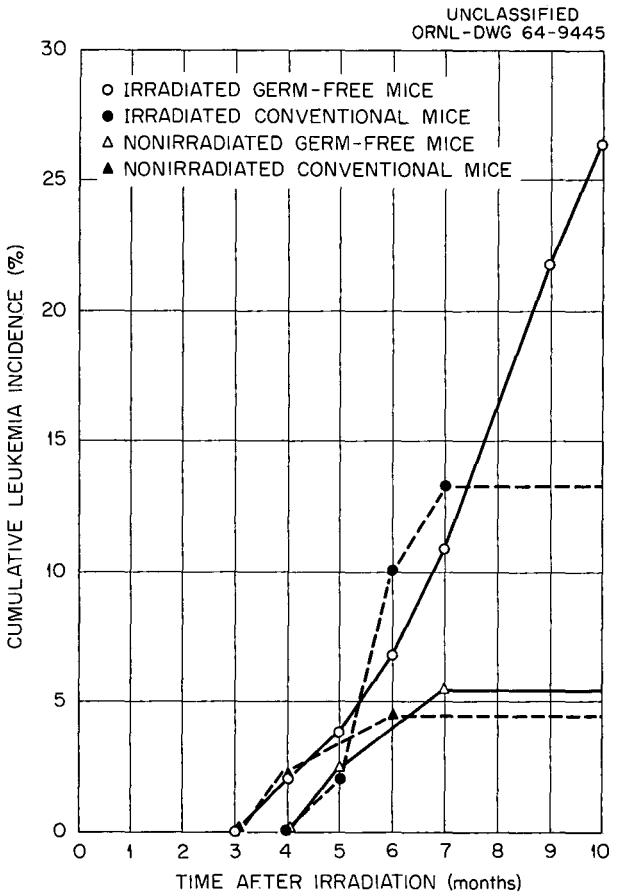

Fig. 11.5.1. Cumulative Incidence of Leukemia in Germ-Free and Conventional ICR Mice in Relation to Time After Irradiation.

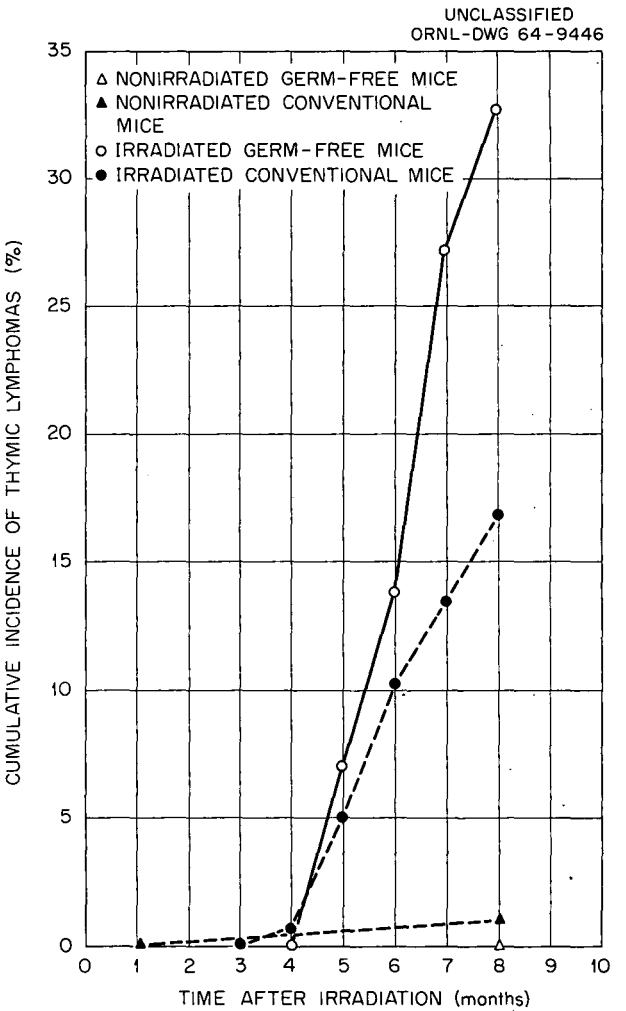

Fig. 11.5.2. Cumulative Incidence of Leukemia in Germ-Free and Conventional RFM/U Mice in Relation to Time After Irradiation. [Data for conventional mice from A. C. Upton *et al.*, *Cancer Research* **18**, 84 (1958)].

particles morphologically similar to the Moloney and Rauscher viruses. Such particles have not been detected in tissues from nonleukemic mice.

Discussion. – The detection of radiation-induced leukemia in germ-free mice has important implications concerning the relationship of the microbial flora to the pathogenesis of leukemia, suggesting transmission of leukemogenic viruses to the first-generation germ-free mice through the ovum or across the placenta of their conventional mother. The interpretation of a viral etiology is supported by the occurrence of characteristic "leukemia-virus-like" particles in the tissues of leukemic germ-free animals, although the cell-free transmission of the leukemias by such particles in these mice remains to be carried out. The relatively poor development of the immunological defense system of germ-free mice, particularly of the lymphatic system,[5] compared with that of conventional mice, may account in part for the higher incidence of radiation-induced leukemia in these animals.

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11.6 CYTOPATHOGENIC EFFECTS OF A MURINE LEUKEMIA VIRUS ON CELLS

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Introduction. – Investigation of the murine leukemia viruses has been hampered by the lack of a continuous cell culture susceptible to infection with these agents. This lack likewise eliminated the possibility of developing an in vitro assay based upon any cytopathogenic effects which might be elicited by the murine leukemia viruses. At present, the only available assay is the development of leukemia per se in the test mice. Recently, however, Wright has reported the development of a continuous cell culture capable of supporting the propagation of the Rauscher leukemia virus.[1] Such infected cultures, although continuously replicating the virus, showed no observable cytopathogenic effects when compared to the uninfected cultures. Cytopathogenic effects have, however, been obtained in such cultures by modifying the medium, as reported in the following.

Results and Discussion. – The culture infected with and propagating the Rauscher leukemia virus and the uninfected control culture were obtained through the courtesy and cooperation of Drs. B. Wright and J. Davenport, Chas. Pfizer and Company, Maywood, New Jersey. These cultures initially revealed no gross differences between infected and control cells in our laboratory. Later, however, when the infected cells were cultured in Eagles' basal medium (BME) instead of the usual modified medium 109, the cells exhibited a pronounced rounding effect. When fresh BME was added to such cells, they appeared to recover and re-formed a semiconfluent cell sheet. It was apparent, however, that cultures recovered in this manner were grossly altered in that there appeared to be some loss of contact inhibition. These cells also exhibited cytopathogenic effects (CPE). The uninfected control culture showed no initial rounding when subcultured on BME medium and no subsequent gross alterations. The CPE exhibited by the infected culture included an increased vacuolization and cytoplasmic eosinophilia including many eosinophilic granules or large eosinophilic inclusion bodies. The nuclei in such cells were smaller, varied in shape, and were more densely stained compared to nuclei of uninfected cells. Cells exhibiting CPE were highly leukemogenic when inoculated into suckling RFM/U mice.

Investigations are now under way to determine the degree of association between the observed CPE and the Rauscher leukemia virus per se and to determine if such CPE can be utilized in the development of an in vitro assay for leukemia viruses.

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11.7 EVIDENCE FOR A POSSIBLE RELATIONSHIP BETWEEN VIRAL RNA AND CELL MESSENGER RNA

R. L. Tyndall K. B. Jacobson[1]
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Introduction. – We previously reported gross differences between RNA fingerprints of total HeLa cell RNA and fingerprints of viral and/or virus-induced RNA. Since total cell RNA represents primarily ribosomal RNA, we have compared fingerprint patterns of cell messenger RNA and transfer RNA with patterns of viral RNA.

Results and Discussion. – Monolayer cultures of HeLa cells were exposed to $^{32}PO_4$ for 1 hr and for 16 hr prior to extraction of RNA-^{32}P with phenol at 60°C. Suspension cultures of HeLa cells were exposed to $^{32}PO_4$ for 5 hr, and soluble RNA-^{32}P was extracted with phenol at 5°C and further fractionated on DEAE cellulose to obtain transfer RNA-^{32}P (ref. 2). The residual interphase was extracted sequentially at 45, 55, and 65°C. To obtain messenger RNA relatively uncontaminated by ribosomal RNA, the 45–55°C fraction was discarded, since the RNA-^{32}P extracted at 45°C under these conditions has been shown to be indicative of ribosomal RNA,[3] and the RNA-^{32}P extracted at 65°C has been shown to be indicative

of messenger RNA.[3] RNA-^{32}P from purified polio virus type I was extracted with phenol at 60°C. The fingerprint patterns of the various RNA's were obtained by two-dimensional chromatography of pancreatic RNase digests.

Pertinent oligonucleotide ratios obtained by densitometric analysis of the fingerprint patterns (Table 11.7.1) reveal that the viral RNA patterns differ grossly from ribosomal patterns, as previously noted. They also differed from patterns of transfer RNA (Table 11.7.1). The viral RNA is characterized by high levels of the AU dinucleotide and low levels of GC and GAC, whereas ribosomal and transfer RNA revealed the reverse pattern. Shifts in HeLa cell RNA-^{32}P toward increased amounts of AU, relative to amounts of GC and GAC, with decreasing labeling time (Table 11.7.1) suggest that messenger RNA has relatively larger amounts of AU. This is further substantiated by the analysis of RNA-^{32}P extracted at 65°C, which shows high levels of AU and low levels of GC and GAC. Thus, the fingerprint pattern of the cell fraction extracted at 65°C, which in other mammalian cells was shown to be messenger RNA-^{32}P, is similar to the patterns obtained with polio virus RNA-^{32}P.

The similarity between viral RNA and cell messenger RNA has several implications: (1) it may account in part for the ability of the virus to gain control of cell synthetic processes and induce virus production; (2) it may denote a degree of complementarity between the two RNA molecules, which might result in binding of cell messenger RNA with viral RNA, leading to inhibition of cell-directed processes, such as has been observed in polio-virus-infected cells.[4]

Table 11.7.1. Relative Oligonucleotide Content of Various RNA Fractions

	U/AU	U/GC	U/GAC
1-hr HeLa cells	1.25	1.43	3.67
16-hr HeLa cells	1.34	0.80	0.85
Transfer RNA	1.74	0.71	0.78
5–45°C RNA (ribosomal)	1.50	0.66	0.75
55–65°C RNA (messenger)	0.99	2.80	2.73
Polio virus	0.71	2.70	2.07

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11.8 SEPARATION AND RELATIVE QUANTITATION OF MOUSE PLASMA ESTERASES WITH DISK ELECTROPHORESIS

R. C. Allen R. A. Popp[1] D. J. Moore

Introduction. – The development of disk electrophoresis[1] in acrylamide gel has provided a method of much higher resolution than electrophoresis in starch gel for the study of plasma esterases. Added advantages are that the pore size of the acrylamide gel may be varied and the supporting medium is transparent, permitting direct densitometric studies of staining reactions. With the recent availability of recording microdensitometers specifically designed for acrylamide, the quantitative study of enzymes with dye-coupled systems is feasible. This work was undertaken to determine optimal conditions for such studies.

Results and Discussion. – Optimal conditions for separation and quantitation of esterases were as follows. Plasma samples containing 500–600 mg of protein were placed in a 50% sucrose solution buffered with 0.47 *M* Tris-phosphate to a pH of 6.9. Spacer gel and lower gel were prepared according to the method of Ornstein and Davis.[2] Electrophoretic separations were carried out at room temperature in 0.05 *M* Tris-glycine buffer at pH 8.3 with a constant current of 3 ma per gel tube for 60 min. Gels were removed from the tubes in an ice bath and transferred to 0.04 *M* Tris-chloride buffer, pH 7.1, at room temperature to 5 min and then into an α-naphthol acetate-fast blue RR salt mixture for 15 min. They were fixed and developed in acid-alcohol overnight and were then placed in 10% glacial acetic acid prior to scanning in a model E recording microdensitometer.

The relative mobilities of plasma esterases are similar in acrylamide and starch gels, but resolution is better in the former. Acrylamide gel resolves mouse plasma into 18 or 19 bands of esterase activity, whereas 10 bands have been observed after starch gel electrophoresis.

Quantitative relationships of the various bands, expressed in percentage of the total activity, as determined by integrating the area under each curve, were reproducible within ±2%. Reaction rates determined for the major bands indicated that the release of α-naphthol was proportional to time up to 15 min.

Increased nonspecific dye coupling to glycine in the electrophoresed gels at pH above 7.2 and changes in the physical properties of gels due to breakdown of acrylamide to acrylic acid on prolonged standing of stock solutions are critical factors to be avoided in obtaining reproducible results in esterase determination carried out in acrylamide gels.

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11.9 SEX-HORMONE-DEPENDENT ESTERASES IN MOUSE PLASMA

R. C. Allen D. J. Moore

Introduction. – In developing techniques for the study of the quantitative relationships of mouse

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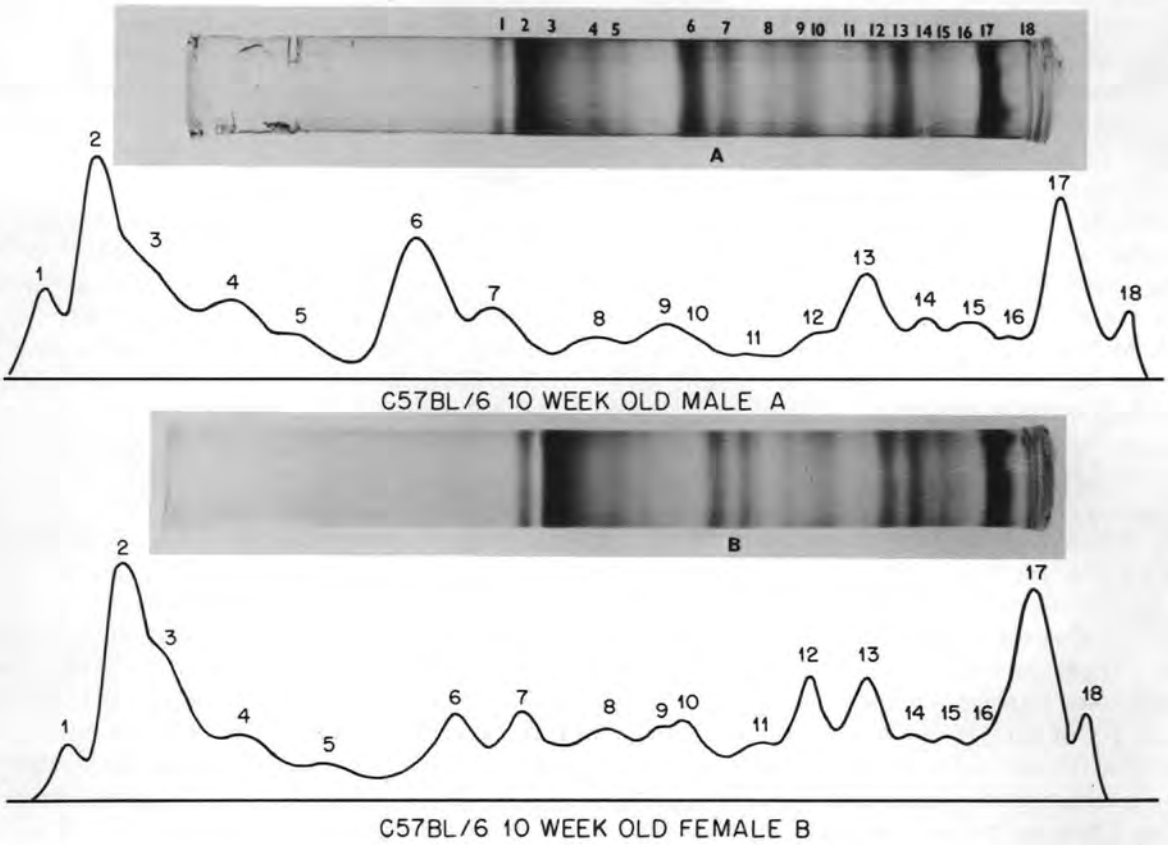

Fig. 11.9.1. Plasma Esterase Profiles of 10-week-old Male and Female C57BL/6 Mice, with α-Naphthyl Butyrate as Substrate. The profiles were obtained by direct densitometric scanning of the reacted gels in a Canalco model E recording microdensitometer.

plasma esterases, differences in the amounts of esterases between sexes were found in six strains of mice, including three hybrid strains. Experiments were carried out to determine whether these differences were under direct control of sex hormones.

Results and Discussion. – C3H/HeJ, C57BL/6, and RFM/U male and female mice were castrated and spayed respectively. Plasma esterase profiles were determined by disk electrophoresis on acrylamide gel.

After castration, bands 6, 7, 12, and 13 in the C57BL/6 (and bands 5, 6, 12, and 13 in C3H/HeJ and RFM/U, not shown), hereafter designated as S1, S2, S3, and S4, respectively, showed marked quantitative differences (Fig. 11.9.1). Band S1 was markedly decreased and S4 only slightly decreased. Band S2 showed a slight decrease and band S3 a marked increase. These changes were apparent within 7 days of castration and a complete reversion to the female type of esterase profile was evident in all mice 14 to 21 days after castration. First changes appeared to be an increase in band S3 followed by a decrease in band S1. On the other hand, no change was detected in female mice up to 40 days after ovariectomy.

Injection of 0.5 mg of testosterone three times weekly caused a return to the normal male pattern in castrated males and a change to normal male esterase profile in spayed females.

Since testosterone dependence of these esterase bands was evident, one might postulate that the sexually immature male mouse would show a female-type pattern; this was indeed so in male mice less than three weeks of age of all three strains. Between the third and fifth weeks of age, the period when the mouse reaches sexual maturity, the pattern changed to the adult male type.

On the other hand, administration of 50 μg of estrogen in the form of Estradiol once a week produced completely unexpected results. There was no apparent change in normal males after three weeks treatment, while in the castrated animal the estrogen caused a reversion to the male-type pattern in bands S3 and S4, with no effect on S1 and S2. In both the intact female and ovariectomized female, a male-type profile in bands S3 and S4 was produced, with no effect on bands S1 and S2.

The unexpected masculinizing effect of estrogen on bands S3 and S4 in the female and the castrated male is being studied further to elucidate the mechanisms responsible for these findings.

11.10 A RAPID METHOD FOR IDENTIFICATION OF THE ENTEROBACTERIACEAE

R. C. Allen R. T. Jones
J. H. Chandler

Introduction. – The systematic biochemical classification of large numbers of enterobacteriaceae as a routine procedure is beyond the scope of the average bacteriology laboratory because of the time, variety of media, and number of personnel required. To aid in identifying large numbers of enteric bacteria in mice, we have sought to develop accurate, rapid, biochemical methods for classification of groups of the enterobacteriaceae.

Results and Discussion. – Sterile de Serres multicompartmented petri dishes were employed in this work. These are sterile 6-in.-square plastic dishes divided into 100 compartments $^{9}/_{16}$ in. square and $^{13}/_{16}$ in. deep and covered by a Mylar film. Standard commercially available media (Difco) were employed. The carbohydrates were adjusted to maintain a pH of 7.2 after sterilization. Triphenyl tetrazolium HCl (0.005%) was added to SIM medium and KCN medium. Sterile media were added to the plates using a Cornwall automatic syringe and a $1\frac{1}{2}$-in. 20-gage needle. The Mylar surface was swabbed with alcohol, the needle inserted to the bottom of the well, and $1\frac{1}{2}$ to $2\frac{1}{2}$ ml of media expelled. Two and one-half milliliters of medium was necessary in KCN tests to avoid overseeding of the medium with resultant false negative reactions. Also, it was found necessary to store this medium at 4°C. After the agar solidified, the plates were dried, and each row was covered with Scotch tape if they were to be stored more than 3 or 4 days. Plates were inoculated with 24-hr broth cultures and with scrapings from agar slants emulsified in broth by inserting a tuberculin syringe with a 1-in. 22-gage needle to the bottom of the well and expressing 0.02 to 0.03 ml of culture.

Representative members of the enterobacteriaceae were used to compare results in the multicompartmented dishes with those in conventional tube media. Readings were made from the reflection of the bottom of the plate held above a mirror.

Carbohydrate fermentation reactions were apparent in 2 to 3 hr after inoculation and gas production was apparent in 6–8 hr, while delayed reactions were observed at 24 to 48 hr. Substitution of 2% carbohydrates for standard 1% carbohydrates prevented complete, early utilization of the carbohydrate and false negatives due to release

of basic amines from peptone in the media, thus allowing overnight incubation of the plates prior to reading carbohydrate fermentations. Similarly, decarboxylase reactions were more reliable with 2% carbohydrate when overnight incubations were employed.

Although this battery of tests was designed primarily to differentiate the enterobacteriaceae, the method is also applicable to other groups of bacteria. Semisolid carbohydrate media work equally well in these dishes and may be employed to determine fermentation reactions of some of the more fastidious organisms such as the *Pasteurella* group. Preliminary results have also indicated the possible use of this technique for the indentification of anaerobes. Fermentation patterns of several *Clostridia* isolates were successfully carried out in a suitably modified medium with a lowered OR potential. Any liquid, solid, or semisolid medium used in standard tube tests, with the exception of those requiring a slant, will work equally well in the de Serres multicompartmented petri dish. The much smaller amounts of media needed, the ease of preparation, and the disposable feature of the dish make it much less costly than standard tube tests. In addition, the time necessary for inoculating and reading plates is greatly reduced.

11.11 PREPARATIONS FOR THE "MEDIUM (LOW) LEVEL EXPERIMENT"

All Members of the Pathology-Physiology Section

Introduction. – The incidence of many late somatic effects of radiation is, in general, dose-dependent, but the data available are not adequate to define precisely the dose-response relation for any effect at relatively low levels of radiation (less than 50 rads). The purpose of this experiment is to determine the effects of low to medium levels of radiation (5 to 400 rads) on the incidence of leukemia and other neoplasms, on longevity, and on the various degenerative changes associated with aging.

Results and Discussion. – Earlier results with RF and LAF_1 mice have served as the basis for estimating the numbers of mice needed. The sample sizes and exposure conditions tentatively selected for use in the experiment (Table 11.11.1) have been chosen to provide reliable data primarily on the incidence of myeloid leukemia, but the quality of data on other effects will be enhanced by the large numbers of animals involved.

The experiment will comprise two series of mice set up concurrently and housed together as a single population: the "autopsy" series and the "serial sacrifice" series. Animals of the autopsy series will be observed until natural death to obtain data on longevity and on the incidence of various diseases in relation to age at death. Animals of the serial sacrifice series will be killed at intervals to obtain longitudinal data on the rate of development of biochemical, physiological, and morphological changes throughout life, as well as to permit continuous microbiological surveillance of the population. All mice will be radiographed after death for detection of bone tumors and other skeletal lesions and will be subjected to complete necropsy.

Table 11.11.1. Tentative Irradiation Dosage Schedule and Sample Sizes for the Low (Medium) Level Experiment

Total Dose (rads)	Dose Rate (rads/min)		
	~50	~1	~0.007
	Number of Mice of Each Sex per Experimental Group		
0	10,000[a]		
	3,000[b]		
5	10,000[a]		
25	1,000[a]	1000[a]	1000[a]
50	1,000[a]		
100	1,000[a]	1000[a]	1000[a]
150	1,000[a]		
200	1,000[a]	1000[a]	1000[a]
	3,000[b]		3000[b]
400	1,000[a]		

[a]Autopsy series.

[b]Serial sacrifice series.

Attempts are being made to minimize the variability among animals by use of a genetically inbred and microbially defined subline of the RF mouse (RFM/Uf) and by rigorously controlling the environment in which the mice are kept. To eliminate pathogens (e.g., arthropods, helminths, protozoa, bacteria, and viruses), mice of the inbred strain have been raised from germ-free foundation stocks. A known microbial flora, free of pathogens and consisting of a few types of bacteria, one of them genetically marked to allow study of microbial variations and interactions, has been established in the mice of the production and experimental stocks. These mice are housed in a facility that is rigorously isolated and maintained under unusually high standards of sanitation. To accumulate the large populations needed (~80,000 animals), mice will be introduced replicatively into each of the experimental groups over a period of several years. To minimize genetic change during the course of study, the production colony will be restocked from the germ-free foundation line after every fifth generation.

A continuing program of bacteriological surveillance of the mice and their quarters has been established to help maintain cleanliness and to monitor the microbial status of the mice. Although the number of mice involved has been relatively small thus far, our results indicate the feasibility of minimizing contamination of the facilities and of the mice by new organisms.

The physical facilities were designed to prevent contamination between rooms within the facility as well as from the outside. Measures for prevention of airborne transfer include several air locks, and a gradient of air pressure from the cleanest to the least clean part of the facility. To reduce contamination by way of fomites or vectors, material and personnel must pass through a sterilizing or sanitizing procedure before entering and then follow a carefully controlled traffic pattern designed to avoid cross-contamination.

Three ^{137}Cs radiation sources, varying in size from 10 to 2200 curies, have been installed and are being calibrated. These will provide a wide range of variation in available dose rate without variation in radiation quality.

A breeding program is now under way to produce mice for the orientation studies. The procedures for management, husbandry, and scientific coordination are being developed and implemented, and the physical plant is being checked for satisfactory operation. Systems for mechanical data coding, collection, and analysis are being adapted for use in the study; apparatus for this purpose is being installed and tested, and operating procedures are being developed.

Pending satisfactory progress on the preliminary phases of the investigation, the main study itself will be under way within the coming year.

12. Co-Carcinogenesis

(Cooperative Program with NIH-NCI)

Co-Carcinogenesis Basic Program

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Inhalation, Chemical, and Microbiological Co-Carcinogenesis

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Biochemistry of Co-Carcinogenesis

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12.1 TESTS FOR UPTAKE AND MUTAGENICITY OF 3,4-BENZPYRENE IN MICROORGANISMS

B. G. Moore A. P. Harrison, Jr.

Introduction. – Although there is at least one report[1] claiming mutagenicity for them, the carcinogenic hydrocarbons are generally considered nonmutagenic. In this regard they stand alone among the various classes of chemical carcinogens. Hydrocarbons such as 3,4-benzpyrene are highly carcinogenic for laboratory animals, are ingredients of smoke from various sources, such as cigarettes, and are implicated as important carcinogenic agents for man. The lack of convincing evidence for mutagenicity has led to other hypotheses[2] for the initial step in carcinogenesis at the cellular level. However, negative results for mutagenicity are equivocal unless it can be shown that the test compound is taken up by the cell. Indeed, with those organisms most suitable for mutagenicity testing, fungi and molds, it is difficult to assure oneself that the compound is even available to them, because these hydrocarbons possess negligible solubility in water. The study reported here is the beginning of a rigorous effort to test 3,4-benzpyrene incorporation by microorganisms together with tests for mutagenicity. Incorporation of the carcinogen without mutation would, in our opinion, be as interesting a find as incorporation with mutation. Should uptake occur with a suitable microorganism, all those advantages that many microorganisms possess as test material (rapid growth to give large populations, ease of manipulation, availability of auxotrophs to permit selective labeling, etc.) would aid in a study of the fate of the hydrocarbon in the cell.

Experimental. – The first solvent system used was 10% sorbitan monooleate (Tween 80) in water.

At this concentration inhibition (reduction in growth rate) for many bacterial species is slight. A stock solution of 0.7% 3,4-benzpyrene was first made up in Tween. All manipulations were either carried out in darkness or under amber lighting, as this hydrocarbon is photodynamic. The stock Tween-hydrocarbon solution was diluted 1:10 in appropriate liquid culture medium as required. The resulting 700-μg/ml benzpyrene solution was perfectly clear. No precipitation occurred, nor could any sediment be detected upon centrifugation at 5000 rpm in the Servall angle-head centrifuge. Above 800 μg/ml, however, benzpyrene precipitated from solution; thus, 700 μg/ml was the upper concentration allowable. Since solutions of Tween are prone to foam badly, aeration during growth was not carried out by the customary bubbling but by means of an air jet a few millimeters above the tubed liquid medium; good gas exchange occurred in the vortex caused by the jet of compressed air, and foaming was not serious. *Aerobacter aerogenes*[3] was the first species tested. The hydrocarbon was in no way inhibitory, and mutation was never higher in the benzpyrene than in the controls (Tween lacking benzpyrene). The mutation under measurement was auxotrophy to prototrophy and also prototrophy to auxotrophy. The latter was tested by direct replicate plating[4] and, after penicillin treatment[5] to enrich for auxotrophys, through selective destruction of wild-type prototrophs. Moreover, employing tritiated 3,4-benzpyrene, uptake could not be detected. Next, two strains of *Bacillus megatherium* were selected. It was thought that a gram-positive bacterium might respond differently. Furthermore, this species contains intracellular deposits of fat. Perhaps if a little hydrocarbon could penetrate the cell, it would accumulate to a potent concentration within the lipid globules. Again, however, the results were negative, both as regards mutagenicity and uptake. Currently, other solvent systems and other species are under test.

Summary. – Using 10% Tween 80 as the solvent, clear aqueous solutions of 3,4-benzpyrene may be acquired up to 700 μg/ml. Foaming of cultures may be minimized by using an air jet *above* the surface of the growth medium rather than the customary bubbling. Incorporation of benzpyrene in the liquid medium caused no inhibition above that slight inhibition due to the solvent, Tween 80. Tritiated benzpyrene was used for uptake measurements. *Aerobacter aerogenes* and *Bacillus megatherium* were tested. Neither mutation nor hydrocarbon uptake was observed.

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12.2 GROWTH AND THYMINE UTILIZATION BY THYMINE-DEFICIENT BACTERIA

A. P. Harrison, Jr.

Introduction. – In the previous report[1] a uv-derived thymine-deficient auxotroph (*Escherichia coli* 15$\overline{T}$) was compared with aminopterin-derived thymine-deficient auxotrophs. Thymine-less bacteria of these two classes were shown to be different and distinct. The present report further characterizes these two classes, describes additional classes of thymine-deficient bacteria, and attempts to delineate the functions involved in thymine utilization and to anticipate the number of associated genetic loci. The immediate goals are to determine the nature of the metabolic "block" in the different thymine-deficient bacteria, and to acquire test organisms for investigating control mechanisms of DNA synthesis and cell division. DNA control and cell division are, of course, central to understanding both normal and cancerous growth. Hopefully, such studies will lead to new and rational approaches in cancer therapy.

Results. – The bacterial strains and some of their characteristics are summarized in Table 12.2.1. Observe that the AM-derived auxotroph, strain 15-1, requires 25 times the amount of thymine as does the uv-derived auxotroph, strain 15$\overline{T}$. Both auxotrophs were derived from a common parent, strain 15. Likewise, AM-derived auxotrophs of *Aerobacter aerogenes* t have the same high requirement. Replicate isolations respond identically, as do AM-derived auxotrophs of *E. coli* K12. (The diphasic growth of the AM-derived auxotrophs has been illustrated already in the previous report.[1]) Although

25 μg/ml thymine is required for a yield of 10^9 viable cells/ml in synthetic liquid medium, this quantity of thymine is not required for DNA synthesis, since most of it remains in the culture filtrate. Apparently, high thymine concentrations are required by AM-derived auxotrophs for thymine uptake. Note in Fig. 12.2.1 that, at 5 μg/ml thymine, the AM-derived 15-1 has the same low uptake as wild-type 15, and that the uv-derived 15$\bar{T}$ has relatively efficient uptake. At 30 μg/ml thymine, however, uptake by 15-1 (Fig. 12.2.2) begins at the low rate characteristic of wild-type, but then abruptly increases (at the onset of the second growth phase) to the high rate characteristic of 15$\bar{T}$. Observe also in Table 12.2.1 that the AM-derived t23 may, with suitable selection, yield clones (represented

Table 12.2.1. Summary Characterization of Bacterial Strains

Species	Strain Designation	Nutrition	Parent	Mode of Isolation	Thymine Needed to Yield 10^9 Cells (μg)	Dihydrofolate Reductase[a]	Thymidylate Synthetase[b]
Aerobacter aerogenes	t	Prototroph (wild-type)			None	+	+
	t23	Auxotroph	t	Aminopterin	25[c]	+	–
	t23B	Auxotroph	t23	Concentration-gradient agar	1	+	–
	t23A	Auxotroph	t23	Concentration-gradient agar	1[d]	+	–
	t23-1	Prototroph (revertant)	t23	Heavy seeding of thymine-less agar	None	n.d.	n.d.
	t23B-7	Prototroph (revertant)	t23B	Heavy seeding of thymine-less agar	None	n.d.	–(?)
Escherichia coli	15[e]	Prototroph (wild-type)			None	+	+
	15-1	Auxotroph	15	Aminopterin	25[c]	+	–
	15-1-1	Prototroph (revertant)	15-1	Heavy seeding of thymine-less agar	None	n.d.	n.d.
	15$\bar{T}$[e]	Auxotroph	15	Fortuitous uv isolation[f]	1	+	–[g]
	15$\bar{T}$-3	Prototroph[h] (revertant)	15$\bar{T}$	Heavy seeding of thymine-less agar	None	n.d.	+ (weak)

[a]Assayed by the method of Misra *et al.*, *Nature* **189**, 39 (1961). Extracts prepared from 10^{11} bacteria with the French pressure cell.

[b]Assayed by the spectrophotometric method described by M. Friedkin in *Methods in Enzymology*, VI, S. P. Colowick (ed.), Academic, New York, 1955. Extracts prepared as indicated above.

[c]Most of the thymine remains unutilized.

[d]Thymine-less death delayed for 3 to 4 hr. An unstable strain (see text).

[e]Acquired from Seymour Cohen.

[f]By Joseph Gots.

[g]First observed by Seymour Cohen.

[h]Unusual growth (see text).

n.d. – Not determined.

by strain t23B) similar to the uv-derived 15$\bar{T}$. Not only is strain t23B able to utilize low thymine concentrations, but it grows like 15$\bar{T}$, lacking the diphasic growth characteristic of AM-derived auxotrophs. The selection technique was as follows. Onto synthetic agar were spread approximately 10^9 cells of t23, then in the middle of the plate there was twisted out a depression in the agar by means of the sharp, sterilized end of a cork borer, and into this was pipetted 0.1 ml water containing 100 μg thymine. The plate was incubated upright for two days. Confluent growth of course surrounded the depression, but at the periphery of this growth, and beyond, a few discrete colonies developed. These were found to be three kinds of variants: revertants to prototrophy (very few), low thymine requirers like t23B, and unstable auxotrophs such as t23A (Table 12.2.1). The latter organism is unusual in that it does not undergo thymine-less death until after 3 or 4 hr when placed in synthetic medium lacking thymine. (With the other auxotrophs thymine-less death ensues within approximately $\frac{1}{2}$ hr.) When a culture of t23A in synthetic medium containing thymine is plated out on complete medium and on synthetic medium lacking thymine, colonies appear on the latter plates at a frequency 10^{-5} that of the former. And *all* the colonies which grow out on the complete medium may be cultivated on synthetic agar lacking thymine. In other words, as long as the organism is held in synthetic medium with thymine most of the cells respond as auxotrophs, but after an exposure

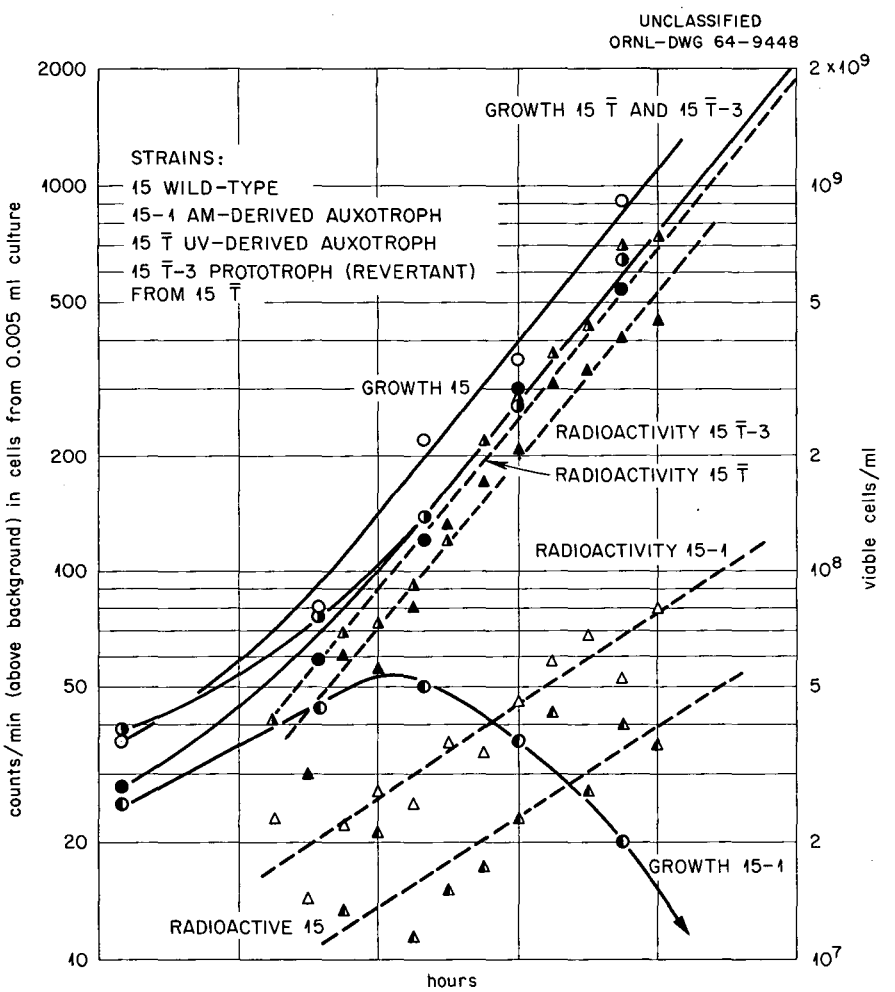

Fig. 12.2.1. Growth and Thymine Uptake by *Escherichia coli*. Medium contains 5 μg (0.74 μc) thymine per ml.

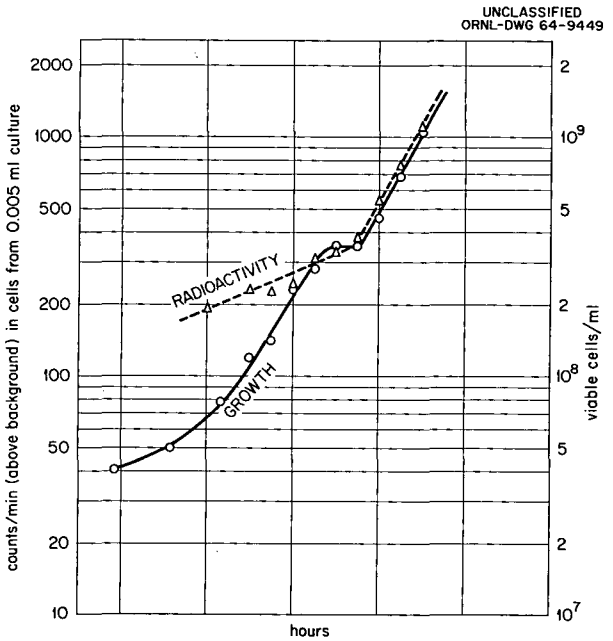

Fig. 12.2.2. Growth and Thymine Uptake by *Escherichia coli* **15-1.** Medium contains 30 μg (4.4 μc) thymine per ml.

to complete medium (yeast extract, peptone, and thymine) all the resulting clones become able to grow as prototrophs.

Two pairs of thymine-deficient bacteria will now be considered. The first pair comprises the two similar strains, 15-1 and t23, both derived by the AM technique. The second pair comprise the two similar strains, 15$\overline{\text{T}}$ and t23B, the former uv-derived and the latter isolated by means of the concentration gradient plating from t23. Consider that the first pair differ from the respective wild-types only in their lack of thymidylate synthetase, whereas the second pair not only differ in this regard but, in addition, differ in their efficient thymine uptake. From this, the following question arose: Would it be possible to revert one of the latter traits but not the other? Could one acquire strains possessing thymidylate synthetase but, unlike wild-type, possessing efficient thymine uptake? With some reservations, the answer is yes. The strains were seeded heavily onto synthetic agar lacking thymine. The very few colonies which developed were ascertained pure by reisolation of colonies from restreaked growth on thymine-less agar. Stocks were held on synthetic thymine-less agar. A collection of six revertants to thymine independence were obtained from each of the four strains. Each new isolate was tested for growth parameters and thymine uptake.[2] All six revertants from 15-1 and all six from t23 possessed normal thymidylate synthetase activity and the low thymine uptake characteristic of wild-type. Revertants of this kind are represented by 15-1-1 and t23-1 in Table 12.2.1. On the other hand, two revertants from 15$\overline{\text{T}}$ and five from t23B, although growing prototrophically, possessed efficient thymine uptake. Representatives of this kind are 15$\overline{\text{T}}$-3 and t23B-7 in Table 12.2.1. The thymine uptake is not spurious, but is used in DNA synthesis. Strain t23B-7 was cultivated in tritiated thymine. The DNA was extracted, hydrolyzed, and chromatographed using isobutyric-isobutyrate. Radioactivity occurred only in the thymidylate. Although t23B-7 can be carried, apparently, indefinitely in synthetic medium lacking thymine, the viable cell yield is only one-tenth that expected at a given culture mass. Moreover, thymidylate synthetase[3] activity must be very low, since it has not been detected. With 15$\overline{\text{T}}$-3 the picture is different. Thymidylate synthetase is produced (whether the cells are grown in presence or absence of thymine), but the activity is less than one-third that of wild-type. Thymine uptake is identical to the parent, 15$\overline{\text{T}}$, thus much higher than wild-type (Fig. 12.2.1). Growth is normal (like 15$\overline{\text{T}}$) in thymine, but in the absence of added thymine is unusual (Fig. 12.2.3). The culture increases in microscopic and plate count about twofold, then the plate count diminishes to the original titer – this first growth phase requires 3 to 4 hr – but then abruptly the elongated cells of which the culture is now exclusively composed appear to fragment; the resulting normal-sized cells increase so that the filaments become successively less prevelant. The slope of the growth curve is steeper than that of any other strain of *E. coli* 15 encountered. This is so, probably because new cells arise from two sources: division of normal cells (at the usual rate) and fragmentation of large cells. The culture is pure; reinoculation starts off the same series of events in a predictable fashion. Strain 15$\overline{\text{T}}$-3 warrants additional study.

Discussion and Summary. – The phenotypic classes of thymine-deficient bacteria under investigation and their interrelationships have led to surmises as to the associated genetic loci. Certainly there is a gene controlling thymidylate

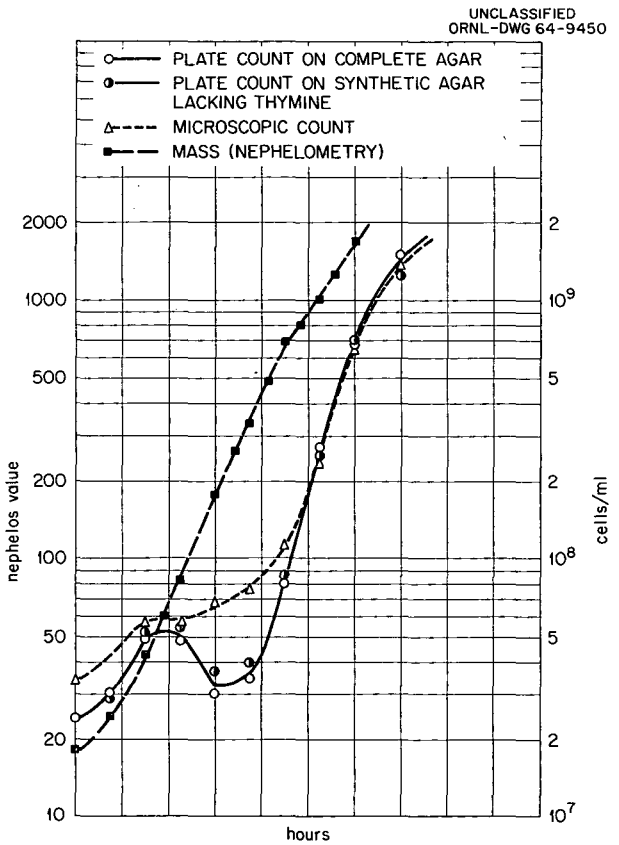

Fig. 12.2.3. Growth of Strain 15$\overline{T}$-3 in Synthetic Medium Lacking Thymine.

synthetase formation. There is at least one gene controlling thymine uptake. Recall the conversion of the inefficient thymine uptaker, t23, to an efficient uptaker, t23B, by a method that one would suppose selects spontaneous mutants. Further, it is likely that at least one more gene is involved, since the similar t23B and 15$\overline{T}$ yield revertants very dissimilar. Obviously, from the demonstrated and proposed steps in thymine utilization by various cells, one can anticipate a certain minimum number of controlling genes. What is referred to in this report are those functions which are represented by available mutants. A genetic study has begun in collaboration with Roy Curtis, where the various thymine markers described herein are to be inserted into suitable polyauxotrophs of *E. coli* K12 recipient bacteria. Also, currently under way are assays for nucleoside transdeoxyribosidase and thymidine hydrolyase in the various classes of thymine-less bacteria and during the different phases of their growth. It is possible that absence or inducibility of one of these enzymes may account for certain anomalies such as, for example, the diphasic growth of the AM-derived auxotrophs. If so, then thymine uptake may become expressible in definite biochemical terms.

References

[1]A. P. Harrison, Jr., and L. E. Franklin, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964*, ORNL-3601, pp. 146–48.

[2]The author is grateful to R. B. Setlow and his group for advice in sampling and fixation of bacterial cells for gas-flow counting.

[3]The tetrahydrofolic acid used in the thymidylate synthetase assays was kindly prepared for the author by D. G. Doherty.

12.3 INHALATION CO-CARCINOGENESIS

D. G. Doherty R. F. Newell[1]
M. G. Hanna, Jr.

Epidemiological surveys have shown a worldwide increase in both the frequency and severity of lung cancer that, in part, seems to be related to environmental conditions. Such factors as virus infection, atmospheric pollution by aerosolized chemicals, and possibly radiation have been implicated. These observations indicate the need for studies aimed at establishing the significance of these factors and elucidating the biological and biochemical changes that finally give rise to lung cancer. The initial objective of the joint AEC-NIH inhalation co-carcinogenesis program is an examination of the role these factors play in the experimental induction of pulmonary tumors in mice. Correlated with this will be a study of the effects of the virus and chemicals on various systems in the animals.

The experimental design is to subject 6-week-old C57BL/6 mice to an initial insult of either sublethal radiation or an influenza virus, or both, and upon recovery expose the animals to an aerosolized gasoline smog or chromic oxide dust in inhalation chambers for daily periods throughout the life-span of the animals. At appropriate intervals animals will be sacrificed and examined for lung tumor induction and progress. The results obtained will

be compared with suitable radiation and virus controls that have been exposed only to clean filtered air. Success of this protocol is dependent upon good control at all levels of the chemical, viral, and biological conditions. Our initial efforts have been directed toward this objective.

A system of instrumentation and control of the chemicals in the aerosol chambers has been developed and is being installed, and methods of sampling and analysis are under study. Specific pathogen-free (SPF) C57BL/6 mice have been obtained, and their bacteriologic and parasitologic profiles determined. The SPF animals, on delivery, were free of *Pseudomonas sp.* and *Salmonella sp.* as well as internal, external, and fecal protozoan parasites. It is planned to try to keep the animals in this condition by a thorough animal health and sanitation program. In addition, a viral profile of the animals will be determined, and these three profiles monitored throughout the chamber program.

The radiation LD50/30 days for x rays of 6-week-old mice was found to be: for males, 654 r (95% limits 621 to 713 r); females, 647 r (95% limits 620 to 688 r); and combined, 650 r (95% limits 631 to 677 r). The parasitologic and bacteriologic tests made on moribund animals and 30-day postirradiation survivors were negative except for two animals that had a nonpigmented *Pseudomonas* infection.

In order to record and correlate all the information that will be obtained in the chamber project, a system of IBM data recording for all aspects is under development.

The program for the production of virus is in abeyance pending completion of construction, as is the shakedown of the operating equipment for the chambers.

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[1]National Institutes of Health.

13. Cell Physiology

N. G. Anderson

Subcellular Physiology (13.1–13.6)

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Mary L. Barber[b]
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S. R. Tipton[b]
P. A. Van Dreal[c]
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[a]Sabbatical leave from the University of California.
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[c]USPHS Fellow.
[d]Visiting investigator from abroad.
[e]Student trainee.

Cell Biophysics (13.7–13.9)

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J. G. Green
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Molecular Physiology (13.10)

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Tom Grizzard
G. R. Randall[e]

Cooperative AEC-NIH Development of an Ultracentrifuge (13.1, 13.2, 13.11)

13.1 VIRAL ANTIGENS IN LEUKEMIC TISSUE

N. G. Anderson

Oncogenic viruses in some instances are not recoverable from the tumors which they initiate. At least part of the viral genome appears to persist since serological evidence for viral protein can be demonstrated. The questions arise: Are these proteins physically as well as serologically identical to the viral protein, is it more likely that the protein can be isolated from the tissue (which is available in quantity) than from the virus (which is generally available in trace amounts), and can immunity to viral infection be produced by injection of isolated viral protein? Previous studies[1] demonstrate that formalinized spleen from Rauscher-virus-infected mouse spleen can be used to effectively immunize mice against the leukemogenic effects of virus challenge and that fluorescent antibodies to the virus stain leukemic spleen cells.[2]

Experimental. – Antisera produced in rhesus monkeys by injection of purified Rauscher virus (gift of Dr. F. J. Rauscher) was absorbed with BALB/c mouse liver powder and coupled with fluorescein isothiocyanate. On Ochterloney plates the antiserum gave a single band when tested against Rauscher leukemic mouse spleen supernatant (RLMSS). Ammonium sulfate precipitation curves for RLMSS and the precipitation range for the antigen were determined. Chromatography of the RLMSS on diethylaminoethyl cellulose columns using a chromatographic method[3] developed specifically for tissue proteins gave a series of fractions as shown in Fig. 13.1.1. When these were concentrated and tested, antigenic activity was found to be associated with the peak indicated.

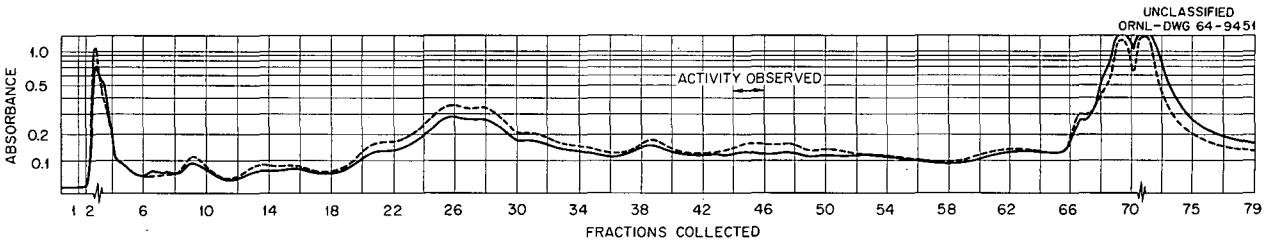

Fig. 13.1.1. DEAE Chromatography of Rauscher Leukemic Mouse Spleen Supernatant.

The antigen is now being tested for immunizing potency in mice. Larger-scale isolation techniques are under investigation to allow the kilogram quantities of mouse spleen currently available to be fractionated. Parallel investigations with human leukemic spleen are also being carried out.

Discussion. – Crude vaccines have been shown by other investigators to be effective in preventing tumor development in response to injection of several oncogenic viruses. If this approach is to be used in man, it is important that the antigen (1) contain no live virus and (2) contain only the antigenic molecular species. Since virus infection often results in the production of excess viral antigen in the infected cell, it appears of interest in such instances to develop methods for isolating viral antigens from cells, rather than attempting to first isolate the virus and then to isolate its subunits.

The definitive identification of the protein isolated here will depend on (1) demonstration of inhibition of immunofluorescence by Rauscher virus treated with appropriate labeled antisera, (2) production of potent antisera as tested by immunofluorescence by using the protein as an antigen, and (3) the results of immunization studies currently being carried out.

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[2]F. J. Rauscher, personal communication.

[3]H. E. Bond, personal communication.

13.2 VIRUS ISOLATION STUDIES USING THE B-V AND B-VIII CONTINUOUS-FLOW ZONAL CENTRIFUGE SYSTEM

G. B. Cline

Introduction. – A basic problem in the determination of biophysical and biochemical parameters of subcellular particles and viruses is purity. Recent advances in rate zonal and density gradient centrifugation with zonal ultracentrifuges indicate that methods are now available for the isolation of these particles in an uncontaminated form. One limitation is the volume of starting sample of the B-IV zonal rotor which is about 50 ml. This becomes limiting when viruses are to be concentrated from large volumes of tissue fluid.

The problem of virus concentration has now been solved with the fabrication of the B-V and B-VIII continuous-flow rotors. The B-V rotor merely collects virus particles on the rotor wall, while the B-VIII rotor traps the particles in a density gradient and bands them isopycnically as the culture fluid is pumped through the rotor. While the material pelleted on the wall of the B-V must be removed after the rotor is decelerated and disassembled, the virus banded in a density zone in the gradient of the B-VIII can be collected by displacing the loaded density gradient from the rotor. The following summary presents experimental data showing that these rotor systems work well for the isolation of viruses such as T-3 phage and live polio virus from large volumes of solution. The data are being utilized to select optimal conditions for the isolation of large quantities of pure Respiratory Syncytial, Adeno-3, and Echo-28 viruses for vaccine production.

Experimental. – Three viruses, Adeno-2, T-3, and polio type IV, have been used to test the cleanout efficiency of the B-V rotor. Adeno-2 cleanout was determined by both electron microscopy and bioassay. A total of 13.7 liters of culture fluid was pumped through the rotor at various rates. The bioassay results do not give sufficient information to plot an accurate curve of percent cleanout vs flow rate. However, the rotor does retain better than 99.9% of Adeno-2 with a flow rate of 2 liters/hr.

More precise determinations of cleanout of virus particles from large volumes of culture fluid have been done using T-3 bacteriophage. The degree of cleanout has been determined at several flow rates and agrees closely with theoretical predictions. Better than 95% cleanout can be obtained at 2.6 liters/hr at a rotor speed of 20,000 rpm. Theoretical calculations predict 95% cleanout at 2.4 liters/hr at this speed.

Live polio virus, type III, has also been used as a test particle under a joint research (contract) program with Eli Lilly and Company, Indianapolis, Indiana. Cleanout of live virus was determined at several flow rates in a B-V rotor at a speed of 35,000 rpm. Bioassay of effluent collections indicate better than 97% cleanout at 3 liters/hr. This is near the theoretical cleanout of 95% cleanout at 3 liters/hr. Fifteen liters were concentrated by the rotor to 150 cc. Further purification of the 100 X virus and cellular debris from this 150-cc fraction was done by rate separation in the B-IV rotor.

The B-VIII continuous-flow rotor utilizes the new principle of flowing a solution containing virus particles over the surface of a density gradient in a centrifugal field. Limited studies have been performed using T-3 bacteriophage as a test particle. Preliminary data show that the virus particle is trapped in the rotor and banded isopycnically in a zone in the density gradient. A crude comparison of rotor cleanout efficiency of the B-V and B-VIII rotors for T-3 is shown in Table 13.2.1.

Top speed with the B-V and B-VIII rotors is 40,000 rpm. Cleanout of any virus at this speed would thus be more complete than the degree of cleanout determined at 20,000 and 35,000 rpm. This is due to the higher centrifugal forces developed at 40,000 rpm. Present studies involve finding ideal conditions for the isolation of the fragile Respiratory Syncytial virus in the B-VIII continuous-flow rotor.

Table 13.2.1. Rotor Cleanout Efficiencies of B-V and B-VIII Rotors for T-3 Phage

Rotor	Rotor Speed (rpm)	Effluent Rate (liters/hr)	T-3 Retained by Rotor (%)
B-V	20,000	5	65
B-V	20,000	3	85
B-VIII	35,000	5	80
B-VIII	35,000	3	99.5

13.3. CAPILLARY COLUMN CHROMATOGRAPHY: PRELIMINARY STUDIES ON NUCLEOTIDE SEPARATIONS

J. G. Green C. E. Nunley
N. G. Anderson

Introduction. – Decreasing bead size of an ion exchange resin improves column resolution markedly, permitting reduction of the column length and elution time in a system. However, the pressure required to maintain a desired flow rate is increased, and the increase is only partially compensated by the reduction in column length. Decreasing the bore of a column increases the resolving capability and necessitates a reduction of flow rate of the eluting buffer and reduces the volume in which the eluted zones appear. An experimental system, discussed here, has been devised to evaluate the performance of capillary columns and the behavior of fine resins at high pressures.

Experimental. – Since pressures exceeding the capabilities of glass columns were expected, the column, shown in Fig. 13.3.1, was fabricated from 316 stainless steel. The column, a steel tube $\frac{1}{8}$ in. ID by $\frac{3}{8}$ in. OD by 30 in. long, supports the resin on a sintered stainless steel disk of 5 μ porosity and is supported and jacketed through the use of heat exchanger fittings. Ready access to

the top of the column without disconnecting the line from the pump is provided, and the eluant is taken from the bottom of the column assembly through stainless hypodermic tubing and AWG No. 22 Teflon tubing. The resin is introduced into the column as a 30% slurry and packed hydraulically in increments. Samples in a volume of 100 μl or less are absorbed on a small cotton pledget which is tamped in the column on the surface of the resin and appear to transfer readily to the resin at the initiation of elution.

The eluting buffer is delivered to the column by a Whitney laboratory feed pump with a rated capacity of 0 to 9 ml/min and an operating pressure of 3500 psi, although delivery rates are reduced at higher pressures. In the preliminary studies, the pump has been adjusted to deliver 0.19 ml/min at 3200 psi. The spectrophotometric monitoring system has been described previously.[1]

Resins with a bead diameter of 5 μ or less are not readily obtainable; therefore, the anion exchange resin employed in this study was hydraulically fractionated from a specially formulated −600-mesh lot obtained from a commercial supplier. Colloidal particles were removed by multiple settling, and the residual particles (mean diameter, 6 μ) were placed on a filter paper disk supported on a sintered glass plate in a large-bore glass column which could be pressurized to encourage liquid flow through the settled resin. The resin in chloride form was washed successively with distilled water, anhydrous methanol, 6 *N* sulfuric

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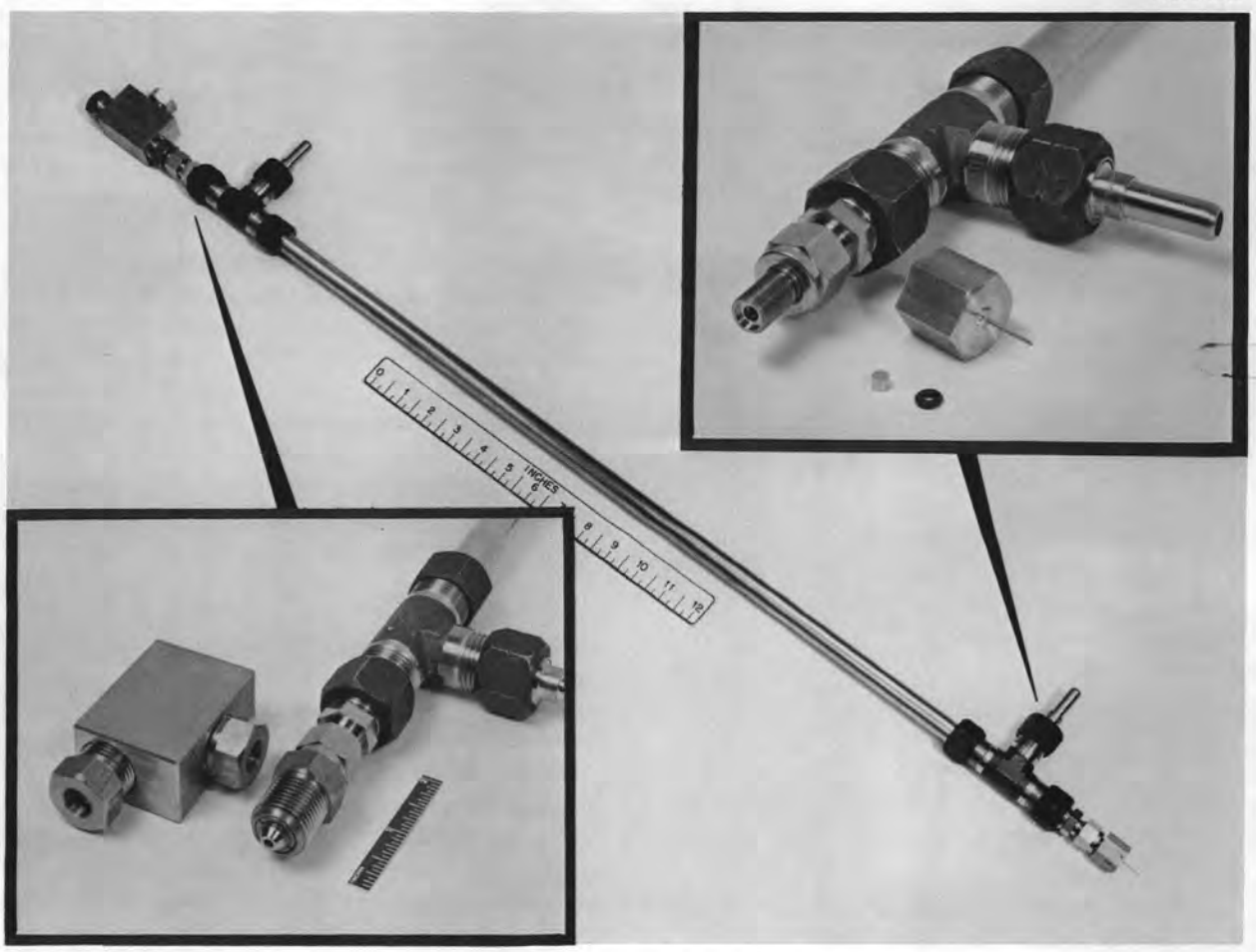

Fig. 13.3.1. Photograph of Assembled and Disassembled Column Used for High-Pressure Elution Studies.

acid, 1 *N* hydrochloric acid, 1 *N* sodium hydroxide, and 3 *M* sodium acetate–acetic acid buffer at pH 4.4. Between reagent washes, the resin was rinsed with large amounts of distilled water.

Initial studies have been conducted with simulated RNA hydrolysate containing the commonly occurring 2′ and 3′ nucleotide monophosphates at a concentration of 10 m*M*; a typical sample contains one micromole of each nucleotide in 100 μl of buffer. Best results have been obtained with acetate gradients progressing from 1.4 to 2.8 *M* at pH 4.6 to a total volume of 64 ml. Before entry into the system the buffer is freed of excess dissolved gas by prewarming in a jacketed gradient cylinder to the column temperature of 45°C.

Results and Discussion. – A typical chromatogram obtained with the experimental system is shown in Fig. 13.3.2. Separation of the nucleotides is acceptable and may be improved with further study. A sample is eluted in 6 hr, rather than 13 hr required in system described previously,[2] and an eluted band appears in 3 to 8 ml of buffer rather than 48 to 100 ml required for the older system.

Preliminary investigation indicates that chromatography with very fine resins, even colloidal forms, appears to be practical and offers advantages in miniaturization, volume and sample reductions, time saving, and resolution over previously adopted systems. Further exploration of these advantages may require systems with capabilities to 10,000 psi and refined monitoring procedures.

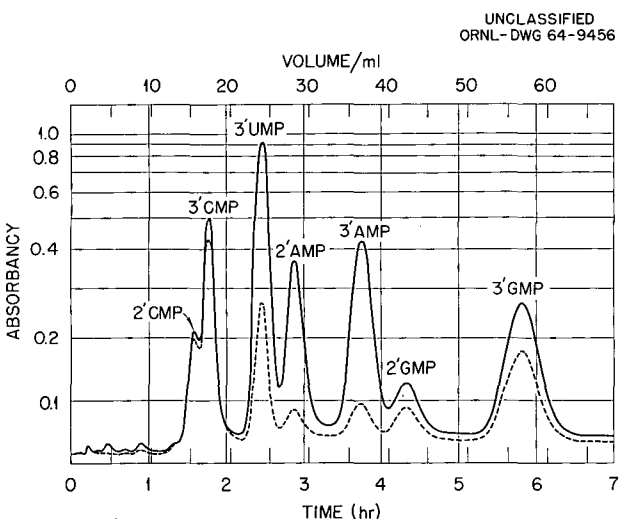

Fig. 13.3.2. Typical Chromatogram Obtained with the Experimental Capillary Column.

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13.4 MORPHOGENESIS, RNA SYNTHESIS, AND ACTINOMYCIN D INHIBITION IN SYNCHRONIZED *TETRAHYMENA PYRIFORMIS*

G. L. Whitson G. M. Padilla
L. H. Elrod

An entrained system of synchronized cell division in *Tetrahymena pyriformis* using a repetitive 12-hr temperature cycle (9.5 hr at 12°C and 2.5 hr at 27°C) has been reported.[1] There is evidence indicating the existence of an actinomycin-D-sensitive period in the synchronized cycle.[2] When added 1 hr before the beginning of the warm period, actinomycin D (10 μg/ml) inhibits division in about 90% of the cells. No forming oral structures are evident in any of the actinomycin-D-treated cells at a time when developing oral anlagen are present in 85% of the control cells. Pulse labeling with ^{3}H-cytidine shows a marked inhibition of nuclear and cytoplasmic incorporation of the ribonucleoside in the actinomycin-D-treated cells. In addition, studies on the kinetics of uptake of ^{14}C-uridine into cold TCA-precipitable material show a marked inhibition in the rate of incorporation by cells treated with actinomycin D. It appears from all these findings that RNA synthesis during the latter portion of the synchronized cycle is necessary for the formation of oral structures and the completion of cell division. Recent findings showed no major differences in total RNA when comparisons were made between changes in total RNA content of control-synchronized and actinomycin-D-treated cells. This evidence suggests that a special kind of RNA (perhaps messenger RNA), which is involved in the formation of oral structures and perhaps cell division, is

being synthesized during this portion of the synchronized cycle. Furthermore, this particular species of RNA contributes very little to the overall total RNA content of the cells.

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13.5 STUDIES ON SYNCHRONIZED *ASTASIA LONGA*

P. A. Van Dreal

Astasia longa has been synchronized on a new cycle using continuous bubbling of 12% CO_2 in air within the medium. In the new cycle, the cells are grown for 18 hr at 12°C and 6 hr at 28.5°C. Under these conditions, the cells begin to divide in 2.5 hr and are finished by 5.5 hr after the onset of the warm period.

This cycle has been used to study the changes in ribosomes as a function of the cell cycle. In general, a single peak is found just after the synchronized burst and during the early hours of the warm period. During the late hours of the cold period, two more rapidly sedimenting peaks appear. The single peak decreases, and the two more rapidly sedimenting peaks increase in size. At the same time, there is a relative increase in amount of membrane fraction. Pulse labeling with ^{32}P shows uptake in the soluble and membrane fractions but not in the other peaks. O. L. Miller is studying these fractions further with the electron microscope to determine their composition.

13.6 CHANGES IN SOLUBLE NUCLEOTIDE POOL OF *VICIA FABA* TIPS EXPOSED TO 5-AMINOURACIL

P. A. Van Dreal J. E. Trosko
J. G. Brewen

A number of chemical synchronization methods have been used with meristematic tissue in recent years. One of the more common of these is 5-aminouracil (5-AU). The mode of action is not directly known, but it is presumed to block uracil incorporation into RNA and DNA. In order to more specifically determine its major effect, we examined the TCA-soluble nucleotide pools of *Vicia faba* root tips, during and after exposure to 5-AU. The nucleotides were separated on an ion exchange column using Dowex 1-X8, as previously described by Burger *et al.* (*Joint NIH-AEC Zonal Centrifuge Develop. Program Semiann. Rept. Jan. 1–June 30, 1963,* ORNL-3502). An acetate gradient (pH 4.4) ranging from 0.15 to 3 *M* is used to elute the nucleotides. Under these conditions we find a peak that gradually disappears and finally is all gone by 24-hr incubation in 5-AU. We have tentatively identified this peak as thymine. Further studies are planned to positively identify this peak.

13.7 WATER IN BACTERIOPHAGE T4B

S. P. Leibo Peter Mazur

Using the data from a variety of sources, it was possible to calculate that 7.7×10^7 A^3 of the internal head volume of bacteriophage could be occupied by H_2O. With the values and the model proposed by Kilkson and Maestre,[1] the "unoccupied" internal head volume of phage was found to be 5.2×10^7 A^3. There are additional, more direct data relating to the hydration of the bacteriophage particle.[2] Nevertheless, the nature and state of the internal water of phage in relation to the organization of DNA remains obscure.

Assuming the presence of internal water in phage, two experimental approaches have been used to study the activity of this water. First, the osmotic sensitivity of phage T4B was studied. Although the results were markedly affected by the conditions of the "osmotic shock," survival remained high (greater than 60%) when the phage were diluted rapidly following suspension in concentrations of NaCl less than 1.5 *m*. Above that critical concentration, survival dropped sharply after rapid dilution. For example, dilution of a suspension in 3.0 *m* NaCl lowered survival to 0.1%. The osmotic sensitivity of phage was strongly dependent upon the total time required for dilution. Survival increased approximately exponentially from 0.06% to 2% as the time for

dilution from 3 *m* NaCl was extended from 0.6 sec to 2.0 sec.

The second approach has been to study the effect on the infectivity of phage of a variety of freeze-thaw regimes. The results of the first experiments are shown in Fig. 13.7.1.

It is tentatively suggested that at least three factors are operating in the inactivation of phage T4B by freezing and thawing: (1) The phase change from the liquid to the frozen state is sufficient to destroy about one-half of the infectivity of the phage population. (2) There appears to be an equivalence between rapid warming from the frozen state and rapid dilution of phage suspended in a concentrated salt solution (i.e., "osmotic shock"). (3) An event occurs during rapid cooling which sensitizes the phage particle for subsequent inactivation during slow warming by some phenomenon such as recrystallization. It is possible that this sequence in phage resembles that which occurs in cells (e.g. yeast) following similar treatment. Work is in progress to test these three proposals experimentally.

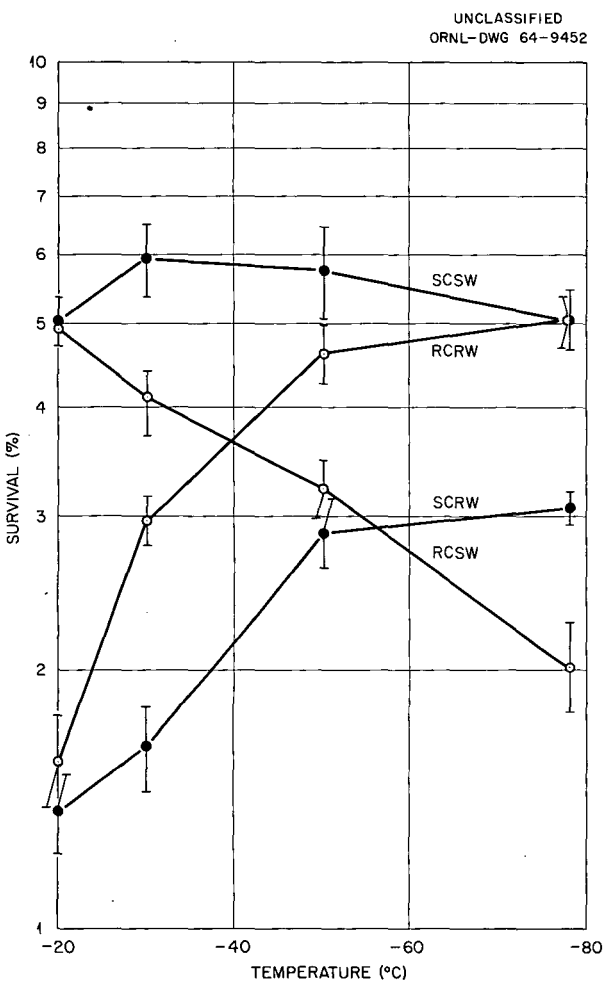

Fig. 13.7.1. Percent Survival of Phage T4B Following Cooling to and Rewarming from Various Subzero Temperatures by Each of the Indicated Treatments. SCSW – slow cooling, slow warming; RCRW – rapid cooling, rapid warming; SCRW – slow cooling, rapid warming; RCSW – rapid cooling, slow warming. Each point represents mean ± standard error of six or nine samples. Controls were held at 25° C.

References

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[2]N. W. Taylor, H. T. Epstein, and M. A. Lauffer, *J. Am. Chem. Soc.* **77**, 1270–73 (1955).

13.8 ROLE OF FREEZING IN THE FORMATION OF THYMINE DIMERS BY ULTRAVIOLET IRRADIATION

Walter Füchtbauer Peter Mazur

Introduction. – In aqueous solutions of thymine, ultraviolet irradiation around 260 mμ forms dimers of thymine only if the solution is frozen. Since the reasons for this fact are not certain, it was decided to follow the thymine dimerization in frozen solutions under accurately controlled conditions.[1]

Methods. – The following setup was used. The thymine solution was introduced into a quartz cell with a 1-mm light path. Rates of freezing ranging from 6 to 1200°C/min were achieved by dipping the cell into baths at temperatures ranging from −3 to −196°C. The temperature of the solution in the cell was measured with a thermocouple. The cell was then inserted into a quartz Dewar vessel. Alcohol flowing around the cell in the Dewar was held at the temperature desired during irradiation. The Dewar has parallel quartz windows which allow ultraviolet light to penetrate the cell. The light source was a GE germicidal lamp. The intensity of the emerging light was

measured by a photovoltaic cell[2] provided with an interference filter for 2537-A light. The photocell was connected to a dc microvolt amplifier which measured the voltage drop and, hence, the current across a variable resistance connected in parallel with the amplifier. The value of the resistance was chosen so as to maintain proportionality between current and light intensity.[3] In this way, the intensity of the transmitted uv light was measured directly by the amplifier, the output of which was connected to a 10-mv recorder.

Results and Discussion. – The light transmission at 2537 A of thymine solutions containing more than 70 mg of thymine per liter is increased by slowly freezing them, whereas the transmission of water decreases when frozen the same way. If a slowly frozen thymine solution is irradiated for successive intervals, and thawed after each interval, the dimerization can be detected as a gradual increase of the light transmission of the thawed solutions. In contrast, the transmission of the frozen solutions remains nearly constant and considerably higher than that of the liquid solution before irradiation (Fig. 13.8.1). Likewise, if solutions containing different amounts of thymine are frozen slowly, their light transmission is constant up to about 200 mg of thymine per liter and decreases only slightly at concentrations higher than 200 mg/liter. These findings are consistent with the view that the thymine is aggregated in large clumps by slow freezing, so that there are areas through which the irradiation can traverse without encountering thymine and, hence, without being absorbed. The successive thawings allowed the clumps to dissolve and rearrange upon refreezing, so that more thymine molecules could be reached by the light; therefore, the final yield of dimers is higher in the case of irradiation interrupted by thawing than in the case of irradiation of material continuously frozen (Fig. 13.8.1). The lack of an influence of a wide range of thymine concentrations on the light absorption is probably due to the fact that the increased concentration enlarges only the size, not the number, of aggregates. The number of the aggregates is probably determined by the number of ice crystals, which in turn is dependent primarily on the rate of freezing and not on the concentration of thymine. Increasing the freezing rate makes the frozen thymine solutions increasingly opaque. This appears to be largely ascribable to the fact that the ice becomes less

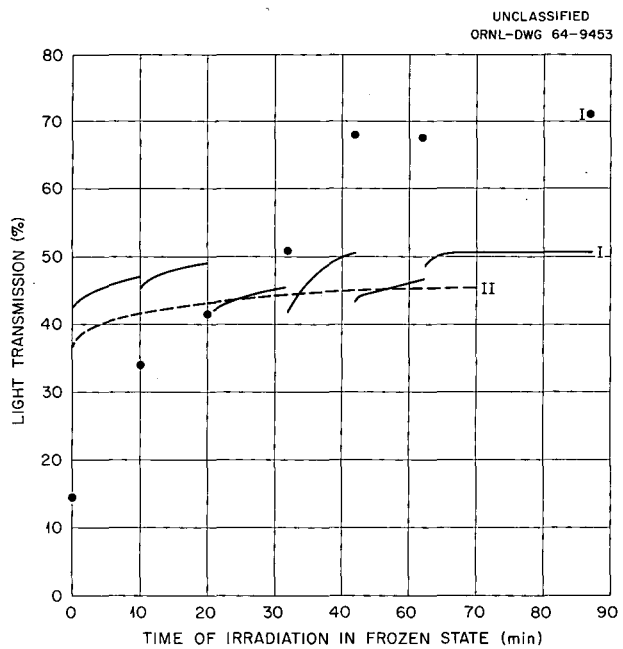

Fig. 13.8.1. Ultraviolet Transmission of Ultraviolet-Irradiated Thymine Solutions. Solution I was frozen and thawed at 10- to 25-min intervals and irradiated only while frozen. The solid lines show the ultraviolet transmission of the frozen sample; the solid points show the transmission of the same solution when thawed. Solution II was kept frozen and irradiated continuously. The broken line shows its ultraviolet transmission. Initial concentration of both solutions, 150 mg of thymine per liter. Final concentration of solution I, 25 mg/liter (i.e., 83% dimerization); of solution II, 80 mg/liter (i.e., 47% dimerization).

transparent to uv when frozen more rapidly; thus, the transmission of pure ice frozen at −3, −5, −10, −30, −110, and −196° and irradiated at −10° is 77, 75, 70, 52, 30, and 20% of the transmission of liquid water. This decreasing transmission is due to increasing scatter. Experiments are in progress to see how the freezing velocity and the temperature during the irradiation influence the rate and extent of thymine dimerization.

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13.9 CARBOHYDRATE ANALYZER: BOUNDARY LAYER DIFFUSOR AND DETERMINATION OF REAGENT FLOW RATES

J. G. Green N. G. Anderson

Introduction. – The concept of the boundary layer diffusor (BLD) was introduced in a previous report,[1] and the operational form of the diffusor is discussed in this report. In addition, accurate determinations of reagent flow rates have been obtained and are reported here.

Experimental. – The BLD, shown in Fig. 13.9.1, consists of a column of glass beads 3 mm in diameter supported in glass tubing which is 0.6 cm in diameter and 67 cm long. The effluent from the column and the chromatographic-grade liquefied phenol enter at the top of the column and mix during vertical passage down the upper portion of the device. Concentrated sulfuric acid is added to the stream at the 22-cm length, and the mixed stream passes an additional 40 cm down the column and is pooled in the base of the diffusor. The 22-cm length allows ample mixing of the phenol and column effluent, and the 40-cm length, coupled with thermal agitation resulting from the addition of the acid, permits thorough mixing and degassing of the reagent stream.

To prevent bubble formation in the process line, gases liberated during passage of the stream

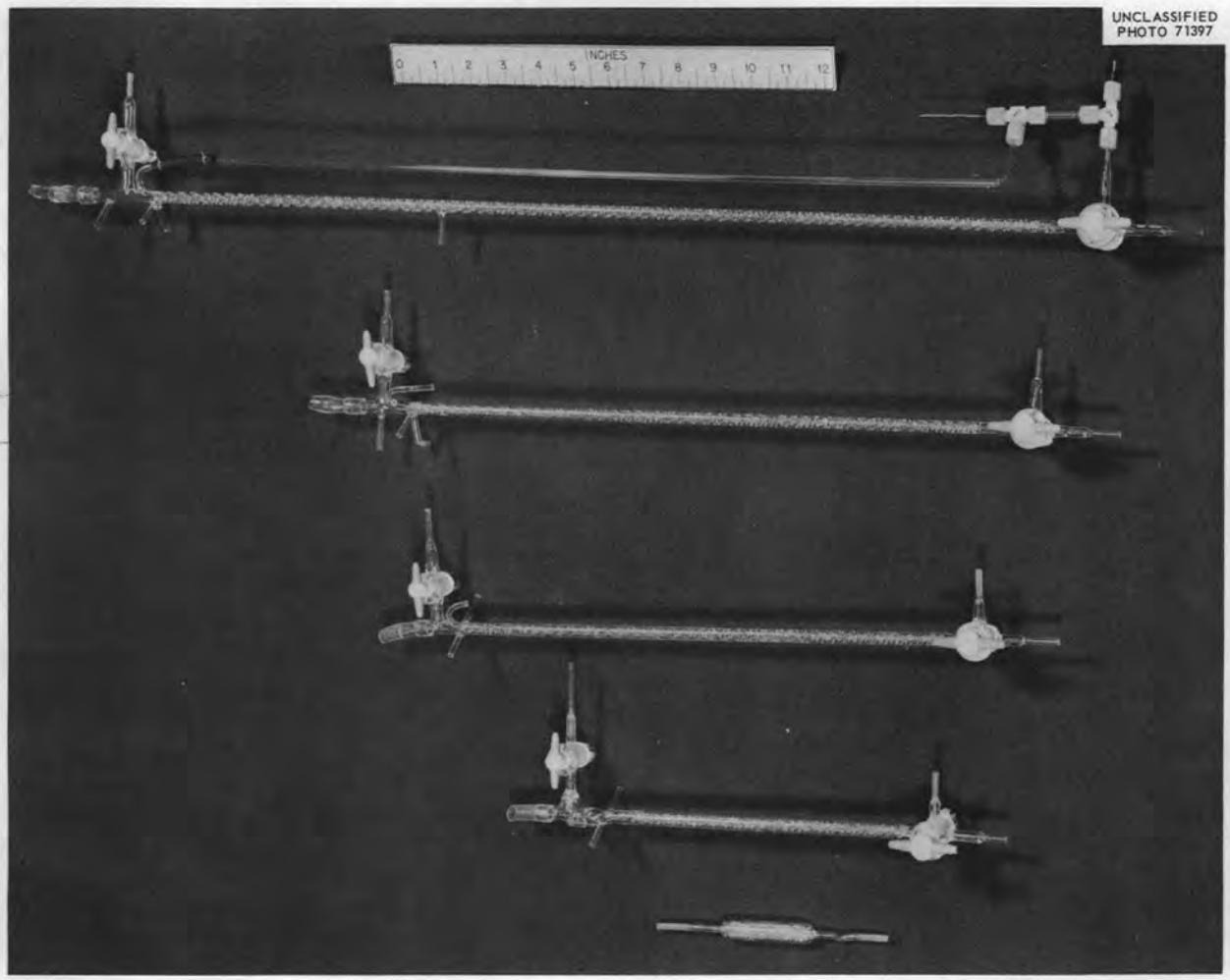

Fig. 13.9.1. Photograph of Evolution and Final Form of the Boundary Layer Diffusor. An intermediate form with manually controlled bleed system is not shown.

through the BLD must be vented from the system. The level of the pool in the base of the diffusor is sensed by a thyratron-controlled, normally open relay system through platinum electrodes introduced into a capillary sidearm system. Opening of the relay circuit by depression of the pool level by accumulated gas opens a solenoid pinch valve, and the gas is bled slowly from the system through a needle valve. When the relay circuit is energized by elevation of the pool level to the upper electrode, the pinch valve is closed and gas release ceases.

Precise determinations of the flow rates of reagents have been made by dispensing them from modified burets. True flow rates yielding best results overall are 1.1 ml/min for column effluent, 2.50 ml/min for concentrated sulfuric acid, and 0.040 ml/min for liquefied phenol. The molar absorbancy index for each processed sugar is dependent upon the rate of addition of the phenol; therefore, that reagent must be delivered precisely and reproducibly.

Results and Discussion. – The development of the BLD system for color processing has solved the principal technical difficulty in the analytical system. Only minor refinements remain to be performed to ready the analyzer for routine laboratory application.

References

[1]J. G. Green and N. G. Anderson, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964*, ORNL-3601, p. 157.

13.10 ISOLATION OF RAT MACROGLOBULIN IN THE ZONAL CENTRIFUGE[1]

W. D. Fisher R. E. Canning
Tom Grizzard

Introduction. – High-molecular-weight antibodies and macroglobulins are widely distributed and of considerable immunological importance. Although good separations of small quantities of material have been obtained by rate zonal centrifugation in swinging-bucket rotors,[2] the separation and purification of macroglobulin in quantity is difficult. This report concerns the application of the zonal centrifuge[3] to the purification of rat macroglobulin. The results of physical and chemical studies on highly purified macroglobulin are also summarized.

Results. – The macroglobulin from 60 ml of pooled rat serum, partially purified by two precipitations in 40% ammonium sulfate and exhaustive dialysis against tap water, was separated in the B-IV zonal rotor on a 1-liter 10–20% sucrose gradient containing phosphate buffer (pH = 7.5, $\mu = 0.1$) by centrifugation to $4 \times 10^{11}\ \omega^2 t$ (approximately $6\frac{1}{2}$ hr at 40,000 rpm). Each run yielded 200–300 mg of macroglobulin. The preparation was ultracentrifugally and immunoelectrophoretically pure. Electrophoresis at pH's from 3.5 to 8.5 gave a single peak. The isoelectric point was 4.38. The carbohydrate content was 10%. The partial specific volume calculated from the amino acid analysis was 0.743. A molecular weight of 859,000 was calculated from diffusion and sedimentation data ($S_{20,w} = 20.0$, $D_{20,w} = 2.22 \times 10^{-7}$ cm^2/sec). Treatment with 0.1 *M* mercaptoethanol dissociated the macroglobulin into smaller units (approximately 7*s*).

Discussion. – The usefulness of the zonal centrifuge for the preparation of large quantities of rat macroglobulin has been demonstrated. The quantities obtained are adequate for physical, chemical, and immunological studies. Preliminary studies demonstrate that the technique is applicable to other protein in the 20*s* size class.

References

[1]Research supported by Joint NIH-AEC Zonal Centrifuge Development Program under contract with the Union Carbide Corporation.

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

13.11 THE JOINT NIH-AEC ZONAL CENTRIFUGE DEVELOPMENT PROGRAM

N. G. Anderson A. A. Barber
W. D. Fisher R. E. Canning

Work on this program is reviewed in detail in semiannual reports having the same distribution as this report. The work is, therefore, presented here in summary form.

Low-Speed Zonal Rotors. – Rotor A-IX has been used to fractionate homogenates of rat liver, *Astasia longa* cells, spinach leaves, and rat muscle. On the basis of this work an improved rotor, designated A-XII, has been designed, built, and tested. A variety of gradients are being tested to find one which will give the best separation of the major cell components.

Intermediate-Speed Rotors. – Rotor B-IV has been used routinely at 40,000 rpm to separate ribosomes, ribosomal subunits, and macroglobulins. Rotors B-V, VI, VII, VIII, and IX have been built and used to study continuous-flow separation of viruses. The B-VIII rotor is designed specifically for the isolation of virus particles which cannot be pelleted without inactivation. Particles are removed from a liquid stream and banded in a density gradient which remains in the rotor. An improved version of this rotor (B-IX) is designed to reduce the back pressure encountered in B-VIII. Two new rotors, B-X and XI, using removable seals and having no upper bearing were built and tested. Preliminary studies suggest that these rotors are capable of separations comparable in resolution to those obtained with B-IV.

Containment. – Routine techniques for the separation of particulate mixtures in completely contained centrifuge systems have been developed and applied to the problem of searching human tumors for virus-like particles.

Rate-Banding Separations. – As previously reported, normal tissue contains very few particles having sedimentation rates and densities in the viral range. To exploit this observation, it is necessary to make separations based on sedimentation rate and then to band isopycnically approximately 30 rate-separated fractions. The rate separations are made in the B-IV zonal centrifuge, and the isopycnic banding is done in CsCl gradients using a series of density beads developed during this report period to give a visual indication of the gradient composition. Light scattering is used to locate the position of banded materials in the tubes. The entire analysis has now been reduced to a three-part diagram such as is shown in Fig. 13.11.1. This technique gives us a new way of looking at a cell, as a two-dimensional distribution of particles. The range of particles separated parallels that observable with the electron microscope. The problem now is to identify the components observed and to determine the sedimentation rates and densities under these conditions. Glycogen and ferritin have been chosen for study initially. Glycogen has been found to band in CsCl at a density of 1.62 and to have a very wide range of sedimentation coefficients. Three levels of organization are distinguishable. These include a subunit approximately 15 A in diameter and a larger subunit approximately 300 A in diameter which may aggregate to form clusters of sizes ranging up to 2000 A in diameter (Fig. 13.11.2).

Computer Program for the B-IV Rotor. – Equivalent sedimentation coefficients (S^*) for a series of densities are now calculated and plotted automatically for each zonal centrifuge run. These plots allow the position of a particle of given density and sedimentation coefficient to be accurately determined. Corrections for osmotic changes have not yet been built into the program.

Computer Analysis of Chromatographic Data. – Under optimal conditions, the analytical tracings of substances eluted from ion exchange columns appear as a series or sum of Gaussian peaks. The nucleotide analyzer developed under this program provides a two-wavelength plot of such peaks, many of which overlap. An iterative, generalized, least-squares nonlinear, fitting routine has been modified for use with such bioanalytical data. Additional restrictions are being added to the program that both 260 and 280 absorbance tracings be resolved into the same number of peaks having the same positions and widths at half height. This program will increase considerably the resolution which may be achieved with presently available chromatographic systems.

Countercurrent Distribution. – Two new countercurrent separation systems have been developed during this report period and tested in prototype form. These will allow the method to be greatly simplified and accelerated. In the second system centrifugation is employed to achieve a more rapid separation of phases. Multistage units are being fabricated for use in RNA and protein separations.

Flame Ionization Analyzer. – The wire-fed flame ionization analyzer has been modified to prevent sample burnoff just before the wire enters the hydrogen flame. The wire is now passed through a cooled jacket arranged to allow the air for combustion to move past the wire and into the flame, carrying with it substances volatilized off the

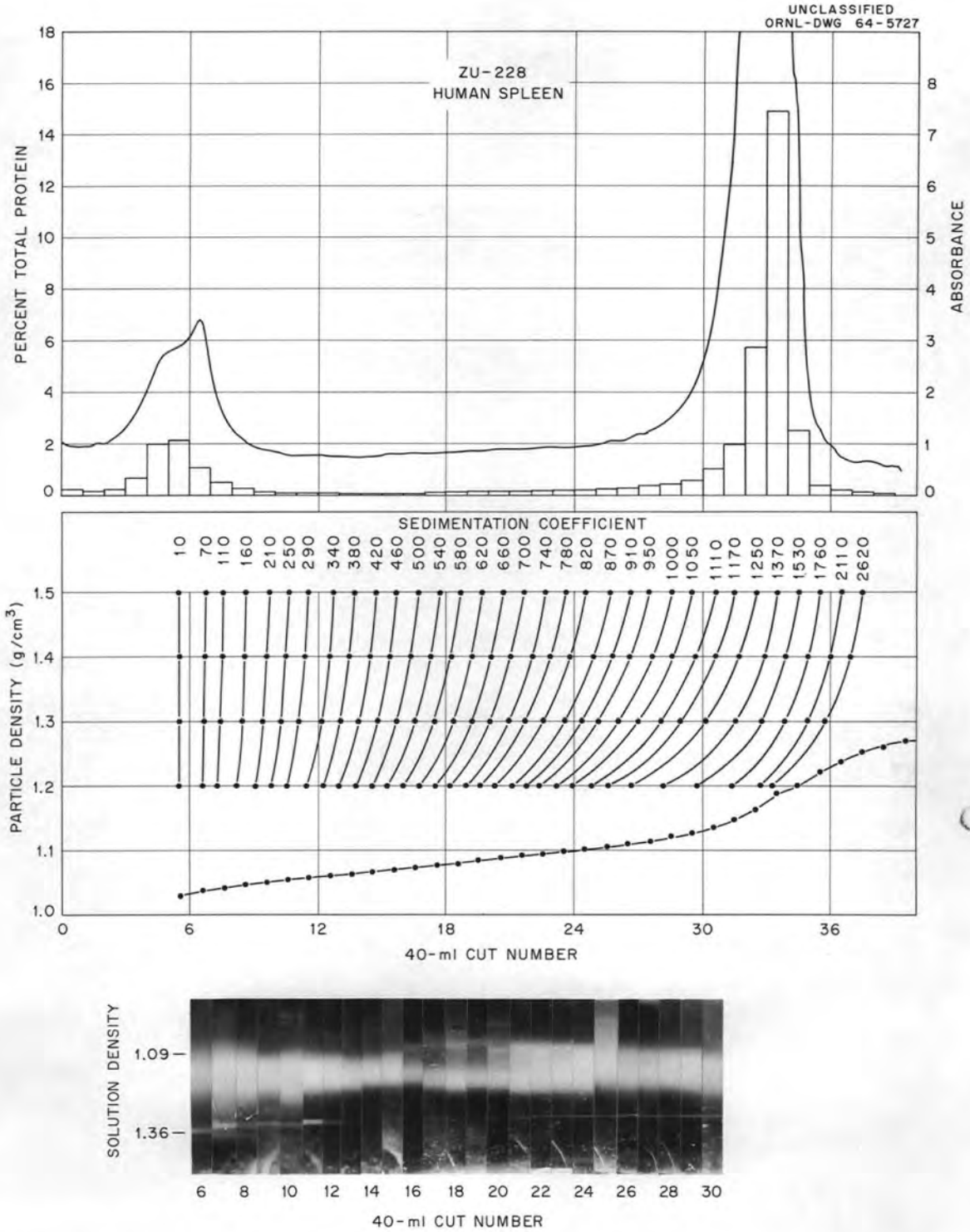

Fig. 13.11.1. Fractionation of Normal Human Spleen. Upper plot shows rate zonal centrifugation separation; computer program plot is shown in center; and isopycnic separations in CsCl are shown at the bottom.

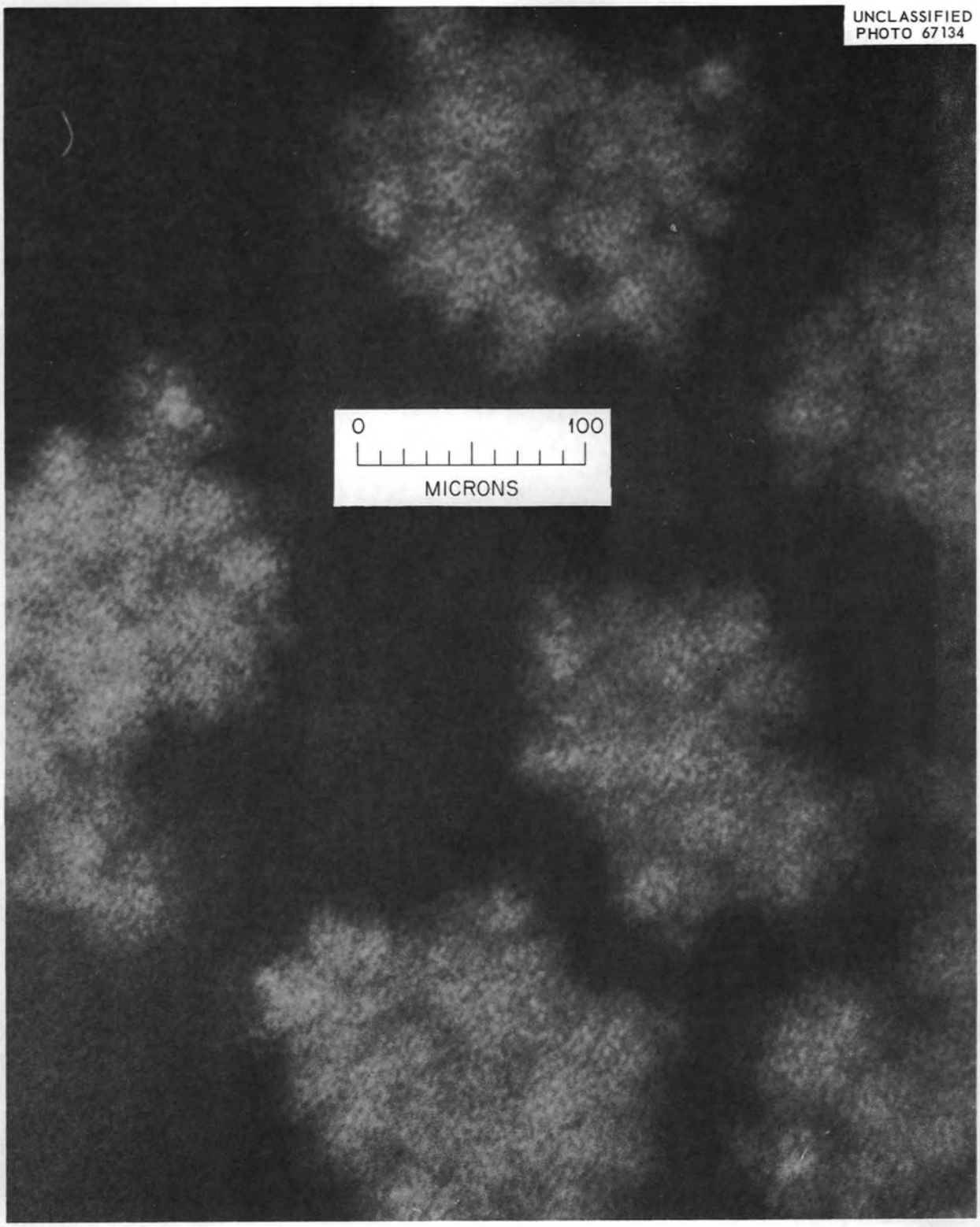

Fig. 13.11.2. Clusters of Glycogen Subunits.

wire before it actually enters the hydrogen flame. This modification improves the sensitivity and reproducibility of the instrument.

Theoretical Studies. – The problem of the capacity of gradients to support particle zones has been approached theoretically and experimentally by previous workers with conflicting results. Studies by Brakke (personal communication) suggest that sucrose density gradients are able to support only a fraction of the mass of viral particles calculated from the Svensson gradient equation. Svensson's treatment has been extended to account for the migration of particles in the centrifugal field, where their concentration is dependent upon time as well as position. The validity of this treatment using 30 *S coli* ribosomes as a test material is being studied, and preliminary analysis suggests agreement between theoretical and experimental results. These studies are intended to make possible the selection of optimum gradients and amounts of material for preparative and analytical separations.

14. Nucleic Acid Chemistry

Nucleotide Chemistry (14.1)		Molecular Biology (14.2, 14.3)	
W. E. Cohn	A. Jeannine Bandy	F. J. Bollum	Janet C. Ring
Mayo Uziel		B. K. Zimmerman	Patricia A. Williams
		Elsa Lukas[a]	Eleanor Groeniger[b]
			G. H. Huffman[c]

[a]Visiting investigator from abroad.
[b]Temporary summer employee.
[c]Student trainee.

14.1 ION EXCHANGE CHROMATOGRAPHY OF OLIGO- AND POLYNUCLEOTIDES

W. E. Cohn A. Jeannine Bandy

The general purpose of the present investigation is to improve the accuracy, speed, and ease of separations of oligo- and polynucleotide mixtures, such as may be encountered in the analysis of nucleic acid molecules. To this end, we are exploring the effects of temperature, pH, replacing ion, counterion (cation, in the case of anion exchange processes), flow rates, etc., on the fractionation of RNase and DNase digests, as well as the ease of recovery and identification of separated constituents by subsequent paper chromatography.

We find that the use of urea in DEAE chromatography can be partially counterbalanced by elevated temperature, and that Mg is preferable to Na as a counterion. However, Mg cannot be used at alkaline pH values; K or NH_4 may be substituted to good advantage for separations under these conditions.

The high resolving power of Dowex 1-X2 has been used to make group separations of the RNase digest. Total elution can be achieved with a single gradient. The tendency of Dowex 1-X2 to shrink at high ionic strength and thus to "trap" longer molecules can be overcome by using acetate instead of chloride as the replacing anion, and Mg again seems to be superior to K or Na, possibly because of its complexing action.

14.2 BIOSYNTHETIC POLY-DEOXYNUCLEOTIDES

F. J. Bollum Patricia A. Williams
M. M. McClelland

Single-chain polydeoxynucleotides, prepared by the action of a *terminal* deoxynucleotidyl transferase from calf thymus gland,[1] may be converted to double-chain polymers containing complementary strands by the action of the *replicative* deoxynucleotidyl transferase (DNA polymerase) of calf thymus gland. In this manner poly dA yields poly dA:dT, and poly dI produces poly dI:dC in replicative synthesis catalyzed by DNA polymerase. The two-stranded helices produced are highly ordered molecules having the structure and physical properties of miniature DNA molecules. These double-chain polymers, being made up of two homopolymer chains, provide simple models of the

more complicated heteropolymer complex we know as DNA. This type of polymer is currently being used for model studies[2] on radiation damage in DNA and for investigations on the enzymic repair of radiation damage.

The double-stranded deoxy-polymers are excellent templates for the RNA polymerases of *Micrococcus lysodeikticus* and *Azotobacter vinelandii*. Their use in the biochemical studies[1] investigating the mechanisms of transcription, translation, and replication, and the regulation of these events in vitro is in progress.

References

[1]F. J. Bollum *et al.*, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964*, ORNL-3601, p. 173.

[2]R. B. Setlow *et al.*, this report, Paper 20.5.

14.3 PURIFICATION AND PROPERTIES OF *MICROCOCCUS LYSODEIKTICUS* DNA POLYMERASE AND STUDIES ON THE PRIMING ACTIVITY OF CROSS-LINKED DNA

B. K. Zimmerman

DNA polymerase has been purified 1000- to 1500-fold from spray-dried *Micrococcus lysodeikticus* cells. The purified enzyme is somewhat unstable but may be stored at −20° in 50% ethylene glycol without loss of activity. In vitro DNA synthesis requires all four deoxynucleoside triphosphates, a DNA or DNA-like primer, and a divalent cation such as Mg^{2+} or Mn^{2+}. Both heated and denatured DNA are effective primers. The unprimed synthesis of dAT occurs, but only in the presence of a stabilizing agent such as bovine gamma globulin. Incorporation of dATP and dTTP occurs only after a lag period of 48 hr at 30°.

Endonuclease activity cannot be detected in the enzyme, and exonuclease activity is detectable only after very long incubation times in the absence of pyrophosphate and deoxynucleoside triphosphates. In the presence of pyrophosphate, nucleotides are slowly liberated from the primer, suggesting a reversal of the polymerase reaction. In the presence of all four deoxynucleoside triphosphates, nucleotides are liberated from the primer at 10–25% of the rate of incorporation of substrates. The implication is that either the enzyme contains a substrate-stimulated exonuclease or that an exchange reaction occurs between the nucleotides of the primer and substrate.

The kinetics of synthesis and characterization of the reaction products are currently under investigation. DNA's covalently cross-linked by nitrous acid or mitomycin C are also being used as primers for the purified enzyme in order to further clarify the mechanism of DNA replication.

15. Nucleic Acid Enzymology

Elliot Volkin	
Microbial Systems (15.1, 15.3–15.5)	**Developmental Biochemistry (15.2, 15.6–15.8)**
Elliot Volkin M. Helen Jones W. E. Barnett R. K. Fujimura J. X. Khym	F. J. Finamore Rose P. Feldman C. G. Mead A. H. Warner[b] Sumiko Iyeiri[a] N. G. Sansing[b]

[a]Visiting investigator from abroad.
[b]USPHS Fellow.

15.1 BIOCHEMICAL TRANSFORMATIONS OF BACTERIOPHAGE T_2 DNA

Elliot Volkin M. Helen Jones

Introduction. – In the previous two ORNL reports,[1,2] experiments were described which indicate that the transfer of parental T_2 DNA to progeny DNA is accompanied by a small, but definite, loss of hydroxymethyldeoxycytidylic acid (HMC) relative to the other three deoxynucleotides. Furthermore, experiments involving labeling of early phage progeny and "second generation" experiments with labeled parent indicated that the phenomenon is associated with certain specific HMC residues. Experiments are under way designed to determine whether HMC nucleotides within specific nucleotide sequences in the parental DNA chain are preferentially removed before transfer to progeny.

Results. – The purines of DNA may be completely removed and the original pyrimidine nucleotide sequences quantitatively retained by treatment of the DNA with diphenylamine–formic acid.[3] Many of the pyrimidine oligonucleotide sequences can be readily separated by chromatographic procedures.

A phage stock labeled in HMC and thymidine was prepared by growing the phage in the presence of deoxyuridine-^{3}H, where the labeled substrate had also been present during logarithmic growth of the host bacteria. The purified phage stock was used to infect unlabeled bacteria, and after 1 hr progeny phage was collected. The DNA's from parental and progeny phage were isolated and subjected to diphenylamine–formic acid hydrolysis and chromatography with Dowex 1 chloride resin. An analysis of the pyrimidine sequences obtained revealed that the HMC *di*nucleotide sequences from progeny DNA acquired significantly less radioactivity from parental material than the HMC mononucleotide sequences. The result indicates that sequences involving two adjacent HMC nucleotides surrounded by at least one purine nucleotide on either side are involved in the "loss" of HMC in parent to progeny transfer. We were unable to separate HMC trinucleotide sequences (or higher homologs, if they exist). Insofar as the extent of labeling was low in these experiments, they are currently being repeated to determine their validity.

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[2]Elliot Volkin and M. Helen Jones, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964,* ORNL-3601, p. 174.

[3]K. Burton and G. B. Peterson, *Biochem. J.* **75**, 17 (1960).

15.2 ENZYMATIC CONDENSATION OF OLIGODEOXYRIBONUCLEOTIDES

C. G. Mead

An enzymatic activity capable of condensing oligodeoxyribonucleotides has been identified[1] and partially purified from extracts of T-2 infected *E. coli* cells. The proposed reaction involves the condensation of ^{32}P-labeled short-chained oligodeoxyribonucleotides derived from T-2 phage DNA with long-chained polynucleotides derived from sonicated T-2 DNA. The system has an absolute requirement for Mg^{2+} and ATP and exhibits maximal activity at pH 9.0. The product of this reaction exhibits the following properties: (1) acid insolubility, (2) ethanol insolubility, (3) sensitivity to enzymatic attack by pancreatic DNase I and snake venom phosphodiesterase, and (4) insensitivity to bacterial alkaline phosphatase hydrolysis and alkaline hydrolysis. It has been demonstrated that DNA polymerase is not involved in this reaction in that the two DNA polymerases present in the crude extract, that is, the *E. coli* DNA polymerase and the T-2 induced DNA polymerase, can both be separated from the active fraction. Gel filtration with Sephadex G-100 results in the separation of two components, both of which are necessary for activity. It has been established that these two components are active in a sequential manner.

References

[1]C. Mead, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964,* ORNL-3601, p. 178.

15.3 ON POLYMERIZATION OF R17 BACTERIOPHAGE RNA

R. K. Fujimura

In the previous ORNL report[1] was described the ^{32}P labeling pattern of oligonucleotides obtained from ribonuclease digests of uniformly labeled and "pulse labeled" RNA samples from R17 bacteriophage. With uniformly labeled RNA the ratio of the specific activities among the oligonucleotides was constant, whereas with pulse labeled RNA this ratio was random. The nonuniform labeling was not due to contamination with non-viral RNA nor to preferential incorporation of the label into certain mononucleotides more than others, but was probably due to partial sequential labeling of viral RNA.

However, in these preparations the homogeneity of only the uniformly labeled sample was determined and it was assumed that under the same isolation procedure the pulse labeled sample was homogeneous also. This is based on the assumption that viral RNA polymerization is completed before RNA becomes associated with coat protein. Thus RNA isolated from nucleoprotein similar in size to mature phage should have complete RNA, and there should be no danger of contamination with partially polymerized RNA.

The first indication that the pulse labeled sample does not have RNA of uniform length was obtained by stepwise hydrolysis of the RNA with snake venom phosphodiesterase. The results indicated the existence of shorter chain polymers rather than partially sequentially labeled RNA. This was confirmed more directly by sucrose density gradient centrifugation of RNA prepared from pulse labeled phage purified by two different methods. In both instances, there were three peaks of which a phage RNA peak and a peak of about 20S were the major components. When these two were fractionated, the base composition of each fraction was the same as viral RNA. No preferential hydrolysis of one component over the other was observed under various RNase concentrations. An attempt to anneal one component with the others has been unsuccessful. Thus the slower sedimenting components are not double-stranded RNA nor a complementary strand of viral RNA. It is not due to transmutation of ^{32}P atoms, since there is no more than one ^{32}P atom incorporated per RNA molecule under these conditions. It is

probably not due to radiation damage, since eliminating the pelleting of phage by high-speed centrifugation, which should cause more damage than in solution, did not have a noticeable effect on heterogeneity of RNA. Two possible explanations are under consideration:

1. It is RNA at an intermediate step in forming a complete viral RNA.
2. It is an artifact of isolation in that phage particles from pulse labeled samples are slightly smaller than mature phage and may contain less protein, making them more sensitive to nuclease activity.

References

[1]R. K. Fujimura, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964*, ORNL-3601, p. 183.

15.4 INTERSPECIES AMINOACYL-sRNA FORMATION

W. E. Barnett K. B. Jacobson

In the previous report[1] it was shown that the phenylalanyl-sRNA synthetase of *Neurospora crassa* attaches L-phenylalanine-^{14}C to three chromatographically (methylated albumin) distinct *E. coli* sRNA's. The *E. coli* enzyme attaches phenylalanine to only one of these three, that is, the normal or correct *E. coli* phenylalanine sRNA ($sRNA^{phe}_{coli}$). We have confirmed and extended these results using countercurrent distribution as a method of separating the various amino-acid-specific sRNA's and identifying the particular sRNA's involved. The *Neurospora* enzyme attaches phenylalanine to the following fractionated coli sRNA's: (1) $sRNA^{phe}_{coli}$, (2) alanine sRNA ($sRNA^{ala}_{coli}$), and (3) an as yet unidentified sRNA ($sRNA^{x}_{coli}$).

Since the sRNA's are fractionated *prior* to charging with the *Neurospora* enzyme fraction, these results preclude the possibility that the differences to chromatographic profile between homologously (*E. coli* enzyme) and heterologously (*Neurospora* enzyme) charged phenylalanyl-sRNA's are due to enzymatic (or non-enzymatic) alteration of the $sRNA^{phe}_{coli}$ during the charging reaction.

It is known that sodium periodate oxidizes the ribose moiety of the terminal nucleosides in sRNA's. This reaction is dependent upon the presence of free hydroxyl groups in both the 2′ and 3′ positions, and destroys the ability of molecules to accept amino acids. Thus aminoacylation, which attaches an amino acid at either the 2′ or 3′ position, protects sRNA's from periodate. We have found that the attachment of L-phenylalanine to $sRNA^{ala}_{coli}$ protects it from periodate and, upon reisolation and retesting, have shown that the ability to accept alanine from the coli enzyme has been retained.

Thus the charging of $sRNA^{ala}_{coli}$ with phenylalanine by the *Neurospora* enzymes in no way destroys the integrity of the molecule, as judged by its capacity to be recognized correctly by, and accept alanine from, the *E. coli* alanyl-sRNA synthetase. The periodate-resistant nature of the heterologously charged sRNA also demonstrates that the amino acid–sRNA linkage is not anomalous.

References

[1]W. E. Barnett and K. B. Jacobson, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964*, ORNL-3601, p. 177.

15.5 FRACTIONATION STUDIES OF sRNA USING PARTITION COLUMNS

J. X. Khym

Introduction. – A series of studies[1,2] have been undertaken to develop the technique of reversed phase partition chromatography as a means to investigate the nucleic acids. Amine salts dissolved in selected organic solvents immobilized on inert supports serve as the column materials. The solvents and amines used in these columns are chosen on the basis of their behavior in single batch extraction experiments.

Results and Discussion. – It was found that tricaprylyl methyl ammonium chloride, a quaternary amine, dissolved in trichlorotrifluoroethane readily extracted sRNA from sodium chloride solutions of concentrations 0.25 *M* or less. The sRNA is recovered from the organic phase by increasing the sodium chloride concentration over narrow ranges to 0.5 *M* salt solution. Phase separation was very rapid compared to other systems studied, and

both the organic and aqueous phases obtained were clear.

The quaternary amine previously mentioned was dissolved in trichlorotrifluoroethane and immobilized on both Saran powder and kieselguhr. Subsequent column runs using sRNA indicated that some fractionation of the sRNA had occurred. In one of the runs a series of fractions analyzed by W. E. Barnett showed some separation of alanine and phenylalanine acceptor sRNA's. In a column run in which Saran was used as support material, the elution profile contained at least five distinct peaks.

References

[1]J. X. Khym, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1963,* ORNL-3427, p. 126.

[2]J. X. Khym, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1963,* ORNL-3498, p. 163.

15.6 STUDIES ON THE STRUCTURE OF AMPHIBIAN EGG RIBONUCLEIC ACIDS

F. J. Finamore Rose P. Feldman

Introduction. – The presence in large quantities of oligoribonucleotides in RNA preparations from ovarian eggs and developing embryos of some amphibia[1] prompted us to investigate the structural relationship, if any, between oligonucleotides and polynucleotides in undeveloped eggs.

Methods and Results. – The total RNA from ovarian eggs of *R. pipiens* was prepared by the procedure of Kay and Dounce.[2] The RNA was analyzed by sucrose density gradient centrifugation and by use of methylated serum albumin columns and was found to be composed almost exclusively of a 4S component. However, 20–25% of this component was observed to be oligonucleotide in nature.

Analysis of the RNA by gel filtration revealed that free or unassociated oligonucleotides could be separated easily from the polynucleotides. However, when polynucleotides, after several passes through columns of Sephadex G-50, were heated at 100° for 10 min and cooled quickly, more free oligonucleotides were produced from polynucleotides. Heating polynucleotides at pH 7.2 for 2 hr at 100° converted about 30% of the polynucleotides to oligonucleotides. DEAE cellulose column chromatography of the heated polynucleotides revealed that olgionucleotides produced by heat were similar to the free or unassociated oligonucleotides present in the original RNA preparation.

These observations led to a more detailed analysis of the liberation of oligonucleotides upon heating ovarian egg RNA preparations. The release of oligonucleotides was linear as a function of temperature between 30° and 60° and was markedly depressed by the addition of Mg^{2+}. Neither Ca^{2+} nor Na^{+} was as efficient as Mg^{2+} in stabilizing the structure of ovarian egg RNA. Heating RNA preparations to release oligonucleotides did *not* result in liberation of more phosphoryl end groups that were susceptible to the action of a bacterial alkaline phosphatase preparation. This suggests that liberation of oligonucleotides by heat is not accompanied by disruption of phosphodiester bonds.

Summary. – The 4S component of ovarian egg RNA appears to be extremely labile to heat, as judged by the release of acid-soluble oligonucleotides. The production of oligonucleotides cannot be accounted for by the disruption of phosphodiester bonds, suggesting that the 4S component is actually a complex of oligonucleotides and polynucleotides held together by relatively weak bonds.

References

[1]F. J. Finamore, *J. Biol. Chem.* **239,** 1882 (1964).

[2]E. R. M. Kay and A. L. Dounce, *J. Am. Chem. Soc.* **75,** 4041 (1953).

15.7 HYDROLYSIS OF P^1,P^4-DIGUANOSINE 5′-TETRAPHOSPHATE BY A NEW PYROPHOSPHOHYDROLASE FROM BRINE SHRIMP EGGS

A. H. Warner F. J. Finamore

Introduction. – A new enzyme, P^1,P^4-diguanosine 5′-tetraphosphate asymmetrical pyrophosphohydrolase (*asym*-di-GDPase), capable of hydrolyzing P^1,P^4-diguanosine 5′-tetraphosphate (di-GDP) has

been isolated from encysted embryos of *Artemia salina* and partially purified as previously described.[1] This enzyme apparently has an absolute substrate specificity and produces equimolar quantities of guanosine monophosphate and guanosine triphosphate from diguanosine 5′-tetraphosphate.

Results. – The heterogeneous appearance of *asym*-di-GDPase activity during fractionation on carboxymethyl cellulose[1] and the fact that all partially purified preparations showed slight nuclease activity prompted the use of acrylamide gel electrophoresis to determine whether multiple forms of the active enzyme are present and to assess the significance of the endogenous nuclease activity. In all electrophoretic experiments only one protein band showed *asym*-di-GDPase activity, while all nuclease activity appeared associated with contaminating protein.

In the presence of the enzyme, di-GDP is completely hydrolyzed to GMP and GTP in equimolar quantities and the reaction appears to be irreversible. Di-GDP hydrolysis can be completely inhibited by pretreatment of the enzyme preparation with heat or chymotrypsin and partially inhibited when assayed in the presence of *p*-hydroxymercuribenzoate.

Maximal rate of di-GDP hydrolysis occurs about pH 8 and 45°C, and the activation energy is approximately 6000 cal/mole. Hydrolysis of di-GDP by *asym*-di-GDPase requires magnesium or manganese for maximal activity while all other cations tested showed little, if any, cofactor activity. When magnesium is used, optimal activity is obtained when (Mg^{2+})/(di-GDP) equals 2, while manganese is optimal when (Mn^{2+})/(di-GDP) equals 1.

When small amounts of GTP are present initially in the reaction mixture, the reaction is inhibited markedly, while initial amounts of GMP have no observable effect on the reaction rate. Under all conditions tested, GMP failed to inhibit the reaction while GTP was effective in micromolar quantities. When the reaction rate is determined in the presence of varying amounts of GTP and substrate and plotted in the double reciprocal form, the inhibition appears to be noncompetitive in nature. This type of inhibition may represent an effective in vivo regulatory mechanism of energy metabolism during embryonic development of *Artemia*.

References

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15.8 PURIFICATION AND PROPERTIES OF CORN ROOT DEOXYRIBONUCLEASE

N. G. Sansing

Studies are continuing on the purification and characterization of a cytoplasmic deoxyribonuclease found in large amounts in the roots of germinating corn. The enzyme chromatographs as a single peak on DEAE-cellulose and carboxymethyl cellulose. The partially purified protein exhibits the following properties: the pH optimum for hydrolysis is 6.0; no requirement for a metal or other activators has been found; the activity is relatively heat labile and is denatured by freezing and thawing when purified. It may be stored at 4°C for extended periods under toluene.

The partially purified enzyme is approximately tenfold more active on heat-denatured than on native DNA. It is not yet known if the activity on native DNA is due to contamination by an enzyme specific for native DNA, if the limited hydrolysis observed removes only portions of the native DNA which have been denatured, or if a single enzyme is present with a reduced specificity for native DNA. This subject is now under investigation.

Analysis of kinetic data during hydrolysis of denatured DNA, assaying on DEAE-cellulose disks, washed with NaCl and urea and on filter paper disks washed with trichloroacetic acid, suggests that the purified enzyme may be acting as an endonuclease. A sensitive test for nucleotidase activity has now been developed and the enzyme preparation has been largely freed of phosphomonoesterase and nucleotidase activity. This makes it possible to characterize the hydrolytic products and determine the mode of action of the enzyme. This problem is now being actively pursued.

16. Chemical Protection and Enzyme Catalysis

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16.1 CHEMICAL PROTECTION AND ENZYME CATALYSIS

D. G. Doherty J. D. Bales, Jr.
Margaret A. Turner

The previous report[1] established that a mixture of AET and glutathione (GSH) injected intraperitoneally into mice 1 hr prior to irradiation with x rays gave a dose reduction factor (DRF) of 2. The prolonged effectiveness made it possible to consider examining the protection afforded by this treatment against fission neutrons. Through the excellent cooperation of the Health Physics Division, we were able to use the Research Reactor (HPRR). The neutron dosimetry was done by members of the Health Physics Division, the power level being set so that the neutron dose rate was approximately 40 rads/min at the position of the mice. The neutron-gamma tissue dose was 10 to 1. The total dose was varied by varying the distance from the plastic mouse holder to the reactor core, the holders being positioned in approximately 40-rad steps. Twenty-four to forty-eight animals were exposed at each position. The radiation LD 50/30 days for $BC3F_1$ male mice was found to be 310 rads as compared to 720 r for x rays, indicating an RBE of 2.3. Treatment of mice with an AET (480 mg/kg)–GSH (1.6 g/kg) mixture intraperitoneally, and exposure to neutron radiation approximately 1 hr later, yielded an LD 50/30 days of 435 rads for a DRF of 1.4. A majority of the protected animals that survived lethal doses of neutrons are alive at three months, although they show signs of accelerated radiation effects.

The protection afforded by glutathione either alone or in mixtures raises the question as to how it functions. If it is acting chemically as a peptide reducing agent and not via an enzymic mechanism, then other cysteine peptides may possess protective activity. However, if it is via an enzymic mechanism, then the glutathione structure is probably unique. This question has received little or no attention in enzymic studies and none in radiation studies, due primarily to the nonavailability of suitable peptides and the difficulties involved in their synthesis. It seemed feasible to attempt the synthesis of several peptides closely related to glutathione in sufficient quantity so that they could be tested for radiation protection in mice. The most promising should be the (until now unknown) optically active D form of GSH in that it might not be metabolized but still retain its protective activity and low toxicity. Other peptides equally interesting are the alpha glutamyl peptide isoglutathione in either L or D optical configuration, as well as the corresponding aspartic acid derivatives, for example, aspartathione. These compounds are of equal value in studying the role of GSH in enzyme systems. A synthetic scheme using suitable blocking

groups that can be easily removed at the proper time has been devised, as has a procedure for the preparation of D cysteine derivatives that can be used in the synthetic sequence of peptide building. The work has proceeded to the stage of tripeptides with one or two blocking groups remaining to be removed for L and D GSH and L and D isoGSH as well as the racemic DL GSH.

References

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17. Enzymology

G. D. Novelli[a]

Protein Synthesis (17.1–17.15)

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Hormonal Control (17.16–17.18)

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Mammalian Cell Regulation (17.19)

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Cooperative NIH-AEC Co-Carcinogenesis Program (17.1, 17.4, 17.6–17.9, 17.16–17.19)

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Protein Synthesis

The reports on protein synthesis describe our efforts to study amino acid incorporation and protein synthesis in a number of cell-free systems (i.e., systems prepared from *Escherichia coli, Clostridium pasteurianum,* rat liver, and maize). Because protein synthesis seems to be dependent on an RNA made by a DNA-dependent RNA polymerase, we are attempting to prepare this enzyme on a large scale to be used in all the cell-free systems currently under investigation.

We have also continued to study the incorporation of amino acids in systems not involving ribosomes. These systems were discovered in this laboratory, and we are trying to assess the significance of such "soluble" systems in relation to the more conventional systems. We have found that arginyl-sRNA can be transferred to serum albumin or edestin as acceptor proteins.

Our studies with the plant protein synthesizing systems have continued. This work is proceeding in two directions: (1) an attempt to understand the effect of preillumination of seedlings on the amino-acid-incorporating activity of the cell-free

system and (2) an attempt to prepare a DNA-dependent RNA polymerase from maize in order to study the control of the biosynthesis of the gene-specific esterases.

As part of our effort in the Co-Carcinogenesis Program, we are continuing our study of the control of protein synthesis in cell-free systems prepared from rat liver at different times after a partial hepatectomy. These studies have suggested that there are possibly two components, one microsomal and one in the cell sap, that are responsible for the increased rate of protein synthesis during liver regeneration. From previous studies, we developed the concept that the protein synthesizing system, that is, the ribosomes with their messenger RNA forming the polysome, is assembled as a complete unit in the nucleus. In an effort to obtain evidence in favor of this concept, we have initiated studies on the effect of the antibiotics actinomycin and puromycin on the synthesis of ribosomal RNA and on the aggregate size of the polysomes. We consider that studying control mechanisms in model systems such as these and others like antibody synthesis and the induced β-galactosidase synthesis will reveal clues to indicate which systems would be most advantageous to study during carcinogenesis.

We have undertaken a cooperative program with Chemical Technology to prepare sRNA from *E. coli* in large amounts in the hope that methods can be developed to separate the individual amino-acid-specific sRNA's in sufficient quantity to permit a detailed analysis of the nucleotide sequence in order to determine the nature of the code. Preliminary results with reverse-phase chromatography using long-chain quaternary amines as the immobile phase indicate that this might be a potent technique for separation of all kinds of nucleic acids. In conjunction with this effort, it is necessary to have available amino-acid-activating enzymes in order to assay the sRNA's. The Analytical Chemistry Division has taken an interest in the assay problem and has assigned two men to help us make the assay more efficient and attempt to automate as much as possible. Also we are trying to purify some of the activating enzymes in sufficient purity and quantity that will permit the study of the manner by which such enzymes recognize the correct species of sRNA. All these activities, as well as others detailed in this report, depend for their success on the pilot-plant operation. In addition to our needs, we have provided other groups in the Division with cells or other materials.

17.1 STUDIES ON THE CELL-FREE SYNTHESIS OF β-GALACTOSIDASE

Lawrence Rosen G. D. Novelli

Introduction. – The inducer-dependent *de novo* synthesis of β-galactosidase in a cell-free system obtained from *E. coli* may be considered to be biphasic,[1,2] In the first phase a messenger RNA (mRNA) containing the "lac" message is copied from the "lac" region of the DNA. The DNA must be obtained from an inducible strain of *E. coli* which has been induced prior to harvesting and isolation of the DNA. This reaction is catalyzed by a DNA-dependent RNA polymerase (RNA poly). In the second phase, the mRNA directs the synthesis of β-galactosidase by a competent protein synthesizing system consisting of a ribosomal fraction (P_{90}), a high-speed supernatant fraction (S_{90}), and added factors. The P_{90} fraction must be obtained from preinduced cells.

In order to determine why it has not been possible to reproduce the β-galactosidase synthesizing system, attention has been paid to the biphasic nature of the reaction.

Accordingly, experiments were run in order to determine the behavior of mRNA in the protein synthesizing system and the response of the protein synthesizing system to added mRNA.

Results and Discussion. – mRNA was synthesized with preinduced DNA from *E. coli* as the template. RNA polymerase was used as the enzyme. The enzyme preparation was free of degradative ability as measured by the lack of formation of acid-soluble fragments from labeled mRNA.

Upon addition of the mRNA to the protein synthesizing system, it was rapidly degraded. This degradative ability was found in the S_{90} fraction and is probably due to polynucleotide phosphorylase.[3,4]

RNA polymerase is finally purified from the S_{90} fraction. Accordingly, another series of experiments were undertaken to follow the synthesis and degradation of mRNA in the protein synthesizing system. This was done by following the incorporation of UMP-^{14}C into an acid-insoluble fraction from UTP-^{14}C in the presence of the

other three ribonucleoside triphosphates. These experiments indicated that there is a rapid synthesis, as well as degradation, of mRNA during phase 2. Under conditions of incubation for protein synthesis, the mRNA formation reaches a maximum in less than 15 min. The addition of both purified RNA polymerase and DNA leads to an increased synthesis of mRNA, but under these conditions, the maximum is also reached in less than 15 min.

Despite the rapid loss of mRNA in the protein synthesizing system, active amino acid incorporation occurs. However, the protein synthesizing system does not respond to either added mRNA polymerase or DNA.

Thus, whether the mRNA synthesized from DNA contains the "lac" message or not, the failure to obtain a response to it by the protein synthesizing system is significant. The lack of β-galactosidase synthesizing ability may be attributed to this lack of response, since the *de novo* synthesis of β-galactosidase was shown to be dependent upon the prior synthesis of mRNA from preinduced DNA. Experiments are in progress to obtain a protein synthesizing system that is responsive to added mRNA.

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17.2 AMINO ACID INCORPORATION BY EXTRACTS OF *ESCHERICHIA COLI*

J. V. Schnell G. D. Novelli

Introduction. – The investigation of the ribosomal-dependent amino-acid-incorporating system obtained from cell-free extracts of *E. coli* is being continued.[1]

Results and Discussion. – Recently it was found that more active ribosomes can be obtained from cells treated as described by Rotman.[2] In this procedure the whole cells are treated with a mixed ion exchange resin before passage through the French pressure cell. The pressure-cell treatment of the deionized cell suspension does not produce extracts showing the usual signs of lysis, that is, darkened color, increased viscosity, and release of subcellular particles such as ribosomes. However, the resin-treated cells are apparently damaged by the pressure-cell treatment, since subsequent adjustment of the suspension to pH 7.2 causes a dramatic increase in viscosity and release of subcellular constituents. When ribosomes are prepared from such extracts, they are up to twice as active in the amino-acid-incorporation assay as are ribosomes prepared in the normal manner. Experiments are now in progress in an attempt to elucidate the reasons for this enhanced activity. Since it is possible that the procedure described results in a large yield of polyribosomes, sucrose density gradient profiles of the ribosomal population are being conducted.

The mechanism of the lysis procedure is not known at this time, but future experiments on the parameters involved are being considered. The lysis observed is accompanied by greatly increased viscosity, and this viscosity can be rapidly reduced by deoxyribonuclease. Hence it is likely that the procedure described may be useful for the isolation of bacterial DNA of high molecular weight.

References

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17.3 AMINO ACID INCORPORATION BY EXTRACTS FROM *CLOSTRIDIUM PASTEURIANUM*

R. D. MacElroy H. D. Peck, Jr.
G. D. Novelli

Introduction. – Cell-free amino acid incorporation by extracts from *Clostridium pasteurianum* was

reported previously.[1] Similar systems have recently been obtained from *Cl. butyricum*. Continuing attempts have been made to detect biosynthesis of ferredoxin, and in conjunction with this some studies have been made on the physical behavior of ferredoxin and ferredoxin derivatives on Sephadex and DEAE columns and in polyacrylamide gels during electrophoresis.

Results and Discussion. – *Clostridium butyricum* cells grown on organic medium are broken in a French pressure cell at about 3000 psi. Cell debris and whole cells are removed by two low-speed centrifugations. The supernatant is then centrifuged at 105,000 × *g* for 2 hr. The resulting supernatant is centrifuged for an additional 2 hr before it is dialyzed. The ribosomal pellet from the first high-speed centrifugation is suspended in buffer containing 10 m*M* Mg, 10 m*M* 2-mercaptoethanol, and 10 m*M* Tris at pH 7.5 (medium A). This suspension is layered over 0.5 *M* sucrose which, in turn, is layered over 1.5 *M* sucrose. The sucrose is dissolved in medium A. This gradient is centrifuged at 105,000 × *g* for 2 hr, and the pellet is resuspended and frozen in liquid nitrogen. About 40% of the ribosomes in this pellet are in the form of polysomes. Single ribosomes have a sedimentation constant of about 70*S*, and in this respect they are very similar to those obtained from *Cl. pasteurianum*.

All of the twelve amino acids tested are incorporated by the system. Incorporation into hot-TCA-insoluble material is dependent on ATP, Mg, Mn, ribosomes, and supernatant. Chloramphenicol and pancreatic ribonuclease inhibit incorporation; deoxyribonuclease has no effect. Addition of a mixture of amino acids stimulates incorporation, as does transfer RNA from either *Cl. butyricum*, *Cl. pasteurianum*, or *E. coli*.

Extracts from frozen *Cl. butyricum* cells show significant incorporation of leucine and phenylalanine into the supernatant alone when it is prepared in a manner similar to that reported previously for *Cl. pasteurianum*.[1] A soluble amino-acid-incorporating system has also been obtained from *Desulfovibrio desulfuricans*.

The behavior of ferredoxin on Sephadex columns in high salt concentrations indicates that it forms a polymer at low salt concentrations, confirming a suggestion made by Mortenson.[2] A mersalyl derivative of ferredoxin chromatographs on DEAE-cellulose in a manner similar to native ferredoxin, except that an additional slower-moving peak is detected. Treatment of mersalyl ferredoxin with 2-mercaptoethanol apparently allows disulfide links to form between adjacent molecules, since the protein cannot then be eluted from DEAE.

Ferredoxin from *Cl. butyricum* moves more slowly than that from *Cl. pasteurianum* on DEAE columns but more rapidly during gel electrophoresis. This reflects the higher concentration of acidic amino acids in the *Cl. butyricum* ferredoxin.

Mersalyl derivatives of both ferredoxins move at the same rates in gel electrophoresis and at the same rate as ferredoxin treated with 2-mercaptoethanol or with acid.

To date, biosynthesis of neither ferredoxin nor any of its derivatives has been detected in either the *Cl. pasteurianum* or the *Cl. butyricum* system. This is possibly caused by our lack of knowledge regarding the nature of the linkages holding the iron and sulfide sulfur in the molecule. Attempts will be continued to synthesize the sulfur- and iron-free protein when we have developed a method for its detection and isolation.

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17.4 PILOT-PLANT FACILITY

E. F. Phares J. T. Davidson
Mary V. Long

Pilot-plant activities have been devoted primarily to bacterial culture in the 15-liter and the 400-liter vat fermenter; the latter has operated about 75% of the time during this period. Satisfactory automatic pH control for the fermenter has been achieved by changing from internal, submerged electrodes to external electrodes fed by a thief stream.

Scaleup of enzyme preparation has been started. RNA polymerase has been prepared from 2 kg of *A. vinlandii*. A published procedure scaled up tenfold was used.[1] Fractionations were carried out in fermenter vessels cooled with ice. Centrifugation of sensitive supernatants was performed

batchwise in a 5-liter Sharples centrifuge; precipitates were harvested with continuous flow in the laboratory Sharples. These operations were performed in times comparable to those in the laboratory on a small scale, indicating that handling of large quantities of labile enzymes is feasible in this equipment even without cold-room facilities.

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17.5 SOLUBLE AMINO-ACID-INCORPORATING SYSTEM FROM RAT LIVER

D. P. Kosow G. D. Novelli

Introduction. – A previous report[1] described an amino-acid-incorporating system isolated from rat liver which does not require a particulate component. In order to obviate some of the difficulties involved in the study of this system, arginyl-sRNA was substituted for arginine, ATP-Mg^{2+}, and rat liver sRNA in the original assay. Some of the results using this improved assay will be discussed.

Results and Discussion. – The protein fraction which catalyzes the incorporation of arginine into a hot trichloroacetic acid–insoluble product has been purified approximately tenfold by a combination of salt precipitation and positive gel adsorption and elution. At this stage of purification, the addition of an acceptor protein is required. Of the proteins tested, bovine plasma albumin and edestin are the most effective. This is not a non-specific protein effect since urease and human glycoprotein fraction V_1 have no effect and phosvitin and pectinase are inhibitory. Evidence has been obtained which indicates that when edestin is present in the assay mixture, arginine becomes bound to the edestin.

Eight other amino acyl–sRNA's have been substituted for arginyl-sRNA without success, indicating that the system is specific for arginyl-sRNA, although there is no detectable difference if arginyl-sRNA synthesized with *E. coli* sRNA is substituted for arginyl-sRNA synthesized with rat liver sRNA.

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17.6 CONTROL OF PROTEIN SYNTHESIS DURING LIVER REGENERATION

Piero Cammarano Bessie G. Lukes[1]
G. D. Novelli

Introduction. – Rat liver cells divide once a year. When a portion of liver parenchyma is extirpated, stringent control mechanisms are temporarily released, and the residual tissue responds with a vigorous burst of mitotic activity after 24 hr. Protein synthesis in vivo increases above normal levels after a lag of about 20 hr. Cell-free preparations from regenerating liver reflect in vitro the increased potential of regenerating liver for protein synthesis. Both particulate and soluble components are more active than their normal counterparts. Because of these characteristics, regenerating liver offers a unique material for the study of mechanisms regulating protein synthesis.

A study of some parameters related to cytoplasmic protein synthesis was reported previously.[2] In the further development of the research, the following additional aspects of the regenerative process have been examined: (1) labeling of microsomal RNA, (2) partition of free and membrane-bound ribosomes, (3) composition of ribosomal populations from regenerating liver, (4) comparative incorporating activity of microsomal and ribosomal populations from regenerating liver, and (5) comparative responsiveness of normal and regenerating liver cell-free systems to synthetic templates.

Results and Discussion. – 1. After labeling in vivo with ^{32}P or ^{14}C-orotic acid for varying periods of time, microsomal RNA from regenerating liver was consistently four to five times more radioactive than microsomal RNA from normal liver. Maximal labeling occurs 17 hr after surgery. A relative decrease of incorporation occurs at 24 hr, probably in relation to the appearance of DNA polymerase and the onset of DNA replication. In normal liver the greatest specific activity after any labeling period is attained by RNA fractions

sedimenting in a sucrose density gradient in the region of 16–4S. During liver regeneration all RNA fractions resolved in a sucrose density gradient attain equal specific activity, suggesting that ribosomal RNA may be the main product of an increased nuclear synthesis.

2. Contrary to reports from other laboratories, the partition of free and membrane-bound ribosomes is not altered during regeneration as revealed by sucrose density gradient of the microsomal fraction according to Hiatt.[3] By using microsomal fractions labeled in vivo with ^{32}P or ^{14}C-orotic acid, it has been established that the ratio of distribution of the newly synthesized RNA between the membrane-bound and the free ribosomes is basically unchanged with respect to normal liver at 16 and 24 hr after surgery. However, 3 hr after hepatectomy less of the newly synthesized radioactivity associates with the membrane components, suggesting the presence at this time of a nonfunctional endoplasmic reticulum. Electron microscopy of regenerating liver at various times after surgery has basically confirmed the lack of any prominent change of ergastoplasmic structures, as revealed by the regular distribution of clustered configurations (presumably polysomes) on the surface of the rough reticulum. No disappearance of the endoplasmic reticulum during early stages of regeneration was ever noticed.

3. The composition of ribosomal population from regenerating liver does not reveal any appreciable change except for a greater concentration of polysomes of size $(83S)_5$. The size of the physiologically present, messenger-free monosomes is essentially unchanged at any time after surgery.

4. Microsomes sedimented in 15 min at 105,000 × *g* were found to be more active and more stable than the usual microsomal preparations obtained after longer centrifugation times. The RNA/protein ratio of these microsomes was strikingly altered during regeneration. The RNA/protein ratio of the microsomes sedimented in the ensuing 60 min was unchanged. The 15-min microsomes represent 70% of the total microsomal RNA sedimented in 90 min under mild homogenization conditions. On an RNA basis these "heavy" microsomes (representing probably the portion of the ergastoplasm relatively less damaged by the preparation procedures) were from 1.5 to 3 times as active in incorporating labeled amino acids as similar preparations from resting liver when tested in the presence of their homologous regenerating supernatant as a source of activating enzymes. When tested in the presence of a supernatant derived from normal liver, the same microsomes were barely more active than normal liver microsomes. However, regenerating liver supernatant did not stimulate normal microsomes to the level of regenerating microsomes.

5. Ribosomal preparations from either source are equally active in incorporating labeled amino acids, provided they are tested in the presence of the same soluble fraction. Normal liver ribosomes are as active as regenerating liver ribosomes in the presence of either supernatant. The different responsiveness of normal microsomes and normal ribosomes to the stimulatory activity of regenerating liver supernatant clearly suggests that modifying circumstances operate at the membrane level restraining the potential ability of normal ribosomes for amino acid incorporation and that these conditions are removed during liver regeneration.

6. Regenerating liver microsomes are at least twice more responsive to polyuridylic (poly-U) acid than similar preparations from normal liver. Ribosomes from either normal or regenerating liver are more stimulable than either microsomal preparation. Ribosomes from either source are equally responsive to poly-U. The poly-U saturation curve for regenerating liver microsomes closely resembles that of ribosomal preparations, whereas normal liver microsomes attain lower maxima and at higher poly-U concentrations.

7. Aqueous extracts from normal liver microsomes strongly inhibit the poly-U-directed incorporation of phenylalanine in a cell-free ribosomal system. Similar extracts from regenerating liver microsomes are less inhibitory.

8. Supernatants derived from normal liver are markedly less active in catalyzing the poly-U-directed incorporation of phenylalanine in a cell-free ribosomal system than similar preparations from regenerating liver. The minor activity of the normal liver supernatant is not overcome by sRNA additions.

The poly-U experiments indicate that the greater activity of the microsomes and supernatants from regenerating liver is not due to increased levels of availability of mRNA. If the increased activity were due to messenger RNA, then the poly-U stimulability would be expected to be nearly similar in both systems. The parallel increase

of the endogenous mRNA-directed incorporation and of the poly-U-directed incorporation suggests that common factors (possibly regulatory components) interfere with the rate of protein synthesis.

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17.7 ASYNCHRONY BETWEEN INHIBITION OF PROTEIN SYNTHESIS AND INHIBITION OF RIBOSOME FORMATION IN RAT LIVER

F. M. Ritossa[1] Marialuisa Melli
G. D. Novelli

Introduction. – Puromycin is known to inhibit protein synthesis by reacting with the growing peptidyl-sRNA and thereby interrupting the growth of the peptide chain.[2] In bacteria, puromycin inhibits protein synthesis but not the synthesis of RNA. The RNA accumulated during treatment with puromycin is stable after removal of the inhibitor and is incorporated into ribosomal particles during recovery from puromycin treatment.[3] Similar studies with mammalian tissue culture cells, on the other hand, revealed that puromycin inhibited both protein and ribosomal RNA synthesis.[4–6]

In preliminary studies with ^{32}P injection into rats, we observed that 2 hr after puromycin injection no further formation of ribosomes occurred in rat liver. The present study was undertaken to determine whether this inhibition of ribosome synthesis by puromycin reflected an inhibition of ribosomal RNA synthesis or an instability of ribosomal RNA synthesized in the absence of protein synthesis.

Results and Discussion. – Rats kept under constant inhibition of protein synthesis by hourly injections of puromycin were injected with ^{32}P. RNA was extracted from microsomes and from the 100,000 × *g* supernatant of a liver homogenate by using the phenol-dodecylsulfate procedure. The specific activity of the sRNA was the same in the control and in the puromycin-treated animals during 4 hr of treatment. On the other hand, although the specific activity of microsomal RNA was the same in the control and treated animals during the first 2 hr, the specific activity of microsomal RNA continued to increase in the controls during the next 2 hr, but it did not do so in the treated animals. This observation suggested that ribosomal synthesis could occur normally when protein synthesis was inhibited by puromycin for a period of 2 hr but thereafter the synthesis stopped.

This experiment could not distinguish between inhibition of ribosomal RNA synthesis and instability of newly synthesized RNA in the absence of the necessary protein. In view of current reports suggesting that ribosomal RNA is synthesized in the nucleus, we decided to repeat the previous experiment and to use a 10-min pulse with ^{14}C-orotic acid and examine the specific activity of the nuclear RNA.

When a 10-min pulse of ^{14}C-orotic acid was given to puromycin-treated animals at any time between 0 and 4 hr of treatment, the specific activity of the RNA obtained from purified nuclei did not differ from that of the control animals. Sucrose density gradients of the pulse-labeled RNA extracted at any time between 0 and 4 hr after puromycin treatment showed a sedimentation profile similar to that of the control. Thus the failure to observe labeling of microsomal RNA between 2 and 4 hr after puromycin treatment in the ^{32}P experiment was not caused by a failure in RNA synthesis, but it seems to suggest that protein synthesis is required for the nuclear ribosomal RNA precursor to appear in the cytoplasm.

In preliminary experiments, rats injected with puromycin for 4 hr are able to recover if the injection of the antibiotic is stopped. The nuclear RNA synthesized during the period of treatment seems to be utilized for ribosome formation during the process of recovery.

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17.8 CHANGES IN THE POLYSOME AGGREGATE SIZE IN RAT LIVER FOLLOWING TREATMENT WITH EITHER PUROMYCIN OR ACTINOMYCIN

Marialuisa Melli F. M. Ritossa[1]
G. D. Novelli

Introduction. – It has been reported that in reticulocytes incubated in the presence of puromycin the ribosomal population sedimenting in a sucrose density gradient showed a 30% decrease in the large polysomal fraction with a corresponding increase in the monosomes,[2,3] During the course of studying the effect of puromycin on RNA synthesis in rat liver, we had occasion to examine the polysome pattern in a sucrose density gradient and discovered the accumulation of a large fraction of dimers.

Results and Discussion. – Rats were injected with puromycin at a level of 10 mg per 100 g of body weight, a dose that inhibits in vivo protein synthesis by 95%. Polysomes were prepared from the postmitochondrial supernatant from control rats and from treated animals at various times after puromycin treatment. The polysome profile was analyzed by centrifugation through sucrose density gradient. As soon as 1 hr after the first injection of puromycin, the sucrose density profile shows a profile different from that of the control. A great majority of ribosomes show a sedimentation constant of about 120s corresponding to the accumulation of dimers, with the polysomal region being sharply decreased. When rats treated for 1 hr with puromycin were allowed to recover, the ribosomal pattern remained essentially the same for at least 1 hr while protein synthesis in vivo had returned to normal. A normal ribosomal population is attained 2 hr after the end of treatment. That this accumulation of dimers is not an artifact of preparation was confirmed by electron microscopy.

In preliminary experiments the injection of actinomycin D seems to cause a similar accumulation of dimers.

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17.9 STUDIES ON ANTIBODY SYNTHESIS

C. J. Wust

Introduction. – The cell-free incorporating system of rat spleen described previously[1] has been examined with respect to its capacity to synthesize hemagglutinin to sheep erythrocytes and to incorporate amino acids maximally. We are preparing ribonucleic acid from spleens of rats immunized with sheep erythrocytes in order to enhance this incorporation and synthesis, as well as to use as informational message in nonimmunized rats.

Results and Discussion. – As reported earlier,[1] ribosomes prepared from rat spleens immunized to sheep erythrocytes were shown to synthesize hemagglutinin as early as 4 hr after injection of antigen. We have examined ribosomes from animals immunized 2, 3, and 5 days. At these times, antibody production in vivo is well established, and the titer of circulating antibody is rising to maximal levels. Ribosomes obtained from animals after 3 to 5 days of immunization seem to have transfer RNA associated with them. In contrast to ribosomes obtained from spleens of nonimmunized rats or rats immunized for less than 48 hr, approximately 75% of maximum incorporation of these ribosomes is found in the absence of added soluble components of rat liver. These ribosomes synthesize hemagglutinin in the cell-free system; however, the ribosomes contain bound antibody when isolated from spleen. Following the incorporation experiment, we are attempting to solubilize hemagglutinin from the ribosomes by dialysis against EDTA or by treatment with 0.5 *M* phosphate, pH 7.0. The latter method seems to be the more effective.

Studies with rat liver microsomes and ribosomes in this laboratory indicated that in terms of amino acid incorporated per milligram of RNA, microsomes were better than ribosomes. It was realized also that pyronin-staining cells of the lymphatic nodule of spleen contained ribosomes with no

definitive endoplasmic reticulum as attested by electron microscope studies of Swartzendruber.[2] Amino acid incorporation was compared by using both microsomes and ribosomes ("natural" polysomes and microsomes treated with deoxycholate). "Natural" polysomes were prepared by sucrose gradient centrifugation of spleen homogenates. Such polysomes were found at the interface between 25 and 36% sucrose and contained 82s, 133s (major component), 194s, and 242s particles. The ratio of RNA to RNA-plus-protein content was about 0.30. Microsomes were found at the interface between 36 and 60% sucrose and contained an RNA to RNA-plus-protein content of 0.14. Ribosomes (microsomes treated with deoxycholate) have an RNA content of about 0.50. In terms of amino acid incorporation per milligram of RNA, there was no significant difference with the three preparations. Use of "natural" polysomes or microsomes from immunized animals in experiments designed to bind these particles to sheep erythrocytes in immunological assay indicated that the particles were not "soluble" above 700 × *g* after incubation. Thus, particles having hemagglutinin and bound to sheep erythrocytes could not be separated from autoagglutinating particles. It was found also that microsomes and polysomes could not be stored in liquid nitrogen for more than a few days. We are abandoning the use of "natural" polysomes, therefore, and are using ribosomes obtained by treatment of microsomes with deoxycholate where these difficulties are not encountered.

High-molecular-weight RNA is being isolated from the nuclear fraction of spleens by the method of Georgiev *et al.*[3] These preparations are being injected into nonimmunized animals. Antibody production in the recipients will be determined in serum and in cells of spleen and lymph node by the method of Jerne and Nordin.[4] These preparations will be tested also in the cell-free system to determine if they contain "message" for antibody synthesis.

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17.10 ISOLATION AND PURIFICATION OF THE ISOLEUCINE-ACTIVATING ENZYME

M. P. Stulberg

Introduction. – Since our last report,[1] we have decided to concentrate our efforts on the purification of the isoleucine-activating enzyme (IAE) from *E. coli*. The purification as yet has not reached a state where we can proceed to answer the questions posed in the last report. However, some of the unique properties of the enzyme have been analyzed, and basic problems of recovery of activity upon fractionation have been resolved.

Results and Discussion. – IAE, as well as amino-acid-activating enzymes in general, is particularly labile during fractionation. The use of high concentrations of mercaptoethanol and EDTA during fractionation and dialysis steps has allowed for increased recoveries. Storage of the enzyme is improved by the addition of 25% glycerol, especially with dilute protein solutions. These stabilization procedures have allowed for recovery of more than 90% of enzyme activity after column chromatography.

An enzyme fraction has been prepared that is approximately 100-fold purified, and this preparation has been utilized for an examination of the IAE reaction properties. In the presence of optimal concentrations of all reagents, the K_m for isoleucine is approximately 2.5×10^{-6} mole/liter at 25°C and pH 8.0. Optimal ATP concentration is 0.001 *M* under the same conditions, where the Mg^{2+}/ATP is 10. At the present time, we are examining the effect of L-O-methylthreonine on activation and transfer of isoleucine to transfer RNA. This analog inhibits the incorporation of isoleucine into protein and may be of use in enzyme mechanism studies.

We have worked out, individually, further steps in the IAE purification and are now in the process of combining these steps for a large-scale high-purification procedure.

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17.11 ISOLATION AND PURIFICATION OF SPECIFIC TRANSFER RNA FROM *E. COLI*

A. D. Kelmers[1] G. D. Novelli
M. P. Stulberg

Introduction. – The objective of this program has been outlined in our last report.[2] Since that time, significant progress has been attained in the resolution of specific amino-acid transfer RNA's (tRNA) of *E. coli*.

Results and Discussion. – Large amounts of crude, active tRNA have been prepared from *E. coli* grown in the pilot-plant facility. A combination of phenol extraction, selective isopropanol precipitation, and DEAE column chromatography has produced sufficient quantities of tRNA to allow for further procedures of purification and isolation.

Preliminary experiments to investigate the applicability of reverse-phase column chromatography to separation of amino-acid-specific tRNA's have been encouraging. The columns consist of an immobile phase of high-molecular-weight quaternary ammonium compounds in an organic solvent fixed to an inert support of treated diatomaceous earth. The mobile phase consists of tRNA, H_2O, and varying concentrations of NaCl. A compatible system for tRNA fractionation was determined only after repeated trials with liquid-liquid partition combinations of ion exchangers, solvents, and extraction solutions.

The most recent fractionation system consisted of a column of dimethyl-dilauryl-ammonium chloride (ion exchanger) dissolved in amyl acetate and fixed on chromosorb G. One hundred milligrams of crude tRNA was added to the column and eluted with a convex-concave gradient of NaCl from 0.24 to 1.00 *M*. The 300-tube fractionation resulted in a significant A_{260} resolution of the tRNA. Biochemical activity assays have shown that some of the specific RNA's have been purified at least twentyfold. Several sRNA's have been separated into more than one peak; for example, leucine tRNA has been resolved into at least five separate peaks of activity. These experiments indicate that this procedure may prove to be more successful than those presently being employed such as countercurrent distribution, molecular sieving, and other chromatography systems.

This collaborative effort between the Chemical Technology and Biology Divisions is now being further supplemented by the assistance of the Analytical Chemistry Division. Their initial efforts are directed towards improving the techniques involved in the biochemical assays of amino acyl–tRNA formation. Since the assay is the most time- and labor-consuming step in the tRNA separation procedure, its simplification and automation will be of immeasurable aid.

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17.12 RNA POLYMERASE FROM CORN TISSUES

R. J. Mans

Introduction. – Studies on the DNA-dependent ribonucleotide-incorporating activity derived from maize seedlings[1] have been extended to include: (1) characterization of the product of the reaction and (2) the appearance of activity in the course of kernel maturation.

Results and Discussion. – 1. Incubation of 1.5 mg of high-speed supernatant protein from maize seedlings with 5 micromoles of each of the ribonucleoside triphosphates (ATP-8-^{14}C as label), 500 μg of calf thymus DNA, 50 micromoles of magnesium acetate, 50 micromoles $(NH_4)_2SO_4$, 100 micromoles 2-mercaptoethanol, and 200 micromoles Tris (pH 8.0) at 30° results in the incorporation of 1 millimicromole labeled nucleotide into acid-precipitable material in 30 min. All of the acid-precipitated radioactivity is rendered acid soluble after incubation in 0.3 *M* KOH at 37° for 9 hr. The addition of 50 μg ribonuclease to the incubation mixture after 30 min will release 94% of the radioactivity after 30 min additional incubation. The lability of the radioactivity of enzymatic degradation and alkaline conditions suggests the

product may be a polyribonucleotide. More definitive information was obtained by preparing a reaction mixture containing α-labeled $GT^{32}P$ as the radioactive nucleotide. After 30 min incubation, 2 mg of yeast RNA was added as carrier and the radioactive product isolated by phenol extraction and alcohol precipitation.[2] After exhaustive dialysis the product was hydrolyzed with 0.3 *M* KOH at 37° for 18 hr. The acidified hydrolysate was passed over activated charcoal[3] and resolved by paper electrophoresis.[4] Radioactivity was recovered in each of the four ribonucleoside-2′,3′-phosphates derived from the product. This result suggests, but does not prove, that the incorporation is into inner linkages of an oligonucleotide chain. It is inferred from the retention of the product inside a dialysis bag for 48 hr and by its lack of retention on G-50 Sephadex that the product has a molecular weight greater than 5000. Preliminary nearest-neighbor analyses on products formed in the presence of various DNA's indicate that the nucleotide composition of the product complements that of the added DNA. These data on the nature of the product, taken with the requirements for and characteristics of the reaction,[5] indicate that the DNA-dependent nucleotide-incorporating activity is an RNA polymerase.

2. It has been observed that the ability of preparations from corn kernels to incorporate amino acids is related to the degree of maturation of the pollinated kernel.[6] Preliminary experiments indicate that a similar relationship between age and level of activity pertains to the RNA polymerase detected in this tissue. An experiment designed to determine the sequence of appearance of the nucleotide and amino-acid-incorporating activities in developing kernels is now in progress.

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17.13 STUDIES ON THE EFFECTS OF LIGHT ON PROTEIN SYNTHESIS IN PLANTS

G. R. Williams G. D. Novelli

Introduction. – Effects of preillumination on the activity of a subsequently prepared cell-free amino-acid-incorporating system from etiolated seedlings have been studied further. Much of the earlier work with maize[1] has been confirmed in bean and soybean.[2] In addition, some effects of light on RNA metabolism in etiolated bean leaves have been examined.

Results and Discussion. – The amino-acid-incorporating activity of ribosomal preparations from the leaves of dark-grown beans or soybeans is stimulated almost 200% by exposing the plants to an hour of illumination 3 hr before preparing the particles. Under similar conditions, a stimulation of 60 to 70% has been routinely found when ribosomes are obtained from treated maize shoots.[1] Repeated attempts to demonstrate a similar light effect upon detached maize shoots and detached bean leaves were all unsuccessful regardless of the medium used for tissue incubation. Parallel measurements of chlorophyll synthesis under conditions of continuous light showed that the rate and extent of chlorophyll development were seriously impaired in the detached tissue.

The effect of light on RNA synthesis has been examined using ^{32}P. After injection into the stem, the isotope was rapidly translocated into dark-grown bean leaves, and significant labeling of the leaf RNA was found in less than 2 hr. Preliminary experiments using the thermal phenol method[3] for RNA fractionation showed that the level of an RNA fraction of high specific activity (obtained at 85° in the presence of detergent) was enhanced by light treatment. Base analysis indicated that this fraction had a higher (A + U)/(G + C) ratio than any other RNA fraction obtained. Upon density gradient centrifugation, this portion of the RNA was found to sediment in a broad band which peaked between 7S and 18S.

Depending upon the duration of the treatment period, the major portion of the extracted RNA (obtained at 4°) may or may not be labeled significantly, and the extent of labeling may or may not be increased by light treatment. Base analysis of this fraction indicated a low (A + U)/(G + C) ratio, and the sedimentation profile indicated that

three major peaks were obtained at approximately 4S, 18S, and 29S.

The effect of light on RNA synthesis will be pursued further in an attempt to characterize the nature of each fraction and the kinetics of the light effect upon its synthesis. In addition, an attempt will be made to localize the site of RNA synthesis in response to light treatment and to assess the respective roles (if any) of nuclear and chloroplast DNA in the process.

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17.14 PHYSICAL CHEMICAL STUDIES ON ADENOSINE 5′-PHOSPHOSULFATE REDUCTASE

A. P. Pfuderer H. D. Peck, Jr.
D. R. Holladay

Introduction. – The enzyme adenosine 5′-phosphosulfate reductase (APS reductase) is an iron-containing flavoprotein from *Desulfovibrio desulfuricans* which catalyzes the reduction of APS to AMP + sulfite. Information on the physical parameters of APS reductase is of interest because of the uniqueness of this reaction.

Results and Discussion. – The partial specific volume of APS reductase, a necessary prelude to sedimentation analysis, is listed in the table that follows. Each point is the average of two determinations.

Determination	Concentration (mg/ml)	$\bar{V}$ (ml/g)
1	2.9	0.747
2	4.4	0.760
3	25.8	0.756

Average = 0.754 ± 0.010

The sedimentation coefficient of the enzyme was found to increase from 10.5 to 12.5S as the temperature was lowered over the range 20°–0°C, but to be essentially constant from 20 to 30°C. The value of the sedimentation constant at infinite dilution at 26.3°C was $S^0_{20,w}$ = 10.6S.

Two molecular weight determinations at a protein concentration of 4.5 mg/ml on a preparation essentially free of aggregate[1] by using the short column equilibrium procedure of Van Holde and Baldwin[2] yielded a distribution of molecular weights from 125,000 to 150,000, with the preponderant species being 149,000. Further dilutions indicate that this value is concentration dependent.

Flavin analyses on the enzyme indicate 1 FAD molecule per 140,000–150,000 molecular weight, while a diffusion constant determined previously[1] combined with the above sedimentation constant yields an $M_{s,D}$ = 149,000.

In the presence of *p*-hydroxymercuribenzoate (pCMB), sodium dodecyl sulfate (SDS), or low pH, APS reductase loses its characteristic yellow color, and its sedimentation constant decreases markedly to values near 4S. This evidence was taken to indicate the presence of subunits.

Molecular weight studies in 0.01 *M* SDS led to a value of 20,000 for the molecular weight of the subunit at low ionic strengths. Comparable studies on crystalline ribonuclease and bovine serum albumin indicate that the apparent molecular weight of the highly charged detergent-protein complex is markedly dependent on ionic strength, so the above value should be regarded as a lower estimate of the subunit size.

All molecular weights have been corrected for bound SDS, using the synthetic boundary cell in the ultracentrifuge as a precision refractometer to determine SDS binding.

Iron analyses of the enzyme indicate from five to six irons per FAD, leading to approximately 24,000 to 30,000 for the smallest iron-containing unit.

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17.15 INACTIVATION OF MESSENGER RNA BY ULTRAVIOLET IRRADIATION

P. A. Swenson R. B. Setlow[1]

Exposure of *E. coli* cells for a short time to an inducer for β-galactosidase is known to cause the formation of a specific messenger RNA (mRNA) for this enzyme. The mRNA expresses itself in enzyme formation in the absence of inducer and also decays away with a half-life of 1 min. We reasoned that after withdrawal of inducer, low-dose ultraviolet radiation effects on β-galactosidase formation should be the result of damage to mRNA. *E. coli* B/r cells were exposed for 20 sec to a high concentration (0.4 m*M*) of inducer (IPTG). The inducer was effectively removed by a 50-fold dilution of the cells, and the suspension was irradiated immediately with various doses of monochromatic (2650 A) radiation. The ultraviolet prevents the mRNA from expressing itself in β-galactosidase formation. The 1/*e* dose for inactivation of what we call mRNA is 180 ergs/mm^2. This compares with a value of 100 ergs/mm^2 for inhibition of β-galactosidase formation by cells irradiated prior to induction – a condition where one is presumably measuring the inhibition of the DNA-directed synthesis of specific mRNA. At the low doses used in these experiments, it seems unlikely that ribosomal or sRNA is affected. The activity of mRNA that survives irradiation decays away with the same kinetics as for unirradiated cells.

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Hormonal Control

Our analyses of the effect of steroid hormones on RNA synthesis in vivo have now demonstrated that, in the two systems examined, the hormones effect a generalized increase in RNA. As discussed in detail below, synthesis of both ribosomal and DNA-like RNA responds to hormonal stimulation, and that of transfer RNA may also be affected. Thus hypotheses invoking the gene-activation model of Jacob and Monod[1] as the locus of hormone action[2] are incorrect, or at least incomplete. These findings have prompted a reinvestigation of the specificity of adrenocortical hormones as enzyme inducers in liver and the derived Reuber hepatoma. Preliminary results suggest that induction may be simply a reflection of hormonal stimulation of general protein synthesis.

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17.16 HYDROCORTISONE STIMULATION OF NUCLEAR RNA SYNTHESIS[1]

D. L. Greenman F. T. Kenney

Introduction. – Previous work has shown[2] that the rate of nuclear RNA synthesis is stimulated in the liver by the administration of hydrocortisone to adrenalectomized rats and that this stimulation precedes the hormonal induction of synthesis of certain hepatic enzymes. If hormonal induction of enzyme synthesis is a specific gene-activation phenomenon, one might expect the hormonal stimulation of RNA synthesis to be rather specific in nature. However, data obtained in the study cited above suggested that overall synthesis of RNA is stimulated by administration of the hormone. In the present study, we have further analyzed the types of RNA that are synthesized as a result of hormone stimulation.

Results and Discussion. – Liver nuclear fractions obtained from rats injected with ^{32}P (1 mc) 20 min before killing were extracted by the phenol extraction method of Georgiev *et al.*[3] Briefly, the four fractions have been characterized as follows: fraction 1 (extracted at 4°) contains 5% of the radioactivity, most of which sediments with the 4s RNA component. The base composition is like ribosomal RNA. Fraction 2 (extracted at 45°) contains 25% of the radioactivity, which sediments with material that is 10s or greater. Base composition of this fraction is also like ribosomal RNA.

Fraction 3 (extracted at 65°) contains 40% of the ^{32}P label, which has a sedimentation coefficient of 20s or greater. The base composition appears to be a combination of ribosomal-like and DNA-like RNA with a predominance of ribosomal-like RNA material. Fraction 4 (extracted at 85° in the presence of sodium dodecyl sulfate) contains 30% of the ^{32}P, most of which sediments faster than 30s material. Base analysis suggests that 50% of this fraction is DNA-like in composition. The administration of hydrocortisone 2 hr before killing causes a twofold increase in the labeling of each fraction, confirming the previous observation that suggested that hormonal stimulation of RNA synthesis is a generalized rather than specific effect. Thus, it appears that hydrocortisone stimulates the synthesis of both ribosomal-like and DNA-like RNA. Presently work is being done to identify the functional nature of those types of RNA that are stimulated by hydrocortisone. Although fraction 4 appears to contain a large percentage of DNA-like RNA, attempts at functional definition of this fraction as containing messenger RNA-like activity in an in vitro amino-acid-incorporating system have failed. Attempts to identify the labeled material of fraction 1 through the use of DEAE-Cl^- paper chromatography have shown that, while most of the material sediments with 4s RNA in a sucrose density gradient, only 10–20% of the label migrates in 0.7 *M* NaCl on DEAE paper. Consequently, a large percentage of this rapidly labeled material appears to be something other than transfer RNA.

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17.17 EFFECTS OF TESTOSTERONE ON RNA SYNTHESIS IN RAT SEMINAL VESICLE[1]

W. D. Wicks F. T. Kenney

Introduction. – Previous work has shown that a large increase in the synthesis of RNA in the seminal vesicle of rats castrated 12–20 hr previously can be induced as early as 20 min after the injection of testosterone. Recent work has concentrated on fractionating the RNA of this organ in an attempt to determine the nature of the RNA species synthesized under the influence of hormone.

Results and Discussion. – The procedures employed for castration and for the injection of testosterone and ^{32}P have been described previously.[2]

After establishing the time course of the hormone effect on the synthesis of total RNA, the effect of actinomycin on this response was examined. Actinomycin D (20, 100, and 500 μg) was injected intraperitoneally 180 min before killing, some 130 min prior to ^{32}P. Whereas the lowest dose of actinomycin depressed the labeling of RNA in both control and treated animals to an equivalent extent (~50%), the larger doses obliterated the hormone response. The degree of labeling after 500 μg of inhibitor was given, although low (10–20%), was not significantly lower than that observed with 100 μg. This suggests the existence of some RNA labeling which is both hormone insensitive and actinomycin insensitive. Analysis of the distribution of ^{32}P in the nucleotides of an alkaline digest of this RNA indicated that terminal labeling of sRNA could not account for the insensitive process.

The hot-phenol extraction procedure of Georgiev *et al.*[3] has been modified for our studies on the fractionation of RNA. Considerable difficulties have been encountered, and the idealized separation of newly synthesized RNA into discrete types identifiable by their unique nucleotide distribution has not yet been realized. Nevertheless, fractions of RNA have been obtained which show certain distinct differences. Basically three fractions have been prepared: (1) tissue homogenate prepared in 0.14 *M* NaCl–0.5% deoxycholate extracted six times with 80% phenol at 0–5°, (2) resulting pellet and interphase extracted similarly at 60–80°, and (3) pellet and interphase extracted with 0.14 *M* NaCl–0.5% sodium lauryl sulfate at 80–90° or with 10% NaCl at 100°. Fraction 1 contains the bulk of the RNA (60–80%) and shows three peaks in a sucrose gradient typical of ribosomal and soluble RNA. Most of the ^{32}P is concentrated in the 4s region, but some is detectable in heavier regions. Fraction 2 generally contains 20–30% of the total RNA, and it exhibits a single peak

sedimenting at greater than 20s in a sucrose gradient. The distribution of ^{32}P is virtually the same as the optical density at 260 mμ. Fraction 3 appears to be similar to fraction 2 in a gradient with perhaps a higher percentage of heavier components.

The specific activity of fraction 3 is generally 15–30% higher than fraction 2 and three to six times that of fraction 1. Differences in the distribution of ^{32}P in the nucleotides of each of the three RNA fractions were disappointingly small and difficult to interpret in view of the work of Georgiev *et al.*[3] Although the significance of the lack of large differences in the base composition of the RNA in each fraction is unclear, it is plain that an increase in the labeling of all three fractions is observable 90 min after hormone is given. All of the experiments described have involved the administration of hormone 90 min and ^{32}P 50 min prior to killing. The hormone effect has been consistently greater in fraction 1 than in the other two, generally 75 to 150% above the control values.

Fraction 1 has been further fractionated into material which is soluble in 1 *M* NaCl and that which is not. The salt-precipitated material contains all but the 4s RNA with ^{32}P distributed in all tubes in a gradient but with a peak still at 4s. About 85% of the RNA and about 70% of the counts are insoluble. The salt-soluble RNA exhibits a single peak of RNA and ^{32}P sedimenting at 4s. Analysis of the distribution of ^{32}P in the nucleotides of the salt-soluble RNA indicated that terminal labeling of sRNA could not account for more than a small percentage of the ^{32}P found in this fraction. Analysis of the two fractions for the ability to accept amino acids revealed that 95–100% of the charging capacity of fraction 1 resides in the salt-soluble RNA. Studies involving chromatography of the two fractions on DEAE-Cl paper with 0.7 *M* NaCl yielded results similar to those just described. The injection of testosterone markedly increased the labeling of both fractions, but the effect seemed to be greater on the salt-insoluble RNA. The significance of these observations is currently under investigation.

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17.18 ENZYME INDUCTION IN RAT LIVER AND THE REUBER HEPATOMA[1]

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Introduction. – The results presented above make it clear that steroid hormones affect RNA synthesis in a very general fashion, a result difficult to reconcile with the selectivity of enzyme induction by hydrocortisone. It is possible that this selectivity is only apparent and that the response of specific enzymes is due, not to a specific effect of the hormone on selected regions of the genome, but rather to differences in turnover rates of various enzyme proteins. Thus a protein with a rapid turnover would respond to a general stimulation of protein synthesis by a rapid increase in amount relative to the total protein that turns over much more slowly. If this interpretation is correct, any means of increasing protein synthesis should lead to an induction of the same enzyme induced by hydrocortisone.

Results and Discussion. – The minimal deviation Reuber hepatoma responds to addition of hydrocortisone to the culture medium in much the same fashion as rat liver; specifically, there is a rapid induction of tyrosine-α-ketoglutarate transaminase.[3] In a study of this response it was found that some induction (two- to threefold) was observed if ethanol, the vehicle in which the steroid was dissolved, was added without the steroid. This induction by ethanol has been studied further in both the hepatoma in culture and in adrenalectomized rats. The response is very similar in these two test systems. Within 1½–2 hr after adding (or injecting) ethanol, the transaminase level is doubled. This phase of the response is very similar to that which follows hormone treatment. However, while induction continues at an increasing rate for 4 to 5 hr when hydrocortisone is given, induction by ethanol stops after 2 to 3 hr, and the enzyme level falls. Increasing the dose of ethanol or repeated administration does not change this result, and administration of pyruvate (to

assist in removal of acetaldehyde) is similarly ineffective. Actinomycin (100 μg/100 g) given before ethanol completely prevents induction, implicating RNA synthesis in the response. Experiments are in progress to determine if (1) ethanol administration increases RNA synthesis in the same fashion as hydrocortisone and (2) ethanol induces other enzymes known to be induced by hydrocortisone. In addition, a search is being made for other nonsteroid substances capable of inducing the rapid turnover enzymes of rat liver.

Interpretation of these experiments is difficult at this time. It would appear that in some unknown fashion ethanol is capable of stimulating hepatic protein synthesis, perhaps by a general increase in energy-yielding reactions. As predicted, this increase in protein synthesis leads to a relatively large increase in tyrosine transaminase because of the rapid turnover of this enzyme. Thus we tentatively conclude that hydrocortisone is not a specific enzyme inducer. Rather, the specificity of the response of various enzymes is "built in," being impressed upon the system by differing rates of enzyme turnover.

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Mammalian Cell Regulation

The reports on mammalian cell regulation constitute a continuing study of the lactic dehydrogenase isozymes. We are attempting to understand the reasons why some of the isozymes break up into subbands, as part of a study of the control mechanism responsible for the different distribution of the five major isozymes in different tissues during development.

17.19 LACTATE DEHYDROGENASE: MULTIPLE MOLECULAR FORMS

K. B. Jacobson P. J. Fritz
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Introduction. – Lactate dehydrogenase from mouse skeletal muscle consists of 15 or more electrophoretically separable forms.[1] The resolution of these multiple bands was shown to depend on pH, concentration of acrylamide in the gel polymer, and concentration of mercaptoethanol. The main question now is whether these many bands of enzyme activity are the result of alteration of the protein structure into a series of closely related forms or whether they indicate a series of proteins whose differences are due to an altered primary structure.

Results and Discussion. – Mouse muscle LDH has been purified by fractionation with ammonium sulfate and chromatography on DEAE cellulose and then on cellulose phosphate. Changes in the electrophoretic behavior or LDH were observed after exposure of the enzymes to ammonium sulfate. The migration rate was slower, and the pattern of subbands was altered. Subsequent fractionation on the ion exchange columns yielded a group of LDH subbands that were resolved adequately by electrophoresis but that again did not correspond to the subbands seen in patterns obtained from the LDH in muscle homogenates. These shifts of patterns indicate that the various forms of mouse muscle LDH are not stable structures but are subject to alteration by various treatments.

The purity of the LDH is such that some of the bands of LDH activity are matched by protein bands.

Another factor which affects the electrophoretic migration of some of the LDH bands was discovered. When an extract of liver was added to the purified mouse muscle LDH, there was an alteration in the electrophoretic migration of several of the subbands. In addition, there was also an appearance of LDH activity in an area of the gel not normally occupied by the purified enzyme. If the effects caused by such extracts are either organ specific or are specific for the LDH from certain organs, this observation may help explain the organ specificity of the LDH isozyme patterns.

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18. Microbiology

Enzyme and Tracer Studies on Bacterial Metabolism (18.1–18.3)

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18.1 EFFECT OF NEAR-ULTRAVIOLET IRRADIATION ON ELECTRON TRANSPORT IN *STREPTOCOCCUS FAECALIS*

M. I. Dolin

In an attempt to gain some insight into the function of *Streptococcus faecalis* quinone (SFQ), the ultraviolet sensitivities of several electron transport reactions of *S. faecalis* have been investigated. Such studies can yield presumptive evidence for quinone function, since quinones are among the most ultraviolet-labile of the electron transport cofactors.[1]

Earlier work from this laboratory[2] had shown that the DPNH-oxidizing enzymes of *S. faecalis* are labile to irradiation at 365 mμ and that the inactivations do not seem to be attributable to flavin destruction. In the present investigation, it has been shown that the energy-yielding keto acid oxidation system of *S. faecalis* is also labile to irradiation at 330–365 mμ. The inactivations are not reversed by any of the soluble cofactors of the system. Inactivation is not restricted to the DPNH oxidase portion of the oxidase system, since the inactivations are also obtained under conditions in which lipoic dehydrogenase is the terminal enzyme of the electron transport chain.

A particulate DPNH–cytochrome c reductase is approximately four times as labile to irradiation at 365 mμ as the keto acid oxidase system. In preliminary experiments, performed with the help of J. Jagger, it has been possible to obtain the photochemical inactivation spectrum for the particulate reductase. The spectrum does not match that of protein, flavin, or SFQ, but shows a peak at approximately 300 mμ. This is of interest since irradiation of pure SFQ in alcohol results in the rapid formation of a derivative showing absorption in the region of 300 mμ. The derivative has lost the β-alkenyl linkage, but still has some ability to act as an electron transport cofactor.

The data show that the photochemical inactivation spectrum of SFQ need not match the absorption spectrum of the intact quinone. It is possible that irradiation of an SFQ-containing enzyme could result in the rapid formation of an altered quinone which might still have electron transport activity. Loss of this function would occur more slowly. Inactivation of the particulate cytochrome c reductase would be consistent with such a mechanism; however, the ultraviolet-labile component of the system has not yet been identified unequivocally.

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18.2 LACTIC-MALIC TRANSHYDROGENASE

M. I. Dolin

Studies on lactic-malic transhydrogenase[1] have been continued. Fluorescence emission and excitation spectra have been determined and fluorescence titrations carried out with substrates and substrate analogs.

The data reveal that lactic-malic transhydrogenase is unique among pyridinoproteins in two respects. First, the NADH is firmly bound[1] and is not displaced from the enzyme by NAD or substrates. Second, the bound NADH does not undergo alternate oxidation to NAD and reduction to NADH during a catalytic cycle. (NAD can be formed when the enzyme is heated at 100°C at pH 10–11 for several minutes in the presence of bound keto acid.) The physiological mechanism of hydrogen transfer, however, involves the formation of a nonfluorescent NADH–keto acid complex, which is dissociated when the equivalent of a hydride ion is transferred from the reductant (a hydroxy acid) to the oxidant (a keto acid). The complex between keto acid and bound NADH may also be dissociated by prolonged dialysis, without the addition of a reductant. Either way of dissociating the complex restores the initial spectrum, fluorescence, and NADH content of the enzyme. Nonfluorescent complexes can also be formed with keto acids which are substrate analogs, but not substrates for the transhydrogenase activity of the enzyme.

Analysis of fluorescence titration data strongly suggests that the keto acid binding sites are not independent. The sites interact in such a manner that as the concentration of free keto acid increases, there is a progressive increase in either (1) the dissociation constant for the keto acid or (2) the quantum yield for fluorescence of the uncomplexed NADH. Since the transhydrogenase contains 3 to 4 moles of bound NADH per mole of enzyme,[1] that is, three to four keto acid binding sites, the implication is that three to four catalytic centers are located at a single "active site."

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18.3 FURTHER CHARACTERIZATION AND LARGE-SCALE PREPARATION OF A MONOSUBSTITUTED NAPHTHOQUINONE FROM *STREPTOCOCCUS FAECALIS* 10C1

R. H. Baum M. I. Dolin

Through the use of the Pilot Plant Facility[1] in this Division, larger amounts of the monosubstituted naphthoquinone, found in this laboratory[2,3] in *Streptococcus faecalis*, strain 10C1, have been isolated and purified. By passing twice through columns of Decalso, wetted with 1% (w/w) water, the quinone was obtained in a form pure enough to crystallize in the cold from absolute ethanol. This procedure has yielded about 14 mg of pure crystalline compound. This preparation will be used, shortly, in nuclear magnetic resonance and infrared spectroscopy studies to confirm the structure of the compound and reveal the nature of the side chain beyond the β-alkenyl linkage.

A redetermination of the molecular weight of the quinone gives a maximum molecular weight of 770. Reversed-phase chromatography[4] of the purified compound, compared to standards, indicates a chain length 45 carbons long – corresponding well with the observed molecular weight.

Preliminary evidence on yield of quinone obtained from the large-scale growth and isolation experiments indicates a 62% greater yield of quinone from cells grown aerobically than from cultures grown fermentatively. The quinone isolated from cultures grown both ways appears to be the same, chromatographically and spectrally.

We are currently undertaking experiments aimed at determining the location of the quinone within the *S. faecalis* cell. A membrane fraction has been prepared by standard procedures;[5–7] this fraction, as well as the supernatant fraction, is being assayed for quinone content. A comparison of the location of the quinone with that of several DPNH-oxidizing enzymes of *S. faecalis* may provide a clue as to the physiological role of the quinone in the cell.

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19. Plant Physiology and Photosynthesis

Plant Physiology (19.1–19.3)		Photosynthesis (19.4)	
A. H. Haber	Sarah A. Angel	W. A. Arnold	J. R. Azzi
D. E. Foard	Pat C. Kerr	W. F. Bertsch	C. W. Smith
T. J. Long[a]	N. Ann Payne	M. A. Kamrin[c]	Helen H. Arnold[d]
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19.1 DECREASED CAPACITY FOR GERMINATION AND INCREASED CAPACITY FOR MITOSIS WITHOUT GERMINATION AFTER STORAGE OF A SINGLE BATCH OF LETTUCE SEED

A. H. Haber Helen J. Luippold
Pat C. Kerr

Introduction. – Germination and cell division can be experimentally separated in moistened lettuce seeds.[1] Certain conditions can prevent germination without preventing mitosis and cell division in the "dormant" seeds.[2] This report describes the effects of aging of a single batch of stored lettuce seeds on their capacity to germinate under conditions favorable for germination and on their capacity to undergo mitotic activity under conditions where none of the seeds germinate.

Results and Discussion. – On each of five successive years (ending in 1964), seeds from the same batch of air-dry New York lettuce, stored in a closed bottle in the laboratory, were sown at 24°C under 60 ft-c of white light and at 28°C in darkness. On each of the first four days after sowing, percentage germination was counted under both conditions. On the first year 90% of the seeds had germinated within the four-day period at 24° under the white light. In succeeding years the germination percentages decreased steadily until by the fifth year none of the seeds germinated at 24°C under white light. At 28°C in darkness none of the seeds germinated on any of the five years. Mitotic activity was examined in the terminal 0.5 mm of the radicle tips in these ungerminated seeds at 28° in darkness. The occurrence of this mitotic activity increased as a function of the duration of seed storage. Whereas on the first year there was an average of 0.16 mitotic figure per radicle, by the fifth year there was an average of 5.1 mitotic figures per radicle. On the third day after sowing, the day of greatest mitotic activity, this frequency went from 0.45 figure per radicle on the first year to 10 figures per radicle on the fifth year. These results confirm that changes that occur as lettuce seeds age during storage can result in decreased germination percentage (sometimes called decreased "viability"). However, the results also show that during this aging, changes occur which actually enhance the capacity for mitotic activity under conditions where such mitotic activity cannot be causally influenced by germination. Thus the apparent "deterioration" of stored seeds need not necessarily represent a general deterioration of all cytologic processes. In addition to pointing to the complexities of the phenomena underlying aging in stored seed, these results provide additional evi-

dence for the lack of correlation between capacity of a given seed to germinate and capacity to undergo mitotic activity in the absence of germination.[3]

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19.2 INITIATION OF LATERAL ROOT PRIMORDIA WITHOUT ANY INCREASE IN CELL NUMBER

D. E. Foard N. Ann Payne

Introduction. – When wheat is germinated and grown on 0.03% colchicine, mitosis does not proceed beyond metaphase and no nuclear and cell divisions occur. Within the primary roots of these seedlings appear structures, called "primordiomorphs," that have the shape of lateral root primordia.[1] In primordiomorphs some nuclei repeatedly enter, but none complete mitosis. The primordiomorphs become large enough to compress the cortical parenchyma and make bulges in the epidermis of the parent root. In typical lateral root primordia comparable in size with primordiomorphs occur hundreds of cell divisions that are absent in the primordiomorphs. Primordiomorphs are found only in those precise regions where lateral roots are typically formed, that is, entirely in the pericycle and endodermis (each uniseriate) and only between successive protoxylem poles and facing a sieve tube. Thus primordiomorphs have a distinctive and necessary morphological characteristic of typical lateral root primordia.[1] The present study was undertaken to learn whether primordiomorphs have the definitive characteristic of lateral root primordia, namely, the potentiality to develop into lateral roots.

Results and Discussion. – After removal of colchicine the primordiomorphs develop with normal cell divisions into polyploid lateral roots as shown by the following findings: (1) stages of development intermediate between primordiomorphs and polyploid lateral roots having typical histological organization are detected; (2) the number of these polyploid lateral roots formed can be accounted for by the number of primordiomorphs which disappear; and (3) no "c-mitoses" occur (i.e., the diploid and polyploid nuclei undergo only normal mitoses). Since primordiomorphs have the potentiality (realized after removal of colchicine) to develop into lateral roots, primordiomorphs are lateral root primordia that developed without completion of mitosis and without cytokinesis. Since the parent root pericycle remains uniseriate, even in that region that comprises the primordiomorph, no periclinal cell division within the pericycle itself could have altered the polarization of growth to set up the apical-basal axis of the primordiomorph. These results demonstrate that the earliest detectable changes in form involved in the initiation of a lateral root primordium can occur without completion of any mitosis and without any cytokinesis.

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19.3 GIBBERELLIN ACTION ON GERMINATION OF EXCISED WHEAT EMBRYOS

T. J. Long Pat C. Kerr

Introduction. – Treatment with gibberellic acid (GA) increases the activity of α-amylase in wheat endosperm,[1] probably by stimulating *de novo* enzyme synthesis.[2] Because α-amylase converts the starch in the endosperm into carbohydrate more readily utilizable by the growing embryo, it has been suggested that GA promotes seedling growth in germinating cereals by means of the stimulation of α-amylase activity.[1] This report deals with GA effects on embryos in absence of endosperm.

Results and Discussion. – Embryos exclusive of scutella were excised from wheat grains and grown in darkness on a basal medium (mineral salts plus vitamins) supplemented with glucose. When no carbohydrate was added to the basal medium, no growth occurred in the excised embryos. Addition of GA to the basal medium plus glucose increased the growth rate of the excised embryos in culture. In embryos excised from grains that had been given 525 kr of ^{60}Co gamma rays, growth occurs without cell divisions. Thus with embryos excised from the irradiated grain, final size can also be studied

in culture. Addition of GA to the basal medium plus glucose increased the final size of the plantlets that developed from the embryos excised from the irradiated grains. These results indicate that GA can increase both growth rate and final size in absence of its actions on endosperm α-amylase and endosperm starch.

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19.4 PHOTOSYNTHESIS

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J. R. Azzi

For many years work in this laboratory has been aimed at understanding the energy-conversion steps of photosynthesis. Over the last three years we have been engaged in an intensive study of the details of the delayed light emission of living plants, because this dim glow affords a means of studying electron transitions over a wide time range within the functioning photosynthetic apparatus. We have now studied this emission over eight orders of magnitude in time, from 5×10^{-5} to 6×10^{3} sec. Although the physical mechanism of delayed light emission remains as unclear as the mechanism of photosynthetic energy conversion, there is ample evidence that the delayed light is closely associated with the mechanism of photosynthesis. Both processes have the same action spectrum. Both processes have an absorption cross section equal to 300–400 chlorophyll molecules, implying the existence of groups of chlorophyll molecules which act together as units. Both processes are heat inactivated at the same temperature, and poisons of photosynthetic energy conversion invariably have a drastic effect on the kinetics of delayed light emission. The turnover time for a photosynthetic unit is reflected in the effects of temperature on the delayed light decay. Both processes show a complex interaction between two pigment systems. It thus seems clear that an understanding of the delayed light would give deep insight into the physical basis of photosynthetic energy conversion.

We have just completed an emission spectrum of the delayed light emitted from the green alga, *Chlorella*, at 1.5×10^{-3} sec after the middle of a flash of exciting light. Since the delayed light is more intense at short times, this emission spectrum is more precise than the earlier ones, which were taken in the 1-sec time range. The spectrum is identical to that of fluorescence and shows a single peak of emission at 685 mμ, in agreement with the earlier work. The delayed light emission spectrum does not change on differential excitation of the two photosynthetic pigment systems. On the face of it, these results are most easily explained by suggesting that the delayed light is emitted from the bulk of chlorophyll *a*.

Although delayed light is closely associated with photosynthesis, we did not, until recently, have direct evidence that a complete functioning photosynthetic unit was necessary for the emission to take place. In collaboration with Dr. R. C. Clayton, Kettering Research Laboratory, Yellow Springs, Ohio, we have studied the delayed light emission at $1–5.5 \times 10^{-3}$ sec from a mutant of the photosynthetic bacterium, *Rhodopseudomonas spheroides*. This mutant, isolated by Sistrom, has a full complement of photosynthetic pigments but cannot perform photosynthesis because it lacks functional photosynthetic reaction centers. We found no delayed light from the mutant, although our instrument was capable of measuring 1/600 of the intensity which was emitted by the wild type. We interpret this finding as implying that photosynthetic reaction centers are part of the mechanism which results in delayed light emission, although our emission spectrum clearly shows that delayed light is not emitted from the reaction centers themselves at room temperature.

We have recently carried out quantum biochemical calculations by the method of molecular orbitals on a series of substituted chloroanilides which are powerful inhibitors of photosynthetic energy conversion. From delayed light measurements, and other evidence, these compounds all appear to act at a single site, which is very close to the short-wavelength photoreaction. Two of the compounds which are part of this series, CMU [Monuron, or 3-(*p*-chlorophenyl)-1,1-dimethylurea] and DCMU [Diuron, or 3-(3,4-dichlorophenyl)-1,1-dimethylurea], have been studied extensively by various workers in photosynthesis. It appears that these poisons block electron flow out of the short-wavelength photoreaction, but the mechanism of this action

has been completely unclear. We have been attempting to correlate various quantum mechanical parameters at different sites on these molecules with the effectiveness of the molecules in poisoning photosynthesis. Since we had about 100 different compounds which have been quantitatively assayed for effectiveness, and which cover a range of 10^5 in effectiveness, our correlations rest on an experimental foundation which is unusually complete for the field of quantum biochemistry. Using five quantum mechanical parameters, we found correlations with effectiveness of poisoning at only one site. The free valence of the number 2 position of the chlorobenzene ring of these compounds correlates with the logarithm of effectiveness as a poison. This indicates that the chemical reactivity of this site is an important factor in determining the effectiveness of a given compound as an inhibitor of photosynthesis. The charge distribution at this site was also correlated with effectiveness, but this correlation was less strong than with free valence. We suspect, therefore, that the chemical reaction which takes place at the number 2 position is with a charged moiety. We are now doing calculations in an attempt to determine what kind of moiety might be reacting at the number 2 position of these poisons.

References

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20. Biophysics

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Radiation Biophysics (20.1–20.4) M. L. Randolph John Jagger Sohei Kondo[a,b] J. L. Hosszu R. S. Stafford	**Molecular Biophysics (20.5–20.7)** R. B. Setlow[a] L. G. Caro J. E. Donnellan, Jr. R. A. McGrath W. L. Carrier R. W. Williams Maria Schnös Carolyn R. Shaffer[c]
[a]Dual assignment. [b]Visiting investigator from abroad. [c]Student trainee.	**Cooperative AEC-NASA Biology Program (20.2)**

20.1 ELECTRON SPIN RESONANCE

M. L. Randolph

Introduction. – In preparation for more intensive studies of electron spin resonance (ESR) phenomena in native and irradiated biological and biochemical systems, instrumentation studies have been initiated primarily to increase the sensitivity of the Varian ESR spectrometer. The promising initial results indicate that much lower spin concentrations may be measured than had previously seemed possible.

Results and Discussion. – Several instrumental developments have been shown to work. (1) The circuitry for analog computation of areas under the absorption curves of ESR spectra has been modified from previous circuitry[1] to be compatible with the modern Varian instrument. For comparison of samples under identical conditions of microwave power, cavity Q, magnetic field, temperature, etc., a dual cavity with two equivalent sample positions and modulation coils run at different frequencies are used. Hence provisions have been made in the analog computer setup to perform simultaneous double integration of each of two signals and to furnish output values for base-line corrections. (2) A large increase in signal-to-noise ratio for stable radicals has been achieved by summing the signals of repetitive scans with a multichannel analyzer, as suggested by Klein and Barton.[2] (3) Operation of the dual sample cavity with both modulation coils run at the same frequency has been shown to have merit. If the coils are run in phase, the cavity packing factor is essentially doubled and signal-to-noise ratio almost doubled. Out-of-phase modulation has two uses: (*a*) with a sample in one section and matching sample tube in the other, extraneous signals arising from cavity imperfections or sample tube are eliminated; (*b*) using two signal containing samples, the difference in signals may be readily detected; for example, a small radiation-induced signal may be resolved from a large naturally occurring signal. A problem in phasing between modulation and detection circuits remains, but has been solved on paper. (4) A new solid-state diode detector (Philco L4154) in preliminary tests has yielded a small improvement in signal to noise. Further empirical adjustment of circuit parameters is needed to optimize use of this detector.

Conclusions. – Highly profitable ESR instrumental development has demonstrated greatly increased sensitivity and versatility of the spectrometer. The developments cited are undergoing final engineering and tests, as well as combinations of the specific improvements. As the overall result, the range of ESR experiments will be greatly extended.

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20.2 DOSIMETRY OF BETA-RAY IRRADIATION DEVICE FOR RADIATION SPACE-FLIGHT INTERACTION EXPERIMENT[1]

Sohei Kondo J. L. Hosszu

Introduction. – Dosimetric studies with the irradiation device for the radiation space-flight interaction experiment to be performed in the Gemini III flight are under way, both in terms of planned and standby doses to the test blood samples and in terms of external radiation to astronauts.

Description of Device. – The principal dosimetric feature of the device for irradiation of blood cells in the Gemini III vehicle[2] is that blood samples in 3-mm-deep plastic containers are sandwiched between pairs of ^{32}P plaques which consist of thin ^{32}P layers deposited on platinum disks, 40 mm in diameter and 0.076 mm thick, backed by 0.76-mm-thick aluminum plates.

Four graded activities (2.5, 1.875, 1.25, and 0.625 mc/plaque) were tested; duplicated sets were made for each activity, and the whole sandwich-type beta irradiators, 25 mc in total activity, were covered by the 1.25-mm-thick aluminum housing.

Results and Conclusions. – The total thickness of β-ray shielding materials is no less than 0.7 g/cm^2; hence no more than 2×10^{-3} of β particles could escape from the irradiation box. The efficiency of production of x photons by β particles absorbed in a target material is usually a few percent. Let the efficiency be 0.05. Then, combining with the above mentioned, we can estimate that the ratio of the number of β particles to that of x photons outside the irradiation box is no more than 0.03.

Figure 20.2.1 shows the spectrum of x rays emitted outside the irradiation box; the peak centering at 67 kev is the characteristic x rays from platinum, and the rest is mainly due to bremsstrahlung from platinum and partly due to x rays from aluminum. The β-ray spectrum did not show up under the present experimental conditions.

The assumption of 5% for the yield of x photon per β decay and the average energy of about 100 kev obtained from the x-ray spectrum lead to the estimate of 6 mr/hr at 10 cm from the box. This is in agreement with the exposure dose of 5.1 mr/hr measured by a 0.25-r Victoreen condenser chamber. The relative dose at x from a ^{32}P plaque was found to be approximated by $e^{-\nu x}$, ν being an effective absorption coefficient, so far as x is larger than the thickness (56 mg/cm^2) of the cover of the blood sample holder. The preliminary ν value obtained by 0.2-mm-thick silver-phosphate glass (made by Toshiba, Japan) was about 10 cm^2/g.

Although the compared reading of the Fricke solution against that of a calibrated Radocon chamber was 0.97 for ^{137}Cs gamma rays (E. B. Darden, Jr.), application of the Fricke dosimeter

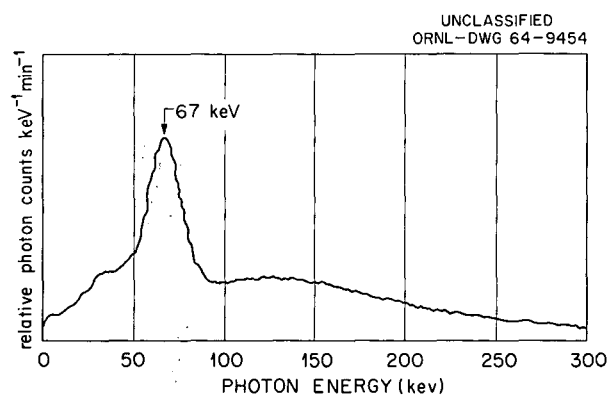

Fig. 20.2.1. Spectrum of X Rays Produced by ^{32}P Beta Particles in the Beta-Irradiation Box. Measurement was carried out by a ($1\frac{1}{2}$ in. in diameter and 1 in. long) NaI(Tl) scintillation detector with a 400-channel analyzer.

to the β-irradiation device has been unsuccessful because of the chemical interaction of the plastic used for the sample holders with the Fricke solution.

The theoretical calculation based on Loevinger's empirical formulas leads to about 500 rads/hr for the pair of 2.5-mc ^{32}P plaques, if backscattering effects of platinum disks and edge effects of ^{32}P plaques are neglected.

The dose rate in the sample holders when they are at standby positionings was about 10 rads/day.

References

[1]AEC-NASA Space Biology Program.

[2]M. A Bender, P. C. Gooch, and S. Kondo, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1964,* ORNL-3601, p. 114.

20.3 BIOPHYSICAL CONSIDERATION OF VARIATION IN RADIATION RESISTANCE WITH GENOME COMPLEXITY AND EVOLUTION

Sohei Kondo

Could there be any general principle specific to the biological field? Let us imagine that a primitive replicating system arose by chance at an early age of the Earth in the primitive sea under the influence of the solar uv. Such an event, which could be neglected in the statistical-mechanical sense because of its negligible probability of occurrence, cannot be neglected simply because the primitive cell under proper conditions could become, later on, one of the major components on the Earth through replication. Therefore, any effect on the capacity for cellular replication could have played a vital role in the history of evolution, especially, at the time of origin of life on the Earth.

A general biological principle might be found in the variation in resistance to radiation damage of the replication capacity among genomes of different complexity. It should, however, be noted that even if there was a single series of causes to give rise to a general law, such a biological law in the present-day form would be masked, more or less, by secondary biological variations because increase in variability is the characteristic of the Darwinian selection.

Since the natural selection works not at the molecular level but through the phenotypic characters of individual organisms, radiation resistance is compared in terms of 37% survival dose, D_{37}. The x-ray D_{37} decreases from 3×10^5 r (ϕX174) to 10^2 r (human cells) with increasing genome complexity while variation of uv D_{37} among genomes of different complexities is much smaller. Concerning the uv resistance, the Maximal Capacity Model and Solar UV Selection Model are proposed.

The details will be published soon (*Japan. J. Genetics,* suppl., 1964). In the prepared paper, variation in mutagenicity with genome complexity has also been discussed from similar evolutionary viewpoints, with emphasis on the introduction of the recent work carried out in Japan.

20.4 FURTHER STUDIES ON DIRECT AND INDIRECT PHOTOREACTIVATION IN VARIOUS STRAINS OF *ESCHERICHIA COLI*

John Jagger R. S. Stafford

Introduction. – It was earlier reported[1] that a mutant of *Escherichia coli* (strain B phr$^-$), which lacks the photoreactivating enzyme, shows a type of photoreactivation (PR) that occurs at 3341 A but not at 4047 A and that is indistinguishable in mechanism from the phenomenon of photoprotection. We have called this "indirect PR" because it probably acts indirectly by inactivating energy-supplying systems in the cell, thus inducing a growth-division delay, which in turn permits more time for dark recovery of ultraviolet (uv) damage to DNA. Evidence was also presented for the existence of indirect PR in the parent strain, B (Harm).

The present studies were undertaken in an effort to determine the relative contributions of direct PR (i.e., that which uses the PR enzyme) and indirect PR in various strains of *E. coli.* These studies are still under way, but some interesting findings have already been made.

Results. – *Thymine-Dimer Studies.* – Cells were grown in thymidine-methyl-^3H (tritiated thymidine) and subjected to various irradiation treatments, after which thymine dimers were extracted and

measured chromatographically. Procedures were similar to those described by Setlow, Swenson, and Carrier.[2]

Photoreactivation of strain B phr$^-$ at 3341 A was found not to decrease the concentration of thymine dimers induced in the bacterial DNA immediately after irradiation. This is consistent with our postulate that PR in this strain operates through an indirect mechanism.

Photoreactivation of the parent strain, B (Harm), at 4047 A causes a great decrease in thymine dimer concentration (eliminating about 75% of the dimers), while the same amount of PR at 3341 A causes only half as much dimer elimination. This suggests that PR at 3341 A is half direct and half indirect. Although these experiments indicate a lower contribution of indirect PR than was suggested by earlier work (which indicated about 80% indirect), it is nevertheless clear that there is a large component of indirect PR at this wavelength in the parent strain. The thymine-dimer studies therefore qualitatively corroborate our earlier conclusions[1] reached on the basis of studies on the macroscopic level.

Kinetic Studies. – Table 20.4.1 summarizes some results obtained by photoreactivation of various strains of *E. coli* at two wavelengths. All cultures were grown by shaking for 18 hr in nutrient broth at 36° from an initial concentration of 10^6 stationary cells per milliliter.

Strain B_{s-1} (Hill) shows no photoprotection and no evidence of dark repair ability. We therefore expect that the rate of PR at 3341 A will be considerably lower than at 4047 A, although the dose-reduction factor (factor of decrease of effective uv dose by maximum PR) should not change. The data confirm this expectation and suggest, as do our earlier studies,[1] that direct PR has a low efficiency at 3341 A.

Table 20.4.1. Photoreactivability of Strains of *Escherichia coli* in the Stationary Growth Phase

Strain	Dose-Reduction Factor		Rate[a] at 3341 A / Rate[a] at 4047 A
	3341 A	4047 A	
B/r (ORNL)	0.48	0.17	1.0
B (Harm)	0.16	0.14	
B_{s-1} (Hill)	0.20	0.20	0.3

[a]Rate at which photoreactivable cells are photoreactivated.

We would expect strain B/r (ORNL) to behave in the same way as B_{s-1} (Hill), because it is in the stationary phase where it shows no photoprotection or liquid holding recovery. One assumes that this strain has sufficiently efficient dark repair mechanisms that it is not aided by treatments, such as photoprotection or indirect PR, that enhance dark repair. Therefore, only direct PR is expected to be effective, and this would be reflected by a lower PR rate at 3341 A than at 4047 A. This lower rate is not observed. The results are complicated by the unexpected high dose-reduction factor at 3341 A, which means that there is much less photoreactivable damage at this wavelength. This behavior is not understood, and these studies are being pursued with the aim of reaching some comprehensive understanding of the varied PR behavior of these strains.

It has been shown[3] that in vitro PR of transforming principle, using an *E. coli* extract, shows an efficiency at 3341 A that is similar to that at 4047 A. Our observation of a lower efficiency of 3341 A in B_{s-1} (Hill) reflects the operation of the PR enzyme within a cell. Whether this means that (1) the enzyme has a different absorption spectrum in the cell, (2) the enzyme has a different action spectrum in the cell, or (3) there are competing processes in the cell is not known.

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20.5 THE DIMERIZATION OF CYTOSINE AND THYMINE RESIDUES IN POLYDEOXYRIBONUCLEOTIDES BY UV IRRADIATION

R. B. Setlow W. L. Carrier

Introduction. – Previous work in this and other laboratories has shown that uv-induced thymine

dimers can account for a large fraction of the biological action of uv on DNA. The dimers act as blocks to DNA synthesis. Radiation-resistant cells seem to owe their resistance to the ability to excise the dimers from their DNA.[1] Recently several model polydeoxyribonucleotides of known composition have become available to us through the courtesy of F. J. Bollum.[2] The analysis of the radiation products obtained by the uv irradiation of these polymers has led to the identification, in acid hydrolysates, of dimers of the form $\hat{UU}$ and $\hat{UT}$. These dimers arise from deamination of $\hat{CC}$ and $\hat{CT}$ in the irradiated polynucleotides.

Results and Discussion. – Two types of polymers, labeled with tritiated cytosine, were investigated: (1) a polymer (dI:dC) consisting of one chain deoxyinosinic and the other deoxycytidylic acid; and (2) synthetic DNA's made with the claf-thymus polymerase using DNA's of known composition as primers. Irradiation of dI:dC with monochromatic uv results in absorbance changes that (with a knowledge of the absorption spectra of various radiation products) indicate that the major reaction is

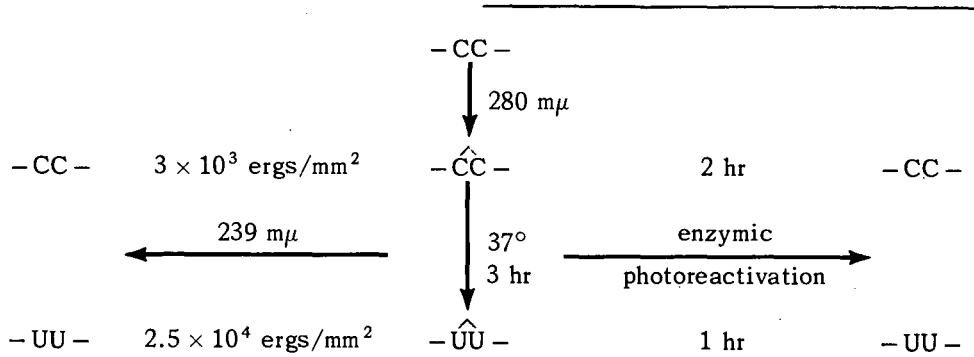
$$\text{—CC—} \xrightarrow{h\nu} \text{—}\hat{CC}\text{———} .$$

Cytosine dimers are not stable but are rather rapidly deaminated so as to yield uracil dimers:

$$\text{—}\hat{CC} \xrightarrow{\text{heat}} \text{—}\hat{UU}\text{—} .$$

Thus the radioactive products observed in an acid hydrolysate of irradiated dI:dC are $\hat{UU}$, U, and C. The uracil arises from the splitting of uracil dimers by radiation and not by a direct photochemical action on cytosine. The photochemical and thermal properties of these dimers are indicated in the following scheme, as is the fact that both $\hat{CC}$ and $\hat{UU}$ (as well as $\hat{TT}$) can be enzymically photoreactivated (work done in collaboration with J. K. Setlow[3]). The photoreactivation of $\hat{UU}$ in cytosine-labeled polymers results in the production of uracil residues, showing that photoreactivation is indeed the splitting of dimers.

The radiation products identified in hydrolysates of DNA's labeled in cytosine and thymine are $\hat{UU}$ (from $\hat{CC}$), $\hat{UT}$ (from $\hat{CT}$), and $\hat{TT}$. The relative abundance of these dimers in *E. coli* DNA irradiated with small doses are: $\hat{TT}$, 50%; $\hat{CT}$, 40%; and $\hat{CC}$, 10%.



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[1]R. B. Setlow and W. L. Carrier, *Proc. Natl. Acad. Sci. U.S.* **51**, 226 (1964).

[2]Molecular Biology Group; see Paper 14.2, this report.

[3]Molecular Photobiology Group; see Paper 1.6, this report.

20.6 PRODUCTION OF THYMINE DIMERS IN UV-IRRADIATED BACTERIAL SPORES

J. E. Donnellan, Jr. R. B. Setlow

Introduction. – Recent studies have implicated thymine dimers produced in the DNA of the bacterial genome as a major lesion in the death of ultraviolet (uv) irradiated bacteria.[1] Since thymine dimers cannot be formed in dry DNA, it appeared possible that the resistance of bacterial spores to uv irradiation might be caused by the lack of production of dimers in their DNA.

Materials and Methods. – The DNA of spores of *Baccilus megaterium* was labeled by growing

vegetative cells in medium containing ^{3}H-thymidine for 36 hr, at which time 80–90% of the population had formed spores. After washing the spores free of medium, a portion of the culture was irradiated with 10^4 ergs/mm^2 at a wavelength of 2652 A, a second portion serving as an unirradiated control. The samples were dried and hydrolyzed with formic acid in the presence of carrier DNA. The resulting hydrolysates were chromatographed and the chromatograms counted in a liquid scintillation counter.

Results. – In addition to radioactivity moving with the thymine spot, the unirradiated control contained three regions of radioactivity, one of which moved just behind the region expected for thymine dimers. These materials may have been produced by degradation and reutilization of the ^{3}H-thymidine by the cells during their long growth period. The irradiated sample contained an additional, poorly resolved, radioactive peak in the region expected for thymine dimers. Attempts to separate and characterize this photoproduct by two-dimensional chromatography have so far proved unsuccessful. While a quantitative estimate of the number of dimers in this region (if indeed they are dimers) had not been possible, the amount is comparable to that usually found in the DNA of vegetative cells so far studied and is certainly greater than would be expected from consideration of the uv resistance of these organisms.

References

[1]R. B. Setlow, "Molecular Changes Responsible for Ultraviolet Inactivation of the Biological Activity of DNA," in *Special Topics in Radiobiology*, Pergamon, New York, 1964.

20.7 DEGRADATION OF DNA IN IRRADIATED BACTERIA

R. A. McGrath R. W. Williams

Introduction. – In this study, we are concerned with the initial damage and the degradative changes which occur within the first cell generation time after x-ray exposure. Two strains of bacteria were used: *E. coli* (B_{s-1}), which is sensitive to x rays, uv light, and radiomimetic compounds; and (B/r), a strain more resistant to each of these insults.

Materials and Methods. – Bacteria were labeled with ^{3}H-TdR and then exposed to x rays. Cells were fixed, washed, and counted on planchets in a gas flow counter. Acid-insoluble radioactivity remaining in the bacterial DNA was used as a measure of the amount of damage produced by irradiation.

Results and Discussion. – During the first 40–50 min (at 37°C) after x-ray exposures between 25 and 100 kv a characteristic amount of the labeled material is lost from each strain. Approximately 80% loss occurs from B_{s-1} and 20–30% is lost from B/r. Results of a typical experiment are shown in Fig. 20.7.1. Chromatographic analysis of the products released into the culture medium after irradiation showed a large amount of material with the chromatographic mobility of thymine and small amounts with that of a TDP. To determine whether losses were a result of lysis the cells were labeled with ^{3}H-uridine (an RNA precursor) and exposed to x rays. Under these conditions a maximum loss of 7% was seen in B_{s-1}, the more sensitive strain. Since RNA was not released from irradiated bacteria in appreciable amounts it is clear that gross lysis of the culture has not occurred. Since degradation of

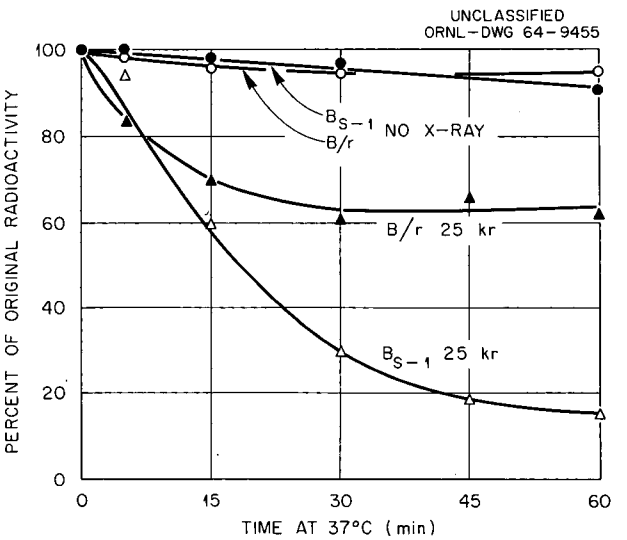

Fig. 20.7.1. Relationship Between Loss of Radioactivity from Prelabeled Bacteria and Time of Incubation at 37°C. One hundred percent radioactivity is equal to approximately 2000 counts per min per 10 μl of sample.

DNA does not occur during 4 hr at ice-bath temperature it is evident that losses are a result of some metabolic process and not a diffusion effect. We speculate that loss of labeled material from DNA is a result of enzymatic degradation which begins at the site(s) of damage. A simple model which assumes that enzymatic degradation begins at any site of damage and continues to the end of the DNA chain is untenable because, as x-ray exposures are increased, this process ultimately results in degradation of all DNA. The possibility that some damaged sites are undergoing a repair-like process or that only certain molecular loci on the DNA can act as foci for enzymatic degradation are possible explanations which are currently being considered.

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ORNL-644	Period Ending February 15, 1950
ORNL-727	Period Ending May 15, 1950
ORNL-807	Period Ending August 15, 1950
ORNL-889	Period Ending November 10, 1950
ORNL-969	Period Ending February 10, 1951
ORNL-1026	Period Ending May 10, 1951
ORNL-989	Period Ending August 10, 1951
ORNL-1167	Period Ending November 10, 1951
ORNL-1244	Period Ending February 10, 1952
ORNL-1297	Period Ending May 10, 1952
ORNL-1393	Period Ending August 10, 1952
ORNL-1456	Period Ending November 10, 1952
ORNL-1497	Period Ending February 10, 1953
ORNL-1614	Period Ending August 15, 1953
ORNL-1693	Period Ending February 15, 1954
ORNL-1776	Period Ending August 15, 1954
ORNL-1863	Period Ending February 15, 1955
ORNL-1953	Period Ending August 15, 1955
ORNL-2060	Period Ending February 15, 1956
ORNL-2155	Period Ending August 15, 1956
ORNL-2267	Period Ending February 15, 1957
ORNL-2390	Period Ending August 15, 1957
ORNL-2481	Period Ending February 15, 1958
ORNL-2593	Period Ending August 15, 1958
ORNL-2702	Period Ending February 15, 1959
ORNL-2813	Period Ending August 15, 1959
ORNL-2913	Period Ending February 15, 1960
ORNL-2997	Period Ending August 15, 1960
ORNL-3095	Period Ending February 15, 1961
ORNL-3201	Period Ending August 15, 1961
ORNL-3267	Period Ending February 15, 1962
ORNL-3352	Period Ending August 15, 1962
ORNL-3427	Period Ending February 15, 1963
ORNL-3498	Period Ending August 15, 1963
ORNL-3601	Period Ending February 15, 1964