BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

FOR PERIOD ENDING AUGUST 15, 1962

ORNL-3352

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

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director

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# Publications and Lectures

## PUBLICATIONS

A total of 350 papers and abstracts and 7 book reviews by members of the Biology Division have been published or are in press during this report period. This figure compares with 318 for the same period in 1961 and with 337 for the February 1962 report period. The 89 published papers and 3 book reviews are listed below. The 45 published abstracts and the 224 papers and abstracts and 4 book reviews in press are not listed.

The 1962 Gatlinburg Symposium, *Specificity of Cell Differentiation and Interaction*, will be published by the Wistar Institute of Anatomy and Biology as an October supplement to the *Journal of Cellular and Comparative Physiology*.

<table>
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<th>AUTHOR(S)</th>
<th>TITLE OF ARTICLE</th>
<th>PUBLICATION</th>
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<tr>
<td>Adler, H. I., and R. K. Clayton</td>
<td>The lethal effects of X-irradiation and of hydrogen peroxide on wild-type and on a high-catalase mutant of <em>Rhodopseudomonas spheroides</em></td>
<td><em>Radiation Research</em> 16, 746-52 (1962)</td>
</tr>
<tr>
<td>Adler, H. I., and J. C. Copeland</td>
<td>Genetic analysis of radiation response in <em>Escherichia coli</em></td>
<td><em>Genetics</em> 47, 701-12 (1962)</td>
</tr>
<tr>
<td>Barnett, W. E., and F. J. de Serres</td>
<td>Modification of EMS-induced reversion frequencies in <em>Neurospora</em> with enriched plating media</td>
<td><em>Neurospora Newsletter</em> 1, 4 (1962)</td>
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<tr>
<td>AUTHOR(S)</td>
<td>TITLE OF ARTICLE</td>
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<tr>
<td>Bezkorovainy, Anatoly, and D. G. Doherty</td>
<td>Isolation and characterization of an acidic glycoprotein from normal bovine plasma</td>
<td>Arch. Biochem. Biophys. 96, 491–99 (1962)</td>
</tr>
<tr>
<td>Brockman, H. E., and F. J. de Serres</td>
<td>Technical notes: Viability of Neurospora conidia from stock cultures on silica gel</td>
<td>Neurospora Newsletter 1, 8 (1962)</td>
</tr>
<tr>
<td>Celada, Franco, and Rachel R. Carter</td>
<td>The radiosensitive nature of homograft-rejecting and agglutinin-forming capacities of isolated spleen cells</td>
<td>J. Immunol. 89, 161–69 (1962)</td>
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<tr>
<td>AUTHOR(S)</td>
<td>TITLE OF ARTICLE</td>
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<tr>
<td>de Serres, F. J. (Editor)</td>
<td>A histologic study of the foreign spleen reaction</td>
<td>Radiation Research 17, 133–39 (1962)</td>
</tr>
<tr>
<td>de Serres, F. J.</td>
<td>Liver changes in sublethally irradiated mice undergoing the foreign spleen reaction</td>
<td>Neurospora Newsletter 1, 9 (1962)</td>
</tr>
<tr>
<td>Detwiler, T. C., T. T. Odell, Jr., and T. P. McDonald</td>
<td>A simple device for rapid preparation of conidial suspensions of Neurospora</td>
<td>Neurospora Newsletter 1, 10 (1962)</td>
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<tr>
<td>AUTHOR(S)</td>
<td>TITLE OF ARTICLE</td>
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<tr>
<td>Goodman, Joan W., and G. S. Hodgson</td>
<td>Evidence for stem cells in the peripheral blood of mice</td>
<td>Blood 19, 702-14 (1962)</td>
</tr>
<tr>
<td>Grell, Rhoda F.</td>
<td>The chromosome</td>
<td>J. Tenn. Acad. Sci. 37, 45-53 (1962)</td>
</tr>
<tr>
<td>Grimm, F. C., and D. G. Doherty</td>
<td>Nature of the coenzyme binding site of bovine malate dehydrogenase (preliminary note)</td>
<td>Biochim. et Biophys. Acta 57, 381-83 (1962)</td>
</tr>
<tr>
<td>Hodgson, G. S.</td>
<td>Erythrocyte Fe(^{59}) uptake as a function of bone marrow dose injected in lethally irradiated mice</td>
<td>Blood 19, 460-67 (1962)</td>
</tr>
<tr>
<td>Hollaender, Alexander</td>
<td>Initial steps in radiation damage to chromosomes and means of preventing this effect</td>
<td>In The Initial Effects of Ionizing Radiations on Cells (ed. by R. J. C. Harris), Academic Press, London, 1961, pp 201-09</td>
</tr>
<tr>
<td></td>
<td>Recent developments in the border fields of basic biology</td>
<td>In Proceedings of the International Symposium on Tissue Transplantation (ed. by Alberto P. Cristofanini and Gustavo Hoecker), University of Chile Press, Santiago, 1962, pp 17-21</td>
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<tr>
<td>AUTHOR(S)</td>
<td>TITLE OF ARTICLE</td>
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<tr>
<td>Kimball, R. F., and D. M. Prescott</td>
<td>Deoxypenturonic acid synthesis and distribution during growth and amitosis of the macronucleus of <em>Euplotes</em></td>
<td><em>J. Protozool.</em> <strong>9</strong>, 88–92 (1962)</td>
</tr>
<tr>
<td>Kluss, B. C.</td>
<td>Electron microscopy of the macronucleus of <em>Euplotes eurystomus</em></td>
<td><em>J. Cell Biol.</em> <strong>13</strong>, 462–65 (1962)</td>
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<tr>
<td>Phares, E. F., Mary V. Long, and S. F. Carson</td>
<td>An intramolecular rearrangement in the methylmalonyl isomerase reaction as demonstrated by positive and negative ion mass analysis of succinic acid</td>
<td>Biochem. Biophys. Research Commun. 8, 142–46 (1962)</td>
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<tr>
<td>AUTHOR(S)</td>
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<tr>
<td>Thompson, P. E.</td>
<td>Asynapsis and mutability in Drosophila melanogaster</td>
<td>Genetics 47, 337–49 (1962)</td>
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<td></td>
<td><em>Radiation Protection</em> [International Commission on Radiological Protection (ICRP), Report of Committee III on Protection Against X-Rays up to Energies of 3 Mev and Beta- and Gamma-Rays from Sealed Sources], Pergamon Press, New York, 1960, vi, 84 pp, 22 illustrations ($3.00)</td>
<td>Am. Scientist 50, 70A (1962)</td>
</tr>
<tr>
<td>Welshons, W. J., and Elizabeth S. Von Halle</td>
<td>Pseudoallelism at the Notch locus in Drosophila</td>
<td>Genetics 47, 743–59 (1962)</td>
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## LECTURES

During the period February to August 1962, members of the Biology Division presented 252 lectures and speeches compared with 168 for the corresponding period of 1961. Of these, 89 were before professional societies at home and abroad, 43 were given as part of the Traveling Lecture Program, and the remainder were presented on invitation of universities, laboratories, and scientific organizations.

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

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<thead>
<tr>
<th>SPEAKER [AND COAUTHOR(S)]</th>
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| Adler, H. I.               | Effects of radiation on microorganisms | 1. Spring Hill College, Mobile, Ala.  
2. Centenary College of Louisiana, Shreveport |
2. Loyola University, New Orleans, La.  
3. Northwestern State College of Louisiana, Natchitoches |
|                            | Physiological aspects of radiation sensitivity in bacteria | 1. Armour Research Foundation, Illinois Institute of Technology, Argonne  
2. University of Delaware, Newark |
|                            | Radiation microbiology (2 lectures) | NSF Summer Institute, Western Kentucky State College, Bowling Green |
|                            | Relationships of catalase, hydrogen peroxide, and ionizing radiation | Symposium on Implications of Organic Peroxides in Radiobiology, Argonne National Laboratory, Argonne, Ill. |
|                            | The response of bacteria to ionizing radiation | University of Richmond, Richmond, Va.  
Randolph-Macon Woman's College, Lynchburg, Va. |
Am. Soc. Microbiol. (62nd Annual Meeting), Kansas City, Mo. |
Forest College, Winston-Salem, N.C.  
Gordon Research Conference on Biochemistry and Agriculture, New Hampton, N.H. |
| Anderson, N. G.            | Countercurrents in biology | Assoc. Southeastern Biologists, Wake Forest College, Winston-Salem, N.C.  
Gordon Research Conference on Colloid and Surface Chemistry, Stanford University, Stanford, Calif.  
University of Michigan, Ann Arbor |
|                            | Integrated analytical systems |  
1. Molecular machines  
2. New approaches to cell fractionation  
3. The structure of living systems |
<p>|                            | The zonal ultracentrifuge: A new instrument for fractionating colloidal mixtures | 36th National Colloid Symposium (Division of Colloid and Surface Chemistry), Stanford University, Stanford, Calif. |</p>
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<th>SPEAKER [AND COAUTHOR(S)]</th>
<th>TITLE</th>
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| Arnold, W. A.              | First step in photosynthesis | 1. University of Florida, Gainesville  
2. University of Texas, Austin  
3. University of Houston, Houston, Tex.  
Solar Energy Symposium, University of Florida, Gainesville  
Vanderbilt University, Nashville, Tenn.  
University of Florida Medical School, Gainesville |
| Bollum, F. J. (and R. B. Setlow) | Nuclease-resistant sequences in UV-irradiated DNA [Federation Proc. 21, 374 (1962)] | Illinois State Normal University, Normal |
| Brockman, H. E.            | Phenotypic reversion in Neurospora | Assoc. Southeastern Biologists, Wake Forest College, Winston-Salem, N.C. |
| Carson, S. F.              | Dependence of ATP yield upon type of fermentation mechanisms: Consequences for available growth energy and current concepts of fermentation pathways (2 lectures) | University of Georgia, Athens |
| Chu, E. H. Y.              | 1. The cytogenetics of man and the mouse  
2. Cytology and evolution in primates  
3. Effects of X rays and ultraviolet on mammalian chromosomes  
Induction of mammalian chromosome aberrations by UV radiation | 1. Division of Medical Genetics, The Johns Hopkins Hospital, Baltimore, Md.  
2. UT-AEC Seminar, UT-AEC Laboratories, Oak Ridge  
College of Notre Dame, Baltimore, Md. |
|                           | 1. Mammalian cytology and cytogenetics  
2. Radiation effects on human chromosomes | 1. Division of Medical Genetics, The Johns Hopkins Hospital, Baltimore, Md.  
2. UT-AEC Seminar, UT-AEC Laboratories, Oak Ridge  
College of Notre Dame, Baltimore, Md. |
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<td></td>
<td>The configuration of pseudouridine and the preparation of its 0-4:C-5' anhydride ([Federation Proc. 21, 375 (1962)])</td>
<td>2. Massachusetts Institute of Technology, Cambridge</td>
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<tr>
<td>Congdon, C. C</td>
<td>Lymphatic tissue recovery in irradiated mice protected with AET</td>
<td>Conference on Bone Marrow Transplantation and Chemical Protection, Long Beach, Calif.</td>
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<td>Lymphatic tissues in radiation chimeras</td>
<td>UCLA, Los Angeles, Calif.</td>
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<td>Research careers in biology</td>
<td>Knoxville College, Knoxville, Tenn.</td>
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<td>Structural changes in lymphatic tissues during an immune response</td>
<td>California Institute of Technology, Pasadena</td>
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<td>Two variables that influence proliferation of germinal center cells during an immune reaction ([Federation Proc. 21, 273 (1962)])</td>
<td>Federation Am. Soc. Exptl. Biol. (Pathology Section), Atlantic City, N.J.</td>
</tr>
<tr>
<td>Congdon, C. C (co-author with A. L. Kretchmar, speaker)</td>
<td>Increased free aspartic acid in liver of mice with grafted homologous bone marrow ([Federation Proc. 21, 424 (1962)])</td>
<td>Federation Am. Soc. Exptl. Biol. (Immunology Section), Atlantic City, N.J.</td>
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<tr>
<td>Congdon, C. C (Joan W. Goodman, and J. W. Ferreebee)</td>
<td>Combined treatment of irradiated mice with bone marrow and thymus cells</td>
<td>Bone Marrow Transplantation and Protection Meeting, Atlantic City, N.J.</td>
</tr>
<tr>
<td>Cosgrove, G. E.</td>
<td>Mammalian Radiobiology</td>
<td>NAS-AEC Summer Institutes in Radiation Biology for Science Teachers</td>
</tr>
<tr>
<td></td>
<td>1. Lectures, demonstrations, laboratory sessions</td>
<td>1. Florida State University, Tallahassee</td>
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<td>2. Lectures, demonstrations, laboratory sessions</td>
<td>2. Wayne State University, Detroit, Mich.</td>
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<tr>
<td>SPEAKER [AND COAUTHOR(S)]</td>
<td>TITLE</td>
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<tr>
<td>Cudkowicz, Gustavo</td>
<td>Homotransplantation of irradiated hemopoietic and antibody-forming cells</td>
<td>University of Tennessee at Memphis</td>
</tr>
<tr>
<td></td>
<td>Studies on transplantation immunity</td>
<td>Institute of Tumor Biology, Stockholm, Sweden</td>
</tr>
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<td></td>
<td>Suppression of the foreign marrow reaction in mouse chimeras by preirradiation of donor cells</td>
<td>2nd International Congress of Radiation Research, Harrogate, England</td>
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<td>Membrane potential and electrolyte changes in irradiated tissues</td>
<td>UT-AEC Seminar, UT-AEC Laboratories, Oak Ridge, Tenn.</td>
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<tr>
<td>Dent, J. N.</td>
<td>Effects of thyroidal depression on the pituitary gland of the newt</td>
<td>Virginia Academy of Sciences, Norfolk</td>
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<tr>
<td>de Serres, F. J.</td>
<td>Factors influencing recombination and interference</td>
<td>University of Texas, Austin</td>
</tr>
<tr>
<td></td>
<td>Fine structure analysis of the ad-3 mutants of Neurospora</td>
<td>1. Dartmouth College, Hanover, N.H.</td>
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<td></td>
<td>Genetic fine structure analysis in Neurospora</td>
<td>2. University of North Carolina, Chapel Hill</td>
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<tr>
<td>Doherty, D. G.</td>
<td>Chemical protection</td>
<td>1. Incarnate Word College, San Antonio, Tex.</td>
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<tr>
<td></td>
<td>Chemical protection against ionizing radiation</td>
<td>2. St. Mary's College, San Antonio, Tex.</td>
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<td></td>
<td>Chymotrypsin studies</td>
<td>Am. Chem. Soc. (Section Meeting), New Orleans, La.</td>
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<td></td>
<td>Enzymes—the biological catalysts</td>
<td>Philadelphia College of Pharmacy and Science, Philadelphia, Pa.</td>
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<td></td>
<td>The hydrolysis of methyl amino- and hydroxyphenylpropionates by chymotrypsin and trypsin</td>
<td>Tulane University, New Orleans, La.</td>
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<td></td>
<td>Federation Proc. 21, 230 (1962)</td>
<td>1. Mississippi Southern College, Hattiesburg</td>
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<td></td>
<td>The role of a specific RNA in the cell-free synthesis of β-galactosidase [Federation Proc. 21, 411 (1962)]</td>
<td>1. Vanderbilt University, Nashville, Tenn.</td>
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<td>2. Oklahoma State University, Stillwater</td>
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<td>3. Tulane University, New Orleans, La.</td>
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<td>SPEAKER [AND COAUTHOR(S)]</td>
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<tr>
<td>Fränz, Jürgen</td>
<td>Free radicals produced by X-ray irradiation of organic acids and their peroxides</td>
<td>2nd International Congress of Radiation Research, Harrogate, England</td>
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<td></td>
<td>Preservation of functional white blood cells</td>
<td>Conference on Bone Marrow Transplantation and Chemical Protection, Long Beach, Calif.</td>
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<td>The proliferative and functional capacities of circulating white blood cells</td>
<td>Stanford University Medical Center, Palo Alto, Calif.</td>
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<td>Transplantation of white blood cells in irradiated mice</td>
<td>2nd International Congress of Radiation Research, Harrogate, England</td>
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<td>Biological effects of radiation</td>
<td>Tennessee Polytechnic Institute, Cookeville</td>
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<td>Gude, W. D. (and T. T. Odell, Jr.)</td>
<td>Megakaryocyte labeling with tritiated thymidine</td>
<td>Conference on Recent Developments in Studies of Blood Platelets, Biology Division, ORNL, Oak Ridge, Tenn.</td>
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<tr>
<td>Hollaender, Alexander</td>
<td>Acceptance speech at the University of Leeds</td>
<td>University of Leeds, England</td>
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<td></td>
<td>General discussion of the Biology Division program with emphasis on dimer formation, chromosome aberrations, mouse genetics, and somatic mutations</td>
<td>Istituto Superiore di Sanita, Rome, Italy</td>
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<tr>
<td></td>
<td><em>Messenger Lectures on Basic Problems in Radiation Biology</em></td>
<td>Cornell University, Ithaca, New York</td>
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<tr>
<td></td>
<td>1. Historical review of radiation biology</td>
<td>Stazione Zoologica, Naples, Italy</td>
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<td></td>
<td>3. Radiation microbiology</td>
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<td>4. Radiation cytology</td>
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<td></td>
<td>5. Mammalian radiation biology</td>
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<td>6. Speculation on the future of radiation biology</td>
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<td>New developments in radiation biology</td>
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<tr>
<td>Jacobson, K. B. (Kazuo Nishio, and A. C. Upton)</td>
<td>Lactic dehydrogenase in plasma of leukemic mice [<em>Federation Proc.</em> 21, 74 (1962)]</td>
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<td>SPEAKER</td>
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<tr>
<td>Jagger, John</td>
<td>How does ultraviolet cause the mutation and death of a cell?</td>
<td>1. Woman's College of the University of North Carolina, Greensboro</td>
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<td>2. Queen's College, Charlotte, N.C.</td>
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<td>3. Winthrop College, Rock Hill, S.C.</td>
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<tr>
<td></td>
<td>Life on other planets</td>
<td>16th Annual Eastern Colleges Sciences Conference, North Carolina State College, Raleigh</td>
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<td>1. Woman's College of the University of North Carolina, Greensboro</td>
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<tr>
<td>Kastenbaum, M. A.</td>
<td>A mathematical model describing two-break chromosome exchanges</td>
<td>Mathematics Panel Information Meeting, ORNL, Oak Ridge, Tenn.</td>
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<tr>
<td>Kenney, F. T.</td>
<td>Hormonal control of protein biosynthesis</td>
<td>St. Louis University School of Medicine, St. Louis, Mo.</td>
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<tr>
<td></td>
<td>2. Quantitative cytochemical studies on Paramecium</td>
<td>International Symposium on Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, The State University of Leiden, The Netherlands</td>
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<td>Plant Evolution Institute, Vanderbilt University, Nashville, Tenn.</td>
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<td>SPEAKER [AND COAUTHOR(S)]</td>
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<tr>
<td>Leonard, Martha R.</td>
<td>Capacity of spleen cells to respond to antigen and to proliferate after sublethal irradiation [ASB Bull. 9, 33 (1962)]</td>
<td>Assoc. Southeastern Biologists, Wake Forest College, Winston-Salem, N.C.</td>
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<tr>
<td>Lindsley, D. L. (Cloé Camba, and Mary Warters)</td>
<td>The correlation between radiation-induced male sterility and reciprocal translocation in Drosophila</td>
<td>2nd International Congress of Radiation Research, Harrogate, England</td>
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<tr>
<td>Makinodan, Takashi</td>
<td>Cellular changes in immune response</td>
<td>Japan National Institute of Health, Tokyo</td>
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<td></td>
<td>Mechanism of antibody formation</td>
<td>Department of Biochemistry and Biophysics, Tokyo University, Tokyo</td>
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<tr>
<td>Makinodan, Takashi (and J. F. Albright)</td>
<td>Cellular variation during immune response; one possible model of cellular differentiation</td>
<td>Symposium on Specificity of Cell Differentiation and Interaction, Gatlinburg, Tenn. (ORNL Biology Division Symposium)</td>
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<tr>
<td>Mans, R. J.</td>
<td>1. Amino acid incorporation by cell-free preparations from maize</td>
<td>Florida State University, Tallahassee</td>
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<tr>
<td></td>
<td>2. Application of radioisotopes to study of biological problems</td>
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<td></td>
<td>3. A liquid scintillation counting technique for macromolecules</td>
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<td>4. Protein synthesis in plants</td>
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<td></td>
<td>Biosynthesis of protein by cell-free systems from plants</td>
<td>Vanderbilt University, Nashville, Tenn.</td>
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<tr>
<td>Mattingly, Sister M. Augustine</td>
<td>Recent advances in human genetics</td>
<td>Spring Hill College, Mobile, Ala.</td>
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<tr>
<td>Mazur, Peter</td>
<td>Mechanisms of injury to microorganisms at subzero temperatures</td>
<td>Am. Soc. Microbiol., Am. Type Culture Collection Round Table Discussion on Freezing and Freeze-Drying of Microorganisms and Tissue Cultures, Kansas City, Mo.</td>
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<tr>
<td>Monesi, Valerio</td>
<td>Variation of the X-ray sensitivity during the cell cycle in spermatogonia of the adult mouse</td>
<td>2nd International Congress of Radiation Research, Harrogate, England</td>
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<tr>
<td>Novelli, G. D.</td>
<td>Biochemical aspects of protein synthesis</td>
<td>Canadian Society of Plant Physiologists, Quebec, Canada</td>
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<td></td>
<td>Protein synthesis in cell-free preparations from bacteria</td>
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<td></td>
<td>1. Recent advances in protein synthesis</td>
<td>1. Amherst College, Amherst, Mass.</td>
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<td>2. The use of microorganisms to study protein synthesis</td>
<td>2. Yale University, New Haven, Conn.</td>
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<td></td>
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<td>Rutgers University, New Brunswick, N.J.</td>
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<td>SPEAKER</td>
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<tr>
<td>Odell, T. T., Jr.</td>
<td>Acute and late somatic effects of radiation in mammals</td>
<td>1. Keuka College, Keuka, N.Y.</td>
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<td></td>
<td>Biological effects of radiation on mammals</td>
<td>2. Alfred University, Alfred, N.Y.</td>
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<td>Radiation effects on mammals</td>
<td>3. Ithaca College, Ithaca, N.Y.</td>
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<td>Studies on blood platelet regulation</td>
<td>4. Wells College, Aurora, N.Y.</td>
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<tr>
<td>Perkins, E. H.</td>
<td>The role of cellular division in antibody formation</td>
<td>US Army Nuclear Science Seminar, ORINS, Oak Ridge</td>
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<td></td>
<td>Studies of radiation-protective and antibody-synthesizing capacities of hematopoietic cells</td>
<td>Conference on Recent Developments in Studies of Blood Platelets, Biology Division, ORNL, Oak Ridge</td>
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<tr>
<td>Popp, R. A.</td>
<td>Hemoglobin as an erythrocyte marker</td>
<td>University of Illinois, Urbana</td>
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<td>2nd International Congress of Radiation Research, Harrogate, England</td>
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<td>Prescott, D. M.</td>
<td>Behavior of proteins of the nucleus during the cell life cycle</td>
<td>International Society for Cell Biology, Meeting on Cell Division and Cell Growth, Liege, Belgium</td>
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<tr>
<td></td>
<td>Biochemistry of cell growth and cell division</td>
<td>1. Medical School, University of Southern California, Los Angeles</td>
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<td></td>
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<td>2. Medical School, University of California, Los Angeles</td>
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<tr>
<td></td>
<td>Nuclear function and nuclear replication</td>
<td>University of Tennessee, Knoxville</td>
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<td>Nucleic acid metabolism</td>
<td>University of Brussels, Belgium</td>
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<td></td>
<td>Problems of cell growth and pyrimidine metabolism</td>
<td>University of Oslo, Norway</td>
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<td></td>
<td>Problems of radiation of tissue culture</td>
<td>Norsk Hydro's Institute for Cancer Research, Oslo, Norway</td>
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<td>Protein synthesis and problems of development</td>
<td>Wenner-Grens Institute, Stockholm, Sweden</td>
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<td>Structure of DNA and chromosomes</td>
<td>University of Geneva, Switzerland</td>
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<td>1. Autoradiographic techniques</td>
<td>Tissue Culture Summer Course (lecture and laboratory sessions), University of Wisconsin, Madison</td>
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<td></td>
<td>2. Biochemistry of cell growth</td>
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<td></td>
<td>3. Chromosome structure and function</td>
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<td>4. Nucleic acid metabolism</td>
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<td>Randolph, M. L.</td>
<td>Biological radiosensitivity for different ionizing radiations</td>
<td>1. Florida State University, Tallahassee</td>
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<td></td>
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<td>2. Georgia Institute of Technology, Atlanta</td>
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<td></td>
<td>1. Physical considerations in radiobiology</td>
<td>University of Florida, Gainesville</td>
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<td>2. Radiation-induced free radicals in seeds and biochemicals</td>
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<td>Electron spin resonances produced by ultraviolet irradiation of amino acids and proteins</td>
<td>2nd International Congress of Radiation Research, Harrogate, England</td>
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<td>Randolph, M. L.</td>
<td>Comparative ESR measurements of the absolute concentration of radiation-produced free radicals</td>
<td>As above</td>
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<td>(co-author with A. Redhardt et al.)</td>
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<td>Roesel, Hilde</td>
<td>Actions of light and gibberellin on wheat coleoptiles</td>
<td>University of Tennessee, Knoxville</td>
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<tr>
<td>Russell, Liane B.</td>
<td>The relative sensitivity of various germ-cell stages of the mouse to radiation-induced nondisjunction, chromosome losses and deficiencies</td>
<td>International Symposium on Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, The State University of Leiden, The Netherlands</td>
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<td></td>
<td>Some biological effects of radiation</td>
<td>13th Annual Naval Nuclear Sciences Seminar, ORINS, Oak Ridge, Tenn.</td>
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<td>SPEAKER [AND COAUTHOR(S)]</td>
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<tr>
<td>Russell, W. L.</td>
<td>The effect of radiation dose rate and fractionalization on mutation in mice</td>
<td>International Symposium on Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, The State University of Leiden, The Netherlands</td>
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<tr>
<td>Russell, W. L. (and E. F. Oakberg)</td>
<td>Radiation genetics in mice</td>
<td>Brown University, Providence, R.I.</td>
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<td>Schuel, Herbert</td>
<td>The role of arbacia sperm surface antigens in fertilization</td>
<td>University of Tennessee, Knoxville</td>
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<td>Setlow, R. B.</td>
<td>Action of ultraviolet light on DNA synthesis</td>
<td>University of Tennessee, Knoxville</td>
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<td>Setlow, R. B. (and F. J. Bollum)</td>
<td>The chemical nature of ultraviolet lesions in DNA</td>
<td>Yale University, New Haven, Conn.</td>
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<td>Setlow, R. B. (and F. J. Bollum)</td>
<td>The effects of UV on DNA (2 lectures)</td>
<td>Stanford University, Stanford, Calif.</td>
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<td>Setlow, R. B. (and F. J. Bollum)</td>
<td>The identification of photochemical changes with biological damage in DNA</td>
<td>Sigma Xi, New Mexico Highlands University, Las Vegas, N.M.</td>
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<td>Suzuki, D. T.</td>
<td>Intercytosomal effects on crossing over in \ [<em>Drosophila melanogaster</em> )</td>
<td>Genetics Society of Canada, Winnipeg</td>
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<td>Suzuki, D. T.</td>
<td>Possible effects of asynapsis on crossing over</td>
<td>University of Chicago, Chicago, Ill.</td>
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<td>Upton, A. C.</td>
<td>Biological effects of radiation</td>
<td>1. ORNL Radiation Safety Training Program, Oak Ridge, Tenn. (3 lectures)</td>
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<td>The experimental pathology of ionizing radiation</td>
<td>2. 86th Session, Radioisotopes Technique School, ORINS, Oak Ridge, Tenn.</td>
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<td>Life-shortening and other population consequences of ionizing radiation</td>
<td>Radiation Ecology Institute, Oak Ridge, Tenn.</td>
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<td>Radiation hazards to man</td>
<td>13th Annual Naval Nuclear Science Seminar, ORINS, Oak Ridge, Tenn.</td>
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<td>Radiogenic leukemia</td>
<td>UT-AEC Seminar, UT-AEC Laboratories, Oak Ridge, Tenn.</td>
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<td>Leukaemogenesis — role of viruses and cytological aspects</td>
<td>Symposium on Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation (jointly sponsored by UNESCO and IAEA), Chester Beatty Research Institute, London, England</td>
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<td>Upton, A. C. (J. W. Conklin, T. P. McDonald, and K. W. Christenberry)</td>
<td>Preliminary studies on late somatic effects of radiomimetic chemicals</td>
<td>As above</td>
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<td>Upton, A. C. (M. A. Kastenbaum, and J. W. Conklin)</td>
<td>Age-specific death rates of mice exposed to ionizing radiation and radiomimetic agents</td>
<td>As above</td>
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<td>von Borstel, R. C.</td>
<td>Species-specific RNA</td>
<td>Duke University, Durham, N.C.</td>
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<td>von Borstel, R. C. (and E. A. Löbbecke)</td>
<td>Killing of cells by radiation</td>
<td>Istituto di Genetica, Universita di Roma, Italy</td>
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<td>Stage sensitivity to X radiation during meiosis and mitosis in the egg of the wasp Habrobracon</td>
<td>International Symposium on Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, The State University of Leiden, The Netherlands</td>
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<td>Wolff, Sheldon</td>
<td>Biochemical aspects of radiation-induced chromosomal changes</td>
<td>1. Georgia Institute of Technology, Atlanta 2. Medical Center Chapter of Sigma Xi, University of Alabama, Birmingham 3. Sigma Xi, University of Alabama, Tuscaloosa UT-AEC Agricultural Research Laboratories, Oak Ridge, Tenn. University of Alabama, Tuscaloosa</td>
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<td>Biophysical aspects of chromosome breakage and rejoining</td>
<td>International Symposium on Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, The State University of Leiden, The Netherlands</td>
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<td>Biophysical aspects of radiation-induced chromosomal changes</td>
<td>2nd International Congress of Radiation Research, Harrogate, England</td>
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<tr>
<td>Yamada, Tuneo</td>
<td>Cellular events controlling differentiation</td>
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</table>
VISITING LECTURERS

During the six months preceding this report, 44 lectures were given on the Biology Division Seminar program by guest speakers from scientific institutions and universities at home and abroad. Visiting lecturers included scientists from Australia, Czechoslovakia, Germany, Italy, Japan, The Netherlands, Scotland, Sweden, Switzerland, and Yugoslavia.

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<thead>
<tr>
<th>SPEAKER</th>
<th>AFFILIATION</th>
<th>SUBJECT</th>
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<tr>
<td>Paolo Amati</td>
<td>Massachusetts Institute of Technology, Cambridge</td>
<td>Transfer of colicinogenic factor to <em>Serratia marcescens</em></td>
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<td>D. E. Beischer</td>
<td>U.S. Naval School of Aviation Medicine, Pensacola, Fla.</td>
<td>Biological effects of magnetic fields</td>
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<td>F. M. Burnet</td>
<td>The Walter and Eliza Hall Institute of Medical Research, Melbourne</td>
<td>An auto-immune disease of mice</td>
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<td>Victoria, Australia</td>
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<td>Stanley Cohen</td>
<td>Vanderbilt University, Nashville, Tenn.</td>
<td>Isolation of a mouse submaxillary gland protein accelerating incisor</td>
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<td>eruption and eyelid opening in the newborn animal</td>
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<tr>
<td>Roy Curtiss III</td>
<td>Department of Microbiology, University of Chicago, Chicago</td>
<td>Studies on the genetics of <em>Escherichia coli</em> K-12</td>
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<tr>
<td>John Davison</td>
<td>Louisiana State University and Agricultural and Mechanical College, Baton</td>
<td>Studies of frog spotting patterns</td>
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<td>Rouge</td>
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<tr>
<td>Christian de Duve</td>
<td>University of Louvain, Louvain, Belgium</td>
<td>Properties and functions of lysosomes (2 lectures)</td>
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<tr>
<td>William C. Dewey</td>
<td>Physics Department, The University of Texas, Houston</td>
<td>Relative radiosensitivity of different phases in the life cycle of</td>
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<td>mammalian cells</td>
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<tr>
<td>E. P. Geiduschek</td>
<td>The University of Chicago, Chicago</td>
<td>Secondary structure and interactions of complementary RNA</td>
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<tr>
<td>Lester Goldstein</td>
<td>Zoological Laboratory, University of Pennsylvania, Philadelphia</td>
<td>Macromolecule movements between nucleus and cytoplasm</td>
</tr>
<tr>
<td>Edmund Guttes</td>
<td>Department of Biology, Brown University, Providence, R.I.</td>
<td><em>Physarum polycephalum</em> — a model organism for cell research</td>
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<tr>
<td>Robert Haynes</td>
<td>Committee on Biophysics, University of Chicago, Chicago</td>
<td>Experimental studies on <em>Physorium</em></td>
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<tr>
<td>P. G. Heytler</td>
<td>Research Supervisor, Central Research Department, E. I. Du Pont de Nemours</td>
<td>Interactions among X-ray, UV and photodynamic inactivation processes in</td>
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<td>&amp; Co., Wilminton, Del.</td>
<td>microorganisms</td>
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<td>Alfred Holtzer</td>
<td>Washington University, St. Louis</td>
<td>Uncoupling of oxidative phosphorylation by the action of carbonyl</td>
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<td>cyanide-phenol-hydrozone</td>
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<tr>
<td>David A. Hopwood</td>
<td>Department of Genetics, The University of Glasgow, Glasgow, Scotland</td>
<td>Two alpha-helical chains</td>
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<tr>
<td>Walter L. Hughes</td>
<td>Medical Research Center, Brookhaven National Laboratory, Upton, L.I., N.Y.</td>
<td>Heterozygotes of <em>Streptomyces coelicolor</em></td>
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<tr>
<td>George S. Hurst</td>
<td>Health Physics Division, Oak Ridge National Laboratory, Oak Ridge</td>
<td>Studies with $^{131}$ deoxyuridine</td>
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<td>Electron interactions in molecular gases</td>
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<td>SPEAKER</td>
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<td>Burke H. Judd</td>
<td>Department of Zoology, University of Texas, Austin</td>
<td>Analysis of products resulting from asymmetrical exchange within the white locus</td>
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<tr>
<td>Martin D. Kamen</td>
<td>Department of Chemistry, School of Science and Engineering, University of California, San Diego, La Jolla</td>
<td>The variant heme protein of photosynthetic bacteria</td>
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<tr>
<td>Edouard Kellenberger</td>
<td>Laboratoire de Biophysique, Université de Genève, Genève, Switzerland</td>
<td>Research on phage at Geneva</td>
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<tr>
<td>Heinrich Kroeger</td>
<td>Eidgenössischen Technische Hochschule, Zürich, Switzerland</td>
<td>Exploring cell differentiation by cytological grafting</td>
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<td>William J. Libby</td>
<td>School of Forestry, North Carolina State College, Raleigh</td>
<td>Genetic studies with forest trees</td>
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<tr>
<td>Robert Livingston</td>
<td>Electronuclear Research Division, Oak Ridge National Laboratory, Oak Ridge</td>
<td>The Oak Ridge isochronous cyclotron</td>
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<tr>
<td>H.-D. Mennigmann</td>
<td>McArde Memorial Laboratory, University of Wisconsin, Madison</td>
<td>Biological activity of DNA from thymineless B. subtilis</td>
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<tr>
<td>A. M. Michelson</td>
<td>Department of Chemistry and Chemical Engineering, The University of Illinois, Urbana</td>
<td>Secondary structure in synthetic oligonucleotides</td>
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<tr>
<td>Alberto Monroy</td>
<td>Università di Palermo, Palermo, Italy</td>
<td>Studies on the protein metabolism during the early development of the sea urchin embryo</td>
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<tr>
<td>Edward Novitski</td>
<td>Department of Biology, University of Oregon, Eugene</td>
<td>An entirely new insight into the mechanism of radiation effects in Drosophila</td>
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<tr>
<td>Fumio Oosawa</td>
<td>Nagoya University, Nagoya, Japan</td>
<td>Mechanochemistry of muscle protein actin</td>
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<tr>
<td>G. C. Perri</td>
<td>Sloan-Kettering Institute for Cancer Research, Rye, N.Y.</td>
<td>Lysozyme and tumor growth</td>
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<tr>
<td>Walter Sauerbier</td>
<td>Institute of Molecular Biology, University of Oregon, Eugene</td>
<td>Photobiology of bacteriophage T1</td>
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<td>Moselio Schaechter</td>
<td>University of Florida, Gainesville</td>
<td>Control mechanisms of cellular and nuclear divisions in bacteria</td>
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<tr>
<td>Miroslav Simic</td>
<td>Institute of Nuclear Sciences, &quot;Boris Kidrich&quot; Institute, Belgrade, Yugoslavia</td>
<td>Survey of radiobiology at Institute of Nuclear Sciences—Boris-Kidrich</td>
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<tr>
<td>Jan Skoda</td>
<td>Assistant Director, Institute of Organic Chemistry and biochemistry, Prague, Czechoslovakia</td>
<td>Studies on polynucleotide phosphorylase with nucleotide analogues</td>
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<tr>
<td>F. H. Sobels</td>
<td>Department of Radiation Genetics, University of Leiden, Leiden, The Netherlands</td>
<td>Modification of the radiation-induced mutation process in Drosophila</td>
</tr>
<tr>
<td>J. Herbert Taylor</td>
<td>Department of Botany and Zoology, Columbia University, New York City</td>
<td>A molecular view of chromosome reproduction and organization</td>
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<tr>
<td>Torsten Teorell</td>
<td>University of Uppsala, Uppsala, Sweden</td>
<td>Membranes and their application to excitability phenomena</td>
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<td>SPEAKER</td>
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<td>K. W. Tucker</td>
<td>Department of Biology, Lake Forest College, Lake Forest, Ill.</td>
<td>Unusual means of sex determination in the honeybee</td>
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<tr>
<td>Adolf Wacker</td>
<td>Universität Frankfurt, Frankfurt am Main, Germany</td>
<td>Molecular mechanisms of radiation effects (UV-, X-ray)</td>
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<tr>
<td>Paul Witfield</td>
<td>Virus Laboratory, University of California, Berkeley</td>
<td>Nucleotide sequence in TMV-RNA</td>
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<tr>
<td>H. Witzel</td>
<td>Marburg University, Chemical Institute, Marburg, Germany</td>
<td>Mechanism and binding sites in the ribonuclease reaction</td>
</tr>
<tr>
<td>Friedrich Zimmermann</td>
<td>Universität Freiburg, Freiburg, Germany</td>
<td>Some problems relating to the use of the ad-2 system in yeast for chemical mutagenesis studies</td>
</tr>
</tbody>
</table>

**FOREIGN TRAVEL**

Twenty-one Biology Division members spoke at meetings or discussed problems of mutual interest with scientists abroad during the period of this report.


Two Division members spoke at the International Symposium on Radiobiology sponsored jointly by UNESCO and IAEA, "The cellular basis and aetiology of late somatic effects of ionizing radiation." The meeting, the third in a series on selected topics on radiobiology so sponsored, was held in March at the Chester Beatty Research Institute in London. W. L. Russell was chairman of a session on Mechanisms of Life-Span Shortening and gave a paper, "The cellular basis and aetiology of the late effects of irradiation on fertility in female mice" (coauthor, E. F. Oakberg). A. C. Upton gave three papers: "Leukaemogenesis – Role of viruses and cytological aspects"; "Preliminary studies of radiomimetic chemicals" (co-authors, J. W. Conklin, T. P. McDonald, and K. W. Christenberry); and "Age-specific death rates of mice exposed to ionizing radiation and radiomimetic agents" (coauthors, M. A. Kastenbaum and J. W. Conklin).

Dr. Upton later consulted with scientists at three other laboratories in England before returning to Oak Ridge.

D. M. Prescott discussed "Behavior of proteins of the nucleus during the cell life cycle" at the meeting on cell growth and cell division in Liege, Belgium, May 19–24. He visited five institutions of higher learning to present lectures subsequently to the meeting. He spoke on "Nucleic acid metabolism" at the University of Brussels, Belgium; "Structure of DNA and chromosomes" at the University of Geneva, Switzerland; and "Protein synthesis and problems of development" at Wenner-Grens Institute, Stockholm, Sweden; and "Problems of cell growth and pyrimidine metabolism" at the University of Oslo, Norway. While in Oslo, Prescott spoke on "Problems of radiation of tissue culture" at Norsk Hydro's Institute for Cancer Research.

Six members of the Biology Division attended an International Symposium on Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, in August at the State University of Leiden, The Netherlands. Division members participating were R. F. Kimball, who gave two papers, S. Wolff, D. L. Lindsley, R. C. von Borstel, W. L. Russell, and L. B. Russell.

Fourteen members of the Division attended the Second International Congress of Radiation Research, August 5–10 in Harrogate, England. Alexander Hollaender, A. C. Upton, and R. A. Popp were chairmen of sessions at the meeting. Drs. Popp and Upton also presented papers. In addition, those presenting papers were: S. Wolff, R. F. Kimball, D. L. Lindsley, E. Capalbo, G. Cudkowicz, H. I. Adler, Joan W. Goodman, M. L. Randolph, R. C. von Borstel, and G. S. Stapleton. M. A Bender also attended the meeting.
Alexander Hollaender received an honorary Degree of Doctor of Science *honoris causa* on occasion of the meeting in Harrogate on August 8 at the University of Leeds. He was one of four scientists so honored. He addressed the congregation for the conferment of honorary degrees at that time at the University of Leeds.

Dr. Hollaender left Oak Ridge June 6 en route to Brussels, Belgium for discussion with R. K. Appleyard, Division of Biology, Communaute Europeenne de l’Energie Atomique, with reference to the Italian radiation biology program. As a member of the Advisory Committee, Division of Biology, Comitato Nazionale per l’Energia nucleare, Hollaender visited Italian laboratories at Rome, Palermo, Naples, Pisa, Bologna, Fiascherino, Turin, and Padua (Venice) to examine and advise on programs in radiation biology. He spoke at Stazione Zoologica, Naples, and at Istituto Superiore di Sanita, Rome. He returned to Oak Ridge June 29.

G. Cudkowicz, after the Harrogate meeting, gave an invited seminar at the Institute of Tumor Biology, Stockholm, Sweden on "Studies on transplantation immunity."

W. E. Cohn is one of three Oak Ridge National Laboratory scientists named as recipients of the Guggenheim Fellowship Award for 1962. This is the second time he has received this award. He again will spend the first six-month period of the Fellowship at Cambridge University conducting work in the field of nucleic acid biochemistry. Dr. Cohn will then journey to the Institute of Biological Chemistry in Paris, France, where he will serve as a visiting professor and work with Marianne Grunberg-Manago.

Takashi Makinodan, Radiation Immunology Group Leader, returned to the Division in June after nine months at the National Institute of Radiological Sciences, Chiba, Japan. His stay there was supported jointly by a NSF Senior Postdoctoral Fellowship and Oak Ridge National Laboratory.

**SIXTEENTH ANNUAL BIOLOGY RESEARCH CONFERENCE**

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

**MACROMOLECULAR ASPECTS OF THE CELL CYCLE**

**Monday, April 8**

**Morning**

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

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

*Synthetic patterns over the bacterial cycle* – Arthur B. Pardee, Department of Biology, Princeton University, Princeton, N.J.

**Afternoon**

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

*Role of RNA and protein synthesies in the initiation of DNA synthesis* – Ole Maaløe, University Institute of Microbiology, Copenhagen, Denmark

*Experiments on the extranuclear control of gene activity in dipteran polytene chromosomes* – Heinrich Kroeger, Zoological Institute, Eidgenössische Technische Hochschule, Zurich, Switzerland
Tuesday, April 9

Morning

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

DNA priming activity during the cell cycle – F. J. Bollum, Biology Division, Oak Ridge National Laboratory

Timing of DNA synthesis during chromosome replication – J. Herbert Taylor, Department of Botany, and Zoology, Columbia University, New York, N.Y.

Afternoon

Open

Wednesday, April 10

Morning

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

Cytochemistry of histones – David P. Bloch, Department of Botany, The University of Texas, Austin

Biochemistry of histones in relation to the cell cycle – Harris Busch, Department of Pharmacology, Baylor University, College of Medicine, Houston

Afternoon

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

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

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

Evening

Round-table discussion – Differential sensitivity of cell life cycle

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

L. G. Lajitha, Department of Radiotherapy, University of Oxford, Oxford, England

Nirmal Das, Department of Zoology, University of California, Berkeley

C. L. Smith, Department of Radiotherapeutics, University of Cambridge, Cambridge, England

L. J. Tolmach, Washington University School of Medicine, St. Louis

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

Thursday, April 11

Morning


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

Correlation of changes in the fine structure of DNA-protein with cell activities – Edouard Kellenberger, Laboratoire de Biophysique, Université de Genève, Geneva, Switzerland.
1962 BIOLOGY SYMPOSIUM

The proceedings of the Fifteenth Annual Biology Symposium, *Specificity of Cell Differentiation and Interaction*, held at Gatlinburg, Tenn., in April, is being published as a supplement to the Journal of Cellular and Comparative Physiology and will be available in October 1962.

BIOMEDICAL PROGRAM DIRECTORS

Several Biology Division members attended the Biomedical Program Directors meeting April 23–24 in Oak Ridge. The Oak Ridge Institute of Nuclear Studies, Medical Division, and the University of Tennessee–AEC Agricultural Research Laboratories were hosts.

BONE MARROW CONFERENCES

Biology Division members took active part in two bone marrow meetings which were held during this report period:

*Discussion on Bone Marrow Transplantation and Irradiation Protection*, April 15, 1962, Atlantic City, N.J. C. C Congdon and Joan W. Goodman presented a coauthored paper.

*Conference on Bone Marrow Transplantation and Chemical Protection in Large Animals and Man*, June 1–2, 1962, Long Beach, Calif. C. C Congdon and D. G. Doherty (coauthors) presented a paper at the session Chemical Protection and Bone Marrow Transplantation. Joan W. Goodman was chairman of the session on White Blood Cell Transplantation and presented a paper.

Other meetings being planned are:

**December 1–3, 1962, New York.** — A conference on *Modification of Radiation Injury by Bone Marrow Transplantation and Chemical Protection*. Members of the Biology Division will present papers at this meeting.

**April, 1963, Atlantic City, N.J.** — Round Table Discussion, *Bone Marrow Transplantation and Protection*. Chairman, L. M. Tocantins.

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


BLOOD PLATELET CONFERENCE

About 85 persons attended a conference on "Recent Developments in Blood Platelets" held June 22–23 at the Biology Division. The meeting was organized by T. T. Odell, Jr., and Mario Baldini. The program included papers on platelet components and their function, immunology, preservation, survival, and on platelet and megakaryocyte dynamics and regulation. Abstracts of the conference were submitted for publication in *Blood*. The abstracts also appeared in a booklet published by the Biology Division. The meeting was supported by Oak Ridge National Laboratory.

EDUCATION

**Student Trainee Program.** — This year 17 juniors from small colleges in 13 states, representing all sections of the United States, and Puerto Rico, were selected on a competitive basis to serve on temporary summer appointments in the Biology Division. This Student Trainee Program, administered in cooperation with the Oak Ridge Institute for Nuclear Studies, provides opportunities for college undergraduates to develop a better comprehension of research methods and their significance.
Each appointee worked on a specific project under the guidance of a research scientist and wrote a paper about his work at the completion of his appointment.

This program, now in its fifth year, has been instrumental in encouraging undergraduate students to continue on with graduate work.

The 1962 students, their affiliations, and the sections to which they were assigned were:

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<tr>
<th>STUDENT TRAINEE</th>
<th>INSTITUTION</th>
<th>ASSIGNED TO</th>
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<tr>
<td>Barbee, Barbara Anne</td>
<td>Centenary College, Shreveport, La.</td>
<td>E. H. Y. Chu, Mammalian Genetics</td>
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<tr>
<td>Brandt, Theresa Phoebe</td>
<td>Our Lady of the Lake College, San Antonio, Tex.</td>
<td>C. J. Wust, Enzymology</td>
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<tr>
<td>Doupark, Anna-Lisa Rebecca</td>
<td>College of Notre Dame of Maryland, Baltimore</td>
<td>K. B. Jacobson, Enzymology</td>
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<td>Drake, Daniel Lee</td>
<td>Reed College, Portland, Ore.</td>
<td>R. B. Setlow, Biophysics</td>
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<td>Griscom, Hilda Ann</td>
<td>Western Maryland College, Westminster</td>
<td>L. H. Smith, Mammalian Recovery</td>
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<td>Groeniger, Eleanor</td>
<td>Mount Holyoke College, South Hadley, Mass.</td>
<td>F. J. Bollum, Nucleic Acid Chemistry</td>
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<td>Joyner, Julia Anne</td>
<td>Winthrop College, Rock Hill, S.C.</td>
<td>L. B. Russell, Mammalian Genetics</td>
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<tr>
<td>LaPrade, Nancy Sims</td>
<td>Mary Washington College, Fredericksburg, Va.</td>
<td>T. T. Odell, Jr., Pathology and Physiology</td>
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<td>McCubbins, Rilla</td>
<td>Phillips University, Enid, Okla.</td>
<td>D. Schwartz, Cytology and Genetics</td>
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<td>Mitchell, Margaret Smith</td>
<td>Wilson College, Chambersburg, Pa.</td>
<td>E. F. Oakberg, Mammalian Genetics</td>
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<td>Ott, Sharon Louise</td>
<td>Mount Union College, Alliance, Ohio</td>
<td>J. F. Albright, Radiation Immunology</td>
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<tr>
<td>Phillips, Thomas Wilson</td>
<td>Oglethorpe University, Atlanta, Ga.</td>
<td>M. S. Gude, Pathology and Physiology</td>
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<td>*Purcell, Caridad</td>
<td>University of Puerto Rico</td>
<td>R. J. Mans, Enzymology</td>
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<tr>
<td>Sparkes, Nancy Tate</td>
<td>Randolph-Macon Woman's College, Lynchburg, Va.</td>
<td>C. C. Congdon, Mammalian Recovery</td>
</tr>
<tr>
<td>Webb, Brenda Carolyn</td>
<td>Pfeiffer College, Misenheimer, N.C.</td>
<td>A. H. Haber, Plant Physiology and Photosynthesis</td>
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<tr>
<td>Wise, William Curtis</td>
<td>Transylvania College, Lexington, Ky.</td>
<td>J. S. Kirby-Smith, Biophysics</td>
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*Supported by Biology Division Funds.
Small College Program. – The Small College Program, set up by the Biology Division, is basically an effort by the Division to offer assistance in the form of lectures to small colleges. Seven Biology Division staff members participated in this program during February–August 1962. They gave 35 lectures at 29 colleges.

H. I. Adler gave a total of ten lectures at nine colleges during the period of February-May. Colleges visited were Spring Hill College (Mobile, Ala.), Centenary College of Louisiana (Shreveport), Loyola University (New Orleans), Northwestern State College of Louisiana (Natchitoches), Longwood College (Farmville, Va.), University of Delaware (Newark), Illinois Institute of Technology (Chicago), Randolph-Macon Woman’s College (Lynchburg, Va.), and University of Richmond (Richmond, Va.).

On May 2-4, F. J. de Serres gave three lectures in Texas. He spoke on "Genetic fine structure analysis in Neurospora" to the Incarnate Word College and St. Mary’s College, San Antonio. He lectured on "Factors influencing recombination and interference" to the Department of Zoology, University of Texas, Austin.

Both Drs. de Serres and Adler spoke at Roane County High School for a Career Day Program in March.


Five lectures were presented by E. H. Y. Chu at two colleges in Maryland during April and May. At the Western Maryland College in Westminster he presented three lectures; at the College of Notre Dame in Baltimore he spoke twice.

D. G. Doherty presented two lectures on "Enzymes—the biological catalysts" to the Northeast Louisiana State College in Monroe, and Mississippi Southern College in Hattiesburg, in March.

John Jagger gave a total of eight lectures to six colleges in North Carolina, Virginia, and South Carolina in February and March. He lectured on "Light and Life" and "How does ultraviolet radiation cause the mutation and death of a cell?" He spoke at Woman's College of the University of North Carolina, Greensboro; Eastern Mennonite College, Harrisonburg, Va.; Winthrop College, Rock Hill, S.C.; Queen's College, Charlotte, N.C.; Madison College, Harrisonburg, Va., and Bridgewater College, Bridgewater, Va.

Messenger Lectures. – Alexander Hollaender was appointed Messenger Lecturer for 1962 by Cornell University. He presented six lectures from April 16–27 on the topic "Basic Problems in Radiation Biology."

This lecture series was instituted more than 35 years ago by Hiram J. Messenger and has centered around the general topic of "Evolution of Civilization." The lectures are an all-campus affair. Lecturers have been authorities from the humanities and social sciences, as well as in the sciences. Previous speakers have included Linus Pauling, R. A. Millikan, Paul Tillich, Howard Mumford Jones, Alfred L. Kroeber, and Roscoe Pound.

Dr. Hollaender lectured on:

- "Historical review of radiation biology"
- "Some problems on the biological effects of ultraviolet radiation"
- "Radiation microbiology"
- "Radiation cytology"
- "Mammalian radiation biology"
- "Speculation of the future of radiation biology"

University Cooperative Program. – S. F. Carson gave two lectures on "Dependence of ATP yield upon type of fermentation mechanisms: Consequences for available growth energy and current concepts of fermentation pathways" at the University of Georgia, Athens, in March as part of the cooperative graduate and postdoctoral teaching program.

Hilde Roesel received a PhD degree in Botany from the University of Tennessee during the period of this report. She worked in the Division under the supervision of A. H. Haber in the Division's cooperative program with UT and ORINS. The thesis is entitled, "Effects of light and gibberellin on elongation of intact wheat coleoptiles."
Courses. – Eleven Biology Division members participated in courses.

T. T. Odell, Jr., spoke on "Radiation effects on mammals" at two Oak Ridge programs. He also spoke on the July 25 ORINS Educational Program, and July 30 at the ORINS Isotope Technology Course.

Five members of the Biology Division took part in the U.S. Army Nuclear Science Seminar held at ORINS on August 6. Those who attended the seminar were given a tour of the Biology Division on August 7. Speakers and titles: W. D. Gude, "Basic radiobiology"
E. B. Darden, "Effects of radiation on biological organisms"
T. T. Odell, Jr., "Biological effects of radiation on mammals"
E. F. Oakberg, "Genetic effects of radiation"
L. H. Smith, "Radiation protection and recovery measures"

A. C. Upton and L. B. Russell spoke at the 13th Annual Nuclear Sciences Seminar June 14 at ORINS. Dr. Upton spoke on "Radiation hazards to man," and Liane B. Russell on "Some biological effects of radiation."

D. R. Krieg taught a portion of a graduate course given by the Department of Biology at the University of California, Berkeley, entitled, Laboratory in General Biology, January 29–March 9. He taught one-third of a semester on bacteriophage while on leave of absence from the Biology Division. Later in March he participated in a West Coast phage meeting at Lake Tahoe sponsored by the University of California.

D. M. Prescott taught at the Tissue Culture Summer Course on June 24–30 at the University of Wisconsin.

S. Wolff taught a course, Seminar in Radiation Biology at The University of Tennessee. Dr. Wolff is a visiting professor in Radiation Biology at the University’s Institute of Radiation Biology.

Training Programs. – Three Biology Division members gave eleven lectures in Training Programs and Institutes.

A. C. Upton gave a talk on "The biological effects of radiation" at ORINS in June. He also spoke at an institute in radiation ecology in Oak Ridge on July 26. The topic of his talk was "Life shortening and other population consequences of ionizing radiation." Dr. Upton gave three lectures on "Biological effects of radiation" as part of a radiation safety training program given in April at Oak Ridge National Laboratory.

T. T. Odell, Jr., spoke August 6 at the Army Summer Training Program in Oak Ridge.

G. E. Cosgrove went to Florida State University (July 30–August 3) and Wayne State University, Detroit (August 7–8) to give lectures and demonstrations in mammalian radiobiology to NAS-AEC Summer Institutes in Radiation Biology for Science Teachers.
Cytology and Genetics

Effects of Radiation on Paramecium
R. F. Kimball  Stella W. Perdue  H. Marine Scandlyn

Maize and Phage Genetics
Drew Schwartz  D. R. Krieg  Jane K. Setlow\(^d\)
C. B. Kincaid  Kathrine H. McGrath  Claire J. Witten  Rilla McCubbins\(^e\)

Timothy
R. T. Brumfield\(^a\)  Donna R. Frantzen\(^b\)

Chromosome Cytology
Sheldon Wolff  H. E. Luippold
Sandra L. Bell\(^c\)  Judith M. Pier
Sister Augustine Mattingly\(^c\)

Fungal Genetics
F. J. de Serres  Ida R. Cox
W. E. Barnett\(^d\)  Arlee P. Teasley
H. E. Brockman\(^f\)
B. B. Webber\(^a\)

Drosophila
D. L. Lindsley, Jr.  J. E. Petty
E. H. Grell  Guthrie T. Pratt
Rhoda F. Grell  Bobbie J. Scandlyn
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[For table data]

THE CONSERVATION OF LABEL FROM TRITIATED RIBONUCLEOSIDES DURING LOG PHASE GROWTH IN PARAMECIIUM AURELIA

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

Introduction. — The interpretation of single cell growth curves and of inheritance in cell lines in Paramecium would be facilitated by as complete a knowledge as possible about the origin and fate of the major cellular components, such as RNA. It has been shown\(^1\) for this species that RNA is formed in and lost from the macronucleus continuously during the cell cycle, the loss presumably being to the cytoplasm. Is this RNA in the cytoplasm broken down or conserved over successive cell generations?

There is evidence for bacteria\(^2\) that ribosomal RNA is conserved during log phase growth, and evidence for \(P.\) \textit{aurelia}\(^3\) that the not-very-specific label, \(^{32}\text{P}\), is also conserved. The present report demonstrates conservation during log phase growth of the label from \(^3\text{H}\)-cytidine and \(^3\text{H}\)-uridine, both of which have been shown to be incorporated into RNA, but not DNA, in this species.\(^1\)


\(^3\)F. Kaudewitz, \textit{Arch. Protistenkunde}, 102, 321 (1958).

Fig. 1. Grains Above Background per Unit Area After the Division at which the Paramecia Were Selected. Data is plotted on a semilog scale. Duration of the successive cell generations and time of the pulse labeling with tritiated ribonucleosides are shown. The solid points are the actual data; the open points the values expected if the counts at each previous cell generation had been halved.
Methods. — Dividing paramecia were collected, pulse-labeled with a mixture of the two tritiated ribonucleosides about 1½ hr after division, washed free of exogenous label, and isolated singly into culture medium. Thereafter, at each of several successive cell divisions, one daughter cell was dried and fixed for autoradiography and the other was kept to maintain the line of descent. Autoradiographs were prepared and grain counts made over standard areas of cytoplasm to measure the amount of label per unit volume.

Results and Discussion. — The data are plotted in Fig. 1. If the label were completely conserved, the amount per unit volume, as measured by the grain counts, should be halved in each cell generation by the addition of newly synthesized, nonlabeled material. In the earlier generations the decrease in label is slower than would be predicted by halving. This apparent "overconservation" is not too surprising because (1) these generations were shorter than normal and (2) it is known that Paramecium does not precisely double its volume in each generation. The data are in good accord with complete conservation of the label with an almost constant growth rate per unit time, though not per cell generation.

Such experiments cannot prove that the RNA itself is conserved, but they show this is a real possibility. If ribosomal RNA is formed continuously, conserved and limiting for cell growth, then the rate of growth should increase throughout the cell cycle, as found in Paramecium. Conservation might also play an appreciable role in the inheritance in cell lines of any other characters that might depend upon the total amount of ribosomal material. The present experiments show that such possibilities deserve further investigation.

STUDIES ON CHEMICAL MUTAGENESIS IN PARAMECIUM

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

Introduction. — Our earlier work with X rays has shown a definite pattern of "sensitivity" to mutation induction during the cell cycle. There are two main periods: a sensitive period extending from midprophase through mitosis to the brief DNA synthesis (S) period midway in the next cell generation, and an insensitive period extending from S to midprophase. The amount of mutation increases the latter in the sensitive period the radiation is given, attaining a maximum value just before S. Almost no mutation can be induced by X irradiation during the insensitive period. The same division into sensitive and insensitive periods is found for mutations induced by 2537 A ultraviolet and by Pu-239 α particles. The base analog mutagen, 5-bromodeoxyuridine, however, acts only when it is present in the medium during the S period. Thus radiation, which may be presumed to alter pre-existing genetic material, has a very different sensitivity pattern from a mutagen that acts by being incorporated during DNA synthesis in place of a normal pyrimidine base. The question arises as to what pattern would be shown by chemical mutagens that, like radiation, would be expected to alter the pre-existing DNA or other chromosomal constituents.

Results. — Attempts to produce mutations with ethyl methanesulfonate, sodium nitrite in acid solution, hydroxylamine, and proflavine in the dark have been unsuccessful. The first two compounds were extremely toxic and concentrations well below those used for mutagenesis in other organisms had to be used. The last two were not as toxic, and the reason for the lack of mutagenicity is unknown.

Nitrogen mustard, however, is an effective mutagen for Paramecium, as first shown by Geckler. Thus several experiments have been carried out with this agent at a concentration of 1 mg/ml in a salt solution buffered at pH 6.0. Dividing paramecia were collected as in previous work and exposed to the mustard for 10 min before being washed thoroughly and returned to culture medium. The results are summarized in Fig. 2. The pattern of sensitivity, as far as it has been checked, is very similar to that found with X rays. There is the same large difference between treating in G1 and postduplication interphase (G2) and a distinct increase in the amount of mutation the closer to S the G1 treatment is

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given. This increase does not depart significantly from the linearity found for X rays, nor is the slope significantly different from that found for X rays. The fit is not too good, however, and it is possible that the marked effects of the mustard treatment on the length of the cell generation and presumably on the time of DNA synthesis, effects which are not found with X rays, modify the shape of the curve.

**Conclusions.**—The "sensitivity" pattern found for nitrogen mustard is essentially the same as that found for various radiations. Thus it seems probable that this pattern is a general one for agents that alter the pre-existing genetic material and is probably the consequence of general processes affecting all such damage, not just a particular kind.

**THE EFFECT OF DOSE RATE AND DOSE FRACTIONATION ON X-RAY-INDUCED MUTATION IN PARAMECIUM**

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

**Introduction.**—In a previous report evidence was given that fewer mutations are induced at low than at high dose rate when log phase paramecia were X irradiated during the preduplication interphase. No fractionation effect was found, however, and it was suggested that the interactions between dose increments, indicated by the dose-rate experiment, were so rapid as to be nearly complete within the period of time required to give a half dose in the fractionation experiments. This conclusion has now been confirmed by experiments at various dose rates. No dose-rate effect was found in the earlier work with stationary phase paramecia, and from this it was concluded that two-hit chromosomal aberrations were not involved in the dose-rate effect in log phase. It is now obvious that this conclusion was premature, and that the interpretation of two-hit chromosomal aberrations fits the data very well indeed.

**Methods.**—The methods for measuring and detecting lethal and slow growth mutations in Paramecium were the same as used in our previous work. X rays were from the Maxitron 250 machine with 3 mm Al added filtration (HVL, 0.44 mm Cu) with the dose rate varied by changing the tube current. The beam was monitored before and after the exposures with a 250-r Victoreen thimble chamber substituted for the container of paramecia and during the exposures with the Radocon integrating dosimeter and two Victoreen thimble chambers in appropriate positions in the beam.

**Results.**—The results of experiments in which a dose of 4.4 kr was given in 1, 3, and 13 min, respectively, are plotted in Fig. 3 together with a much lower dose-rate point from the earlier experiments. This latter was corrected for dose by assuming a linear relation. The curve was obtained by fitting the first three points with the equation, \( M = aD + bGD^2 \), where \( G \) is the function derived by Lea relating the duration of the exposure to the half life of chromosomal breaks. The data give a value for this half life of about 1.3 min, supporting our earlier interpretation that the interaction between increments of the dose is very fast. The low dose-rate point agrees very well with the extrapolation of this curve.

The previous evidence that there is no dose-rate effect for paramecia irradiated in stationary phase has now been shown to be explainable by a very much longer lifetime for the breaks. The previous data

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showed no detectable difference between giving the dose in about 3 min and in about 120 min. It has now been found, however, that appreciably less mutation is produced when the radiation is given in four equal fractions with several hours between them. It would appear that breaks have a very long lifetime under stationary phase conditions, a conclusion that agrees well with Wolff's evidence that energy metabolism and protein synthesis are required for restitutional rejoining.

Conclusions. – Our data can be explained by assuming that the dose rate and dose fraction effects on mutation induction in Paramecium are entirely the consequence of a relatively small contribution from two-hit chromosomal aberrations. The maximum contribution when complete interaction between breaks can occur is about 0.25 M units at 4.4 kr, as judged by the difference between the intercept at zero time and the lower asymptote in Fig. 3. A similar value can be derived from the stationary phase fractionation experiment. Thus the contribution from two-hit aberrations is about 20% of the total mutation in log phase and an appreciably larger fraction in stationary phase since total mutation is less in this phase.

The most important conclusion is that the rate of rejoining of breaks under log phase conditions is much higher than the rate of repair of the bulk of the premutational damage. The latter can be shown from earlier data to be about 16% of the total reparable damage per hr as compared to almost 50% per min for breaks. Thus we are able to distinguish clearly between breaks and a nonbreakage damage accounts for most of the mutation, is reparable at relatively low rates, does not take part in two-hit events, and does not become converted to final mutation until DNA synthesis. Thus it has properties that would make it suitable as premutational precursor of true point mutation.

THE TIME OF CHROMOSOME SPLITTING AS REVEALED BY COMBINED X-RAY AND LABELING EXPERIMENTS

Sheldon Wolff H. E. Luippold Judith M. Pier

Introduction. – If an actively growing population of cells is irradiated and the types of chromosomal aberrations are observed at metaphase at various times after irradiation, it is found that the cells hit in late interphase when close to metaphase exhibit chromatid aberrations, whereas those cells hit in early

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interphase when farther from metaphase exhibit chromosome aberrations. From such timing experiments, correlations were made with the time at which the chromosome split into two chromatids. Such experiments with *Vicia faba* gave results that were consistent with the hypothesis that the chromosome split at the time that DNA synthesis occurred within the cell. Others, however, have stoutly maintained that all chromatid aberrations come from breaks that were induced before the chromosomes split, followed by a partial restitution of only one of the strands after splitting.

The use of H³-thymidine as a specific label for chromosomes that are in the synthetic period of the cell cycle makes it possible to test directly the types of aberrations that are induced either before, during, or after the period of DNA synthesis (S). In the present experiments, actively growing lateral roots of *Vicia* seedlings were labeled for 15 min with 0.016 mc per ml of H³-thymidine. This treatment labeled those cells in S. The roots were then irradiated with 150 r or 250 kvp X rays to induce aberrations. Autoradiographic slides were prepared every 3 hr from this material to see what types of aberrations were present at metaphase and whether or not they were labeled. A similar set of experiments was performed in which the cells were irradiated first and then labeled. In some cases, a delay occurred between the irradiation and the addition of H³-thymidine to allow cells that were in the pre-DNA synthesis (G₁) period of the cell cycle to proceed into S where they could take up the label.

**Results.** — In those experiments in which the cells were labeled and then aberrations induced, in the first 6 hr after irradiation only unlabelled chromatid aberrations were induced. From this point on until 24 hr, a mixture of labeled and unlabeled chromatid aberrations were observed. Subsequent to this, some chromosome aberrations started to reach metaphase but these were always unlabeled. These results have indicated that any labeled cell, i.e., any cell that was in synthesis, when irradiated, would give rise to chromatid aberrations no matter how early that cell was in the synthetic period (which lasts about 6 hr in *Vicia* root tips).

When the experiment was performed by irradiating before labeling and sampling the cells at successively later periods of time to ensure observing cells that were at the beginning of S, no labeled chromosome aberrations were found until a delay of 2 hr ensued between the irradiation and addition of the label, and roots were picked at 24 to 27 hr after X rays. This result indicated that the chromosome might possibly be duplicated and give rise to chromatid aberrations even before the synthetic period. When, however, some autoradiographs from the same experiments were overexposed, it was found that some of the earliest chromosome aberrations to reach metaphase (24 hr) were lightly labeled even with no interval between irradiation and labeling. This indicated that a small percentage of the cells containing chromosome aberrations were at the very beginning of S at the time of irradiation.

**Discussion.** — These results on the time of duplication of the chromosome, as revealed by whether the chromosomes reacted as single or double to radiation, have indicated that the chromosome is double very close to the beginning of the synthetic period. McLeish, by studying the duplication of chromosomal protein by cytophotometric techniques, had concluded that the protein moiety of the chromosome

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duplicates at or just before S. The fact that the chromosome reacts as though double in the very early portions of the synthetic period, even though the majority of the DNA is not added to the chromosome until later in S, suggests that the protein moiety of the chromosomes and not the nucleic acid portion contributes to the linear continuity. Such results are consistent with previous experiments that have shown that protein synthesis is necessary for the rejoining of radiation-induced chromosome breaks.15

Experiments in which attempts were made to label the chromosomal protein as it duplicated were found to be unsuitable in that labeled amino acids are incorporated into nuclear proteins throughout the interphase. This makes it impossible to distinguish, by this means, those cells in which the protein was doubled.

**STUDIES OF THE MECHANISM OF THE EFFECT OF FUDR ON CHROMOSOMES**

Sandra Bell Sheldon Wolff

**Introduction.** — The results of experiments with protein synthesis inhibitors have indicated that protein synthesis is necessary for chromosome repair.16 Other experiments17 in which cells were treated with fluorodeoxyuridine (FUDR) have been interpreted to indicate that DNA synthesis is necessary for repair. In these experiments, FUDR induced chromosome breakage and shattering in the lateral roots of *Vicia faba*. Because FUDR had been found to be a rather specific DNA synthesis inhibitor in *Escherichia coli*,18 it was postulated that it was specific in *V. faba* and that the chromosomes were broken in the latter part of the DNA synthetic (S) portion of the cell cycle. This latter conclusion was drawn from the observation that cells, in order to have broken chromosomes, had to be treated with FUDR for a length of time equivalent to that required for a nontreated cell to proceed from the end of S to metaphase where chromosomes could be visualized.

Since FUDR had not been shown to be a specific inhibitor of DNA synthesis in *V. faba* and since the argument based on timing might not be pertinent because all chemical agents that break chromosomes usually also inhibit mitosis, a series of experiments was undertaken to see if the chromosomes were indeed in late S when broken and if FUDR was a specific DNA synthesis inhibitor in *V. faba*.

**Results.** — In experiments in which roots were treated 15 min with 0.016 mc/ml of H3-thymidine to label cells in S and then treated with 10^{-5} M FUDR plus 10^{-4} M uridine, fragments were found in those cells treated 2 hr and shattering in those treated at least 4 hr. This is consistent with earlier reports.17 FUDR markedly inhibited the mitotic index within 4 hr. Autoradiographs of these cells showed that the chromosomes were not labeled and thus must have been broken in G2 rather than in S as had been previously reported.17

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When lateral roots were treated with FUDR plus uridine preceding treatment in H\textsuperscript{3}-thymidine, autoradiographs showed labeled nuclei. The number of grains over the nuclei was not significantly different from that in untreated cells. This finding suggests that the thymidine reversed the FUDR inhibition just as thymine does in \textit{E. coli}.

In experiments in which protein was labeled with H\textsuperscript{3}-lysine during FUDR treatment, the autoradiographs indicated that FUDR has no effect on protein synthesis.

Experiments in which lateral roots were treated with FUDR plus uridine and then labeled with H\textsuperscript{3}-cytidine followed by removal of RNA prior to the development of autoradiographs indicate that DNA synthesis is only slightly or not at all affected by the presence of FUDR in concentrations which induce chromosome breakage.

**Discussion.** — FUDR is seen to have many effects on the cells of \textit{V. faba}; it delays mitosis, breaks or shatters chromosomes, and perhaps only slightly inhibits DNA synthesis. Cohen \textit{et al.}\textsuperscript{18} reported that FUDR almost completely inhibits DNA synthesis in \textit{E. coli}. On the basis of experiments reported here, this does not seem to be true for \textit{Vicia faba}. The interpretation of the earlier experiments\textsuperscript{17} with FUDR that DNA synthesis, in contrast to protein synthesis, is necessary for repair of chromosome breaks, is now seen to be unwarranted.

**MITOTIC INHIBITION BY MALEIC HYDRAZIDE**

Sister Augustine Mattingly

Chromosome-breaking agents have previously been classified according to the time that the effect first becomes cytologically observable in the cell, \textit{i.e.}, agents with nondelayed effects (\&-ethoxy-cafeine) produce aberrations about 2 hr after the treatment, while those with delayed effects (maleic hydrazide, nitrogen mustard) produce aberrations which appear only 8 to 12 hr after treatment.\textsuperscript{19} It is difficult, however, to assess the true nature of this delay mechanism, since many of these chemicals also produce more or less severe mitotic inhibition. Experiments have been designed, therefore, to measure the extent of this mitotic inhibition, using H\textsuperscript{3}-thymidine and autoradiography.

In the first series of experiments, lateral roots of \textit{Vicia faba} were exposed to H\textsuperscript{3}-thymidine for 15 min and were subsequently transferred to spring water for 6 hr. At the end of this time, when it was expected that the incorporated label would appear in prophase and metaphase chromosomes, half of the roots were placed in maleic hydrazide (0.1 mM) for 2 hr, while the remaining roots were grown in fresh spring water. Roots were thereafter fixed at intervals of 3 hr, and inspection of the resulting autoradiographs showed no distinguishable aberrations in any labeled chromosomes. Aberrations, in fact, only appeared after several hours, in nonlabeled cells. On the other hand, a distinct stickiness was evident in metaphase chromosomes immediately after maleic hydrazide treatment, resulting in the appearance of many labeled micronuclei in the roots. Furthermore, although the presence of

the tritium itself could produce some of the micronuclei, the incidence of these bodies in maleic hydrazide-treated cells was significantly higher than in the controls.

A second series of experiments was run in which lateral roots of V. faba were exposed to H³-thymidine for 15 min and immediately thereafter, half were transferred to maleic hydrazide for 2 hr, and half were placed in spring water. Subsequently, roots were fixed at intervals of 4 hr. While scoring of these slides is still in progress, sufficient data have been accumulated to indicate that the observed mitotic inhibition is quantifiable through the use of autoradiography and that this inhibition involves cells in the S period during the maleic hydrazide treatment. Furthermore, the degree of labeling seen in the aberrations, and the fact that these labeled aberrations are succeeded by nonlabeled ones, supports the conclusion of McLeish and others that the maleic hydrazide-sensitive stage of the V. faba cell occurs before the chromosome is doubled.

REGULATORY MUTANTS IN MAIZE

Drew Schwartz Katherine H. McGrath

The E gene in maize controls the synthesis of the pH 7.5 esterase enzyme. Three alleles of this gene, \( E^F \), \( E^N \), and \( E^S \), have been described. These alleles specify isozymes with different electrophoretic migration rates and are active throughout the development of the endosperm in controlling synthesis of the esterase. We have analyzed mutant forms of the \( E^F \) and \( E^S \) alleles, designated \( E^{F'} \) and \( E^{S'} \), in which the gene is turned off early in the development of the endosperm. These mutant forms are aberrant in the regulatory element which controls gene action rather than in the structural gene, since the enzymes specified by \( E^{F'} \) and \( E^{S'} \) when active cannot be distinguished from those specified by \( E^F \) and \( E^S \), respectively. The regulatory element is very closely linked to the structural gene — no recombination has been observed to date. The regulatory element operates only in the cis condition, controlling the activity of the coupled structural gene on the same chromosome. In heterozygotes for a regulatory mutant and a normally active allele, the activity of the normal allele is not affected by the presence of the mutant in the same nucleus. The normal allele remains fully active while the activity of the regulatory mutant is turned off at around 12 days after fertilization. There appears to be a fairly sharp cutoff of the activity of the regulatory mutant in the developing endosperm. The activity of the structural gene in the \( E^{S'} \) complex is turned off somewhat later than in the \( E^{F'} \). A total of three \( E^{F'} \) type alleles have been found in different strains of maize, however at the moment we don't know whether they were all derived from a common origin or represent independent mutations of the regulatory element in the \( E^F \) complex.

The E alleles also control the synthesis of the pH 7.5 esterase in diploid seedling and mature plant tissue; however, the aberrant behavior of the regulatory mutants is expressed only in the endosperm. In the diploid tissues the activity of the \( E^{F'} \) and \( E^{S'} \) alleles cannot be distinguished from that of their normally acting counterparts.

A TEST OF ALTERNATIVE MECHANISMS OF EMS MUTAGENESIS

D. R. Krieg       E.-A. Löbbecke       Claire J. Witten

Introduction. — The induced reversion of rII mutants from exposure of phage T4 to ethyl methanesulfonate (EMS) has been investigated to characterize and measure the rates of induced mutation at a variety of distinguishable sites within an intensively studied genetic region. Certain mutants which are induced to revert at high rates are interpreted to contain a guanine-hydroxymethylcytosine (GC) pair in the mutant nucleotide sequence corresponding to an adenine-thymine (AT) pair in the wild type sequence; hence EMS induces the GC to AT transition at a high rate. The interpretation has also been advanced that certain other EMS-revertible mutants involve a transversion of GC to a pyrimidine-purine pair.

Two molecular mechanisms may be considered to account for EMS-induced mutations at a GC site; both are based on the production of 7-ethylguanine from guanine in DNA exposed to EMS, but they differ as to the subsequent events leading to the base pair substitution during synthesis. The "gap hypothesis" is based on the increased susceptibility to hydrolysis of the glycoside bond holding 7-ethylguanine to the phosphate-sugar backbone of the polynucleotide strand, and assumes that it is the gap in the nucleotide sequence consequent to the loss of the purine which directly leads to the base substitution during DNA synthesis. Since the base incorporated opposite a gap would not be specified by the template, it is assumed that either a purine or a pyrimidine may be incorporated, resulting in either a transversion or a transition. The "pairing error hypothesis" is based on the increased tendency of the N₁ hydrogen of a 7,9-disubstituted guanine analog to be dissociated; the pK for this dissociation is lowered relative to guanine's by several pH units toward neutrality. After dissociation, the ionized form of the purine can be expected to pair with thymine rather than make the normal cytosine pairing, and this would result in an induced GC to AT transition.

We are attempting to discriminate between these alternative hypotheses to gain a deeper insight into mutagenesis and gene replication generally and particularly to evaluate the somewhat questionable inference of EMS-induced transversions. Our experimental design is based on the fact that the two mechanisms make different predictions as to the effect of a posttreatment to EMS-exposed phage that would promote the hydrolysis of 7-ethylguanine from DNA prior to infection of bacteria and DNA synthesis. If the mutations are due to such gaps, the frequency of induced revertants should be increased; if the mutations are due to pairing errors of the base analog still present within DNA during replication, the frequency of induced revertants would not be increased but should decrease. Since the two mechanisms are not actually mutually contradictory, and transitions may be induced by either while transversions could only be induced by the gap mechanism, we are testing a number of different mutants. The procedure is to terminate the EMS exposure of a phage suspension by diluting into a thiosulfate solution (which rapidly reacts with and eliminates EMS), then posttreating for various times in buffer at 45°C. Two posttreatments have been investigated: phosphate buffer at pH 7 and succinate buffer at pH 4; hydrolysis should be faster at the lower pH. Osmotic shock-resistant phage stocks are used since they are more permeable to ions than normal T4 phage.

Results. – Two mutants have so far been tested at both pH's; rAP72 is a presumed transition mutant and r207 is one which was inferred to be a transversion mutant. The results with the pH 4 post-treatment are given in Table 1. To make conditions for unexposed phage (line 2, Table 1) equivalent to those for exposed phage, thiosulfate-quenched EMS was present during posttreatment. EMS was absent from the other controls (line 1).

<table>
<thead>
<tr>
<th>Treatment of Phage</th>
<th>EMS Exposure (min)</th>
<th>Posttreatment (min)</th>
<th>Revertants per 10^6 Progeny Phage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rAP72</td>
<td>r207</td>
<td>rAP72</td>
</tr>
<tr>
<td>0</td>
<td>0.14 ± 0.02</td>
<td>47 ± 18</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.2 ± 0.1</td>
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<td>77.5 ± 2.8</td>
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<td>84.2 ± 20.8</td>
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<td></td>
</tr>
<tr>
<td>30</td>
<td>51.4 ± 3.1</td>
<td>78 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

dStandard errors are estimated from replicate assays in a single experiment.

Results from a posttreatment at pH 7 are qualitatively in agreement with those shown for pH 4; the reduction in induced rAP72 revertants is more significantly established, while with r207 the spontaneous frequency of reversion is apparently too high to permit easy quantitation of any small change from the posttreatment. In all cases the duration of posttreatment is limited by the progressive killing of EMS-exposed phage, which is greater than for unexposed phage and is much faster at pH 4 than at pH 7.

Discussion. – We have no direct estimates of the incidence of gaps and 7-ethylguanine within phage before and after the posttreatment, but the above data and previous results on EMS-induced mutation and killing of phage are consistent with the following interpretation. The EMS exposure converts an appreciable fraction (between 0.01 and 0.0001) of the guanine in phage to 7-ethylguanine, and a small fraction of this is hydrolyzed to produce gaps during the original exposure. During the posttreatments we use there is a relatively large increase in the incidence of gaps, but most of the 7-ethylguanine is retained in the DNA. We assume that a gap is usually lethal to a phage, and that “pairing error” transitions are induced by 7-ethylguanine present in DNA during synthesis. Phage surviving posttreatment still produce nearly as many induced mutations as without posttreatment, and phage which have acquired gaps are dead and, hence, are not examined for induced mutations. We doubt that r207 reversions are due to transversions and consider it possible that they are due to transitions. The possibility still exists that EMS may induce transversions by the gap mechanism, but we have no evidence for that hypothesis.
and unless other mutants show a posttreatment increase in revertant frequency we will conclude that it is much less important than the pairing error mechanism.

THE NATURE OF THE PHOTOREACTIVABLE ULTRAVIOLET LESION IN DNA

Jane K. Setlow R. B. Setlow

Introduction. — Ultraviolet-inactivated transforming DNA may be photoreactivated with a yeast extract in the presence of light of longer wavelength.\textsuperscript{23} The nature of the ultraviolet-induced lesions that are eliminated by the yeast extract treatment has been elucidated by making use of a newly discovered type of light reactivation whose biological effect has been correlated with a particular photochemical effect. The loss of transforming activity in \textit{Hemophilus influenzae} DNA caused by large doses of 2800 Å radiation may be partially reversed by irradiation at 2390 Å, and this reactivation has been quantitatively correlated with reversal of thymine dimerization by the shorter wavelength.\textsuperscript{24} Thus it is possible to distinguish between biological damage resulting from formation of thymine dimers and biological damage resulting from other photoproducts.

Results and Discussion. — If the yeast extract increases biological activity by reversing thymine dimerization, prior photoreactivation with the yeast extract should eliminate 2390 Å reactivation. The result of this experiment is that prior photoreactivation causes 2390 Å irradiation to have an inactivating rather than a reactivating effect. The conclusion that at least some of the biological effect of photoreactivation involves splitting thymine dimers is in accord with the chemical experiments of Wulff and Rupert\textsuperscript{25} which show that ultraviolet-induced thymine dimers in DNA can be split by yeast extract and light.

The question of whether splitting of thymine dimers is sufficient to account for all the increase in biological activity observed after yeast extract treatment may also be answered by an experiment with combinations of photoreactivation and 2390 Å reactivation after large doses of 2800 Å radiation to transforming DNA. If the yeast photoreactivating extract repairs some type of DNA damage from 2800 Å irradiation other than that repaired by the 2390 Å radiation, we would expect maximum photoreactivation following 2390 Å reactivation to result in a survival higher than that expected from splitting the remaining thymine dimers. Moreover, since we may assume that most types of damage increase with dose, there should be relatively more photoreactivation after larger initial 2800 Å doses, and thus the slope of the 2800 Å inactivation curve after 2390 Å reactivation and photoreactivation should be less steep than that after reactivation by 2390 Å irradiation alone. These slopes are found to be the same. It is concluded that the yeast photoreactivating preparation has little or no biological effect except that resulting from splitting of thymine dimers.


HOMOLOGY TESTS ON X-RAY-INDUCED RECESSIVE LETHAL MUTATIONS IN
THE AD-3 REGION OF NEUROSPORA
F. J. de Serres

Introduction. - Forward-mutation experiments on a balanced heterokaryon between an ad-3A ad-3B double mutant and wild type showed that 76% of the ad-3 mutants induced by X rays are recessive lethal on adenine-supplemented minimal medium. If we assume, a priori, that all mutations within the boundaries of either the ad-3A or ad-3B loci are reparable on adenine-supplemented medium, then the most likely explanation for irreparable (or recessive lethal) mutations is that they include deletion of unrelated genetic material in immediately adjacent regions. Atwood has shown that heterokaryon tests can be used to determine the homology of recessive lethal mutations of independent origin and to determine the extent of the genetic damage in individual mutants. Such "homology tests" have been used to map the ad-3A, ad-3B or ad-3A ad-3B recessive lethal mutations recovered in previous experiments. Tests on such a series of mutations are of particular interest since they provide a unique approach for the analysis of the genetic composition of this region.

Results. - Homology tests were made on 5 ad-3A, 7 ad-3B, and 7 ad-3A ad-3B recessive lethal mutations which are maintained as a dikaryon of the following genetic constitution: component (A) A bist-2 ad-3A ad-3B nic-2 + ; + ; inos ; + and component (B) A + (ad-3) + al-2 ; cot ; + ; pan-2 (where ad-3 can be ad-3A, ad-3B or ad-3A ad-3B). All possible pairwise combinations of these 19 dikaryons were made on adenine-supplemented medium and homology tests were made by plating conidia on medium supplemented with adenine and pantothenic acid at 37°C. Nonhomologous combinations of recessive lethal mutations should be able to show complementation as dikaryons and form colonies homokaryotic for the markers al-2 pan-2 and cot at 37°C. In such a test (a 19 x 19 grid) all pairwise combinations of dikaryons are made in duplicate and the combination of each dikaryon with itself (on the diagonal) serves as a control when conidia from the resulting trikaryons are plated. The interaction matrix derived from these tests has been used to construct the complementation map shown in Fig. 4. Such tests have shown (1) that some of the mutations (2/19) originally classified as recessive lethal mutations are due to the simultaneous occurrence of a viable mutation with a recessive lethal mutation elsewhere in the genome and (2) that all combinations of ad-3 recessive lethal mutations show homology except some of the ad-3A + ad-3B combinations. However the types of homology differ in different mutant combinations.

Discussion. - The present experiments and those described previously have provided a direct test of the usefulness of a genetically marked heterokaryon for forward-mutation studies. In accord with theoretical expectation, a much wider variety of mutational events has been recovered with this system than in comparable experiments on wild-type strains. The use of recessive lethal ad-3A and ad-3B mutations in dikaryons as tester strains in future studies will permit analysis of unknown ad-3 mutations with regard to (1) genotype (ad-3A, ad-3B or ad-3A ad-3B), and (2) the nature of the induced mutation in the ad-3

region (viable or recessive lethal), directly by means of a simplified homology test. This will also eliminate misclassification of viable mutants as recessive lethals because of the presence of recessive lethal damage elsewhere in the genome.

The apparent homology of certain ad-3A + ad-3B mutations is of particular interest especially since all combinations of recessive lethal ad-3A and ad-3B mutations complement in the trikaryon involving the genetically marked ad-3A ad-3B double mutant (component A). All such trikaryons grow on minimal medium, yet no ad-3A + ad-3B dikaryon has been recovered from the combinations shown as overlapping in Fig. 4. Such patterns of interaction suggest that there is an intact essential region (designated region X) present in the ad-3A ad-3B double mutant (component A) that is nonfunctional in either of the overlapping ad-3A and ad-3B recessive lethal mutations. Furthermore, it has not been possible to obtain al-2 pan-2 cot derivatives of these overlapping combinations by plating conidia from the trikaryon on a complete medium, as might be expected if region X contained a locus or loci specifying other biochemical requirements. Damage in this region is apparently irreparable. Other experimental approaches are in progress to verify these general conclusions.
AN ANALYSIS OF GENETIC SITE INSTABILITY

W. E. Barnett        F. J. de Serres

Introduction. — Spontaneously unstable or highly mutable alleles have been described in many genetic systems. Many of these alleles are autonomously unstable and are independent of chromosomal breakage phenomena or mutator genes. Furthermore, it appears almost certain that many such highly mutable alleles arising after exposure to various base analogues or chemical mutagens represent authentic transitional mutations (i.e., substitutions of the purine-for-purine, pyrimidine-for-pyrimidine type), which are unstable. One particularly interesting feature of autonomously unstable alleles is that they mutate to stable forms. This report deals with an analysis of site instability, as opposed to allele instability, in which a genetic site has mutated to a state of permanent high spontaneous mutability in two of its allelic (and phenotypically different) forms.

Results. — Strain 137, an ad-3B mutant of Neurospora, was isolated from conidia of strain 74A following exposure to nitrous acid. The most striking feature of 137 is its high frequency of spontaneous reversion \( \times 10^{-7} \) which is about 1000 times that of normal or stable alleles at this locus. To demonstrate that 137 was autonomously unstable, the strain was crossed with wild type and 120 ad-3 progeny were isolated. Each of these segregants was then tested for instability. They were always highly mutable and indistinguishable from 137. Furthermore, ascospore abortion data from this cross indicated that this allele was not associated with any detectable chromosomal abnormality.

To test further the nature of this instability, spontaneously arising revertants of 137 (s-revertants) were isolated and made homocaryotic by crossing. Table 2 shows a comparison of the forward-mutation frequencies of two s-revertants with that of the parental wild type strain. The 137 site alone in the s-revertants has a spontaneous forward-mutation frequency some 50 times greater than that for the entire ad-3 locus in the parental wild type strain. In one such experiment, 49 ad-3 mutants (s-mutants) were isolated from two s-revertants and compared with the original 137. Forty-eight of the 49 were indistinguishable from 137 on the basis of complementation responses to 19 ad-3 mutant tester strains, and, furthermore, all 48 exhibited the same degree of instability characteristic of 137. The one exception in these experiments undoubtedly is a result of "true" spontaneous mutation as the 49 were selected from approximately \( 3 \times 10^6 \) colonies.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of Colonies Observed</th>
<th>Number of ad-3 mutants Present</th>
<th>Spontaneous Forward Mutation Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>74A (wild type)</td>
<td>( 79.0 \times 10^6 )</td>
<td>23</td>
<td>( 2.9 \times 10^{-7} )</td>
</tr>
<tr>
<td>137-R4</td>
<td>( 2.6 \times 10^6 )</td>
<td>40</td>
<td>( 154.0 \times 10^{-7} )</td>
</tr>
<tr>
<td>137-PR5</td>
<td>( 3.2 \times 10^6 )</td>
<td>51</td>
<td>( 159.4 \times 10^{-7} )</td>
</tr>
</tbody>
</table>
To ascertain whether an unstable suppressor was involved in the s-revertant phenotype, they were crossed with wild type and the progeny assayed for ad-3 mutants. If a suppressor were present one would expect it to recombine with and segregate (or if nonlinked, simply segregate) from the 137 site. Such recombination would contribute to the ad-3 mutants recovered in direct proportion to the distance separating the two. Approximately $2.5 \times 10^6$ progeny were analyzed, and no evidence for suppressors was found.

The molecular configuration of the 137 site is shown to be unstable. Assuming that the genetic information resides in the nucleotide base pair sequences within DNA and that DNA exists as proposed by Watson and Crick, these results suggest that at a given site, certain nucleotide pairs are much more susceptible to the mechanism(s) of spontaneous mutation than others. Thus the mutability of a given base pair may be greatly influenced by its neighboring nucleotides.

Closer examination of these data, however, indicates that, although neighboring nucleotides may influence the probability of mutation of a base pair, they are highly selective in the type of mutation they enhance. This comes from the observation that 137 preferentially mutates to an unstable allele (or nucleotide pair) rather than to the stable wild type allele (or nucleotide pair).

**A STUDY OF CHEMICAL MUTAGENESIS IN NEUROSPORA**

W. E. Barnett  H. E. Brockman  F. J. de Serres

**Introduction.** — In phage and certain bacterial systems, it is now possible to identify a large percentage of the transition mutants as to the particular base pair present at the site of the mutation. This is made possible by analyzing the reversion responses of mutants to treatments with various chemical mutagens and base analogues. The general nonresponsiveness of *Neurospora* to base analogues has made the techniques utilized in bacteria and phage ineffective in this organism. This report describes the development of an analysis, based on reversion responses to nitrous acid (NA) and ethyl methanesulfonate (EMS), which permits one to identify transition mutations of *Neurospora crassa* and to separate these into two classes.

**Results and Discussion.** — Twenty-four nitrous acid-induced ad-3 mutants of *Neurospora* were screened in a typical reverse mutation experiment for reversion induction by NA and by EMS. Both NA and EMS treatments were standardized so that there was approximately 20% survival.

Nitrous acid is believed to produce both types of transition mutations (*i.e.*, AT → GC and GC → AT) by deamination. EMS, on the other hand, reacts preferentially with guanine and may produce either nontransitional (GC → T) mutations due to the hydrolysis of ethylated guanine or transitional (GT → AT) mutations via errors in replication. Table 3 shows that among the nitrous acid-induced mutants which may be classified as transitions (those which revert strongly with NA) approximately 50% also revert strongly with EMS. These results are consistent with the idea that NA induces both transitions with about equal frequency and that the GC sites are susceptible to reversion induction by EMS. No mutants have been found which revert strongly only with EMS. The results also suggest that NA induces a high percentage of nontransition mutations since 25% of the NA mutants do not respond to NA. Failure to respond is not proof however, and certain of the nonresponders may be transition mutants which for other reasons are not susceptible to NA mutation induction.
These results indicate that EMS induces only one type of transitional mutation. One would predict then that EMS-induced mutants would be largely nonresponsive to EMS and that the transitional mutations which induced (if any) would respond to NA only. This prediction is verified in the results shown in Table 4. EMS mutants fall into two main groups: those which revert strongly with nitrous acid only and those which revert with neither. The former group may be considered as having an AT base pair at the site of mutation; the latter can be considered nontransitional.

Thus it appears that the pattern of response to EMS and NA may be indicative of the type of mutational alteration that is present in a given mutant.

**AN IMPROVED MINIMAL MEDIUM FOR NEUROSPORA**

**H. E. Brockman**  **F. J. de Serres**

**Introduction.** — Sucrose is usually used as the carbon source in *Neurospora* minimal medium, and L-sorbose is added to this medium if a colonial morphology is desired. We have shown, however, that a number of factors — autoclaving time of the sucrose, sorbose concentration, type of basal medium, and source of
sorbose—can significantly modify the viability of *Neurospora* conidia on sorbose-sucrose media. Furthermore, maximum viability is achieved either by prolonged autoclaving of the sucrose or by substituting fructose and glucose for sucrose in the minimal medium. Because of these results, we have investigated the use of fructose and glucose as the carbon source for *Neurospora* minimal medium.

**Results and Discussion.**—Wild-type conidia were plated in three common *Neurospora* basal media—Fries’, Westergaard’s, and Vogel’s—which had been supplemented with fructose and glucose in combination with three concentrations of sorbose and autoclaved for 5, 15, 30, 45, and 60 min. The results of this experiment showed that the viability of *Neurospora* wild-type conidia in sorbose-fructose-glucose (S-F-G) medium is completely independent of autoclaving time, sorbose concentration, and types of basal medium. The viabilities of a number of nutritional mutants (niacin, pantotenate, inositol, histidine, lysine, tryptophan, and adenine-requiring) on S-F-G Westergaard’s medium are also independent of autoclaving time (the experimental variable least easily controlled), so the medium appears equally applicable to auxotrophic mutants of *Neurospora*.

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**Table 4. EMS Mutants**

<table>
<thead>
<tr>
<th>Mutant Number</th>
<th>Reversion Frequency (× 10⁻⁷)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spontaneous</td>
</tr>
<tr>
<td>5-3-31</td>
<td>0.0</td>
</tr>
<tr>
<td>34</td>
<td>0.7</td>
</tr>
<tr>
<td>40</td>
<td>0.0</td>
</tr>
<tr>
<td>14</td>
<td>0.0</td>
</tr>
<tr>
<td>13</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>33</td>
<td>0.0</td>
</tr>
<tr>
<td>35</td>
<td>0.1</td>
</tr>
<tr>
<td>38</td>
<td>0.8</td>
</tr>
<tr>
<td>11</td>
<td>0.3</td>
</tr>
<tr>
<td>6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

---

As *Neurospora* is frequently incubated at various temperatures, we studied the effect of this variable on the viability of wild-type conidia in S-F-G and sorbose-sucrose Westergaard's medium. The viability was constant at all temperatures studied (17, 24, 30, and 37°C) with S-F-G medium but decreased with decreases in temperature with sorbose-sucrose medium.

The results of these experiments suggested an explanation of the curious finding by Newmeyer that the viability of *Neurospora* ascospores is enhanced if they are allowed to germinate on sucrose medium prior to overplating with sorbose.\(^{29}\) We reasoned that the overplating method would give an increase in viability on sucrose, but not on fructose-glucose, medium. We have tested this possibility and have found that overplating does not increase viability on S-F-G medium and that this medium gives a higher viability of ascospores than does sorbose-sucrose medium with or without overplating.

Our studies show that the viabilities of *Neurospora* conidia and ascospores in sorbose-sucrose medium are strongly affected by many frequently encountered and difficulty controlled experimental variables. Fortunately, these same variables do not affect the viability on sorbose medium in which fructose and glucose are substituted for sucrose.

**INDUCTION OF AD-3 MUTANTS OF *NEUROSPORA CRASSA* BY 2-AMINOPURINE**

H. E. Brockman F. J. de Serres

**Introduction.**—The base analog, 2-aminopurine (AP), is an effective mutagen in phage and bacteria, and its mutagenic specificity is confined to the induction of transitions (AT→GC or GC→AT).\(^{30}\) The isolation of mutants at various loci of *Neurospora* following base analog treatment has been reported, but data have not been presented indicating whether the mutants are recovered at a rate greater than spontaneous frequency or whether they have any characteristics which distinguish them from spontaneous mutants.\(^{31,32}\) This report presents evidence for AP-induced forward mutation at the ad-3 region of *Neurospora*.

**Methods and Results.**—Wild-type conidia were inoculated into 59 test tubes containing minimal medium and into 64 tubes supplemented with minimal medium plus 1-5 mg/ml AP. After these cultures had grown, \(8 \times 10^5\) conidia from each test tube were inoculated into a flask containing 10 liters of adenine-supplemented minimal medium. Under these conditions, wild-type and ad-3 conidia form white and purple colonies, respectively. The 59 control flasks yielded 18 ad-3 mutants, and the 64 flasks inoculated with conidia from the AP-treated cultures yielded 136 ad-3 mutants. The complementation patterns of these mutants and of 16 spontaneous mutants from the stock collection were determined by testing them in pairwise combination with a set of testers which defines the ad-3A locus and 17 complementing units in the ad-3B locus. As mutants from the same flask could have been repeats replicated from a single mutation, any group of mutants


from a single flask having the same complementation pattern were classified as one mutant. By this criterion, the following number of mutants was isolated: 59 control flasks, 17; 64 AP flasks, 63.

Though the number of mutants recovered from the experimental flasks was clearly higher, it was possible that pre-existing spontaneous mutations had been selected for during growth of the AP-treated cultures. This objection is overcome by the following results from the complementation experiment. The frequency of \textit{ad-3B} mutants which complement at least one \textit{ad-3B} mutant in the tester set was 37\% for all spontaneous mutants and 64\% for the mutants from AP-treated cultures. More striking is the fact that the spectrum of functional defects of the complementing \textit{ad-3B} mutants recovered from AP-treated cultures is clearly different from that of the spontaneous complementing \textit{ad-3B} mutants (Fig. 5).

**Fig. 5.** Complementation Map of Complementing \textit{ad-3B} Mutants of 2-Aminopurine and Spontaneous Origin.

**Discussion** – Three lines of evidence support the conclusion that AP induces forward mutation at the \textit{ad-3} region of \textit{Neurospora}: (1) AP-treated cultures yield a significant increase of \textit{ad-3} mutants over the spontaneous frequency, (2) the percentage of complementing \textit{ad-3B} mutants is greater among the mutants recovered following AP treatment than among those arising spontaneously, and (3) the functional defects, as revealed by complementation mapping, of the mutants isolated from AP-treated cultures are distinctive from those of the spontaneous mutants.
THE ANTIBODY-PRODUCING CAPACITY OF CELL CLONES GROWN IN VIVO

D. L. Lindsley Guthrie T. Pratt J. E. Petty

It was shown by Till and McCulloch\textsuperscript{33} that the injection of small numbers of bone marrow cells into lethally irradiated mice gives rise, after 10 days, to large foci of proliferating cells in the spleens of the recipients. The number of foci formed after the injection of different numbers of cells or following injection of constant numbers of cells that have been given different doses of radiation suggests that these foci represent clones of cells derived from single, injected cells. Cytological observations of Welshons (unpublished data) agree with this interpretation.

We have been producing clones from lymph node and spleen cells from preimmunized donors, and then administering the suspended contents of entire clones to lethally irradiated recipients and testing the ability of the recipients and thus of the clones to produce antibody. The protocol is briefly as follows: Cell suspensions from the spleens or mesenteric lymph nodes of animals that have been preimmunized with \textit{Salmonella choleraesuis} are prepared and doses of ca $5 \times 10^4$ eosin-negative spleen cells or $2 \times 10^6$ eosin-negative mesenteric lymph node cells are administered intravenously into mice that have received 850 r.

At the same time the recipients receive \textit{S. choleraesuis} intraperitoneally. Ten days later the surviving recipients are killed and their spleens removed. The individual nodes are dissected free of surrounding tissue and are then teased apart in Tyrode's solution and strained; the resulting suspension is injected into a single mouse that was treated on the previous day with 900 r and $10^6$ isologous bone marrow cells. At the same time, \textit{S. choleraesuis} is administered intraperitoneally. Ten, 15, or 20 days later the recipients of clones are bled and their sera titrated.

The results of these titrations are summarized in Figure 6 and Table 5. It can be seen that the probability of recovering a clone that produces antibody is considerably greater from lymph node-derived cells than from spleen-derived cells. The proportion of clone producers among lymph node cells is small, but those cells that do produce clones a much higher proportion are capable of producing antibody than is the case of cells derived from spleen. Furthermore the 20-day titers produced by lymph node derived cells are higher than any other case studied.

The observations to date suggest that only a small proportion of the cells in the original dose administered have the capacity to proliferate into macroscopically visible clones, and that when the cells injected are derived from lymph nodes this proportion is much smaller than when they are derived from spleen. Among the clones produced some have the ability to produce antibodies and some have not. This proportion is much higher for cells derived from lymph nodes than for cells derived from spleen.

Fig. 6. Frequency Distribution of Anti-Salmonella Titers Observed in Recipients of in vivo Cultured clones of Spleen- or Lymph Node-Derived Cells.

Table 5. Antibody-Producing Capacity of Produced in vivo Clones of Cells from Spleen Cells and Mesenteric Lymph Node Cells

<table>
<thead>
<tr>
<th>Injected Cells</th>
<th>Number of Clones Per 10^6 Injected Cells</th>
<th>Time Between Transplantation of Clone and Bleeding (days)</th>
<th>Ab-Producing Clones (%)</th>
<th>Ab-Producing Clones Per 10^6 Injected Cells</th>
<th>Mean Log₂ Titer of Ab-Producing Clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x 10^4 Isologous spleen cells</td>
<td>48.9</td>
<td>10</td>
<td>4</td>
<td>2.0</td>
<td>2.9</td>
</tr>
<tr>
<td>5 x 10^4 Isologous spleen cells</td>
<td>90.5</td>
<td>15</td>
<td>16</td>
<td>14.5</td>
<td>2.9</td>
</tr>
<tr>
<td>5-10 x 10^4 Isologous spleen cells</td>
<td>31.8</td>
<td>20</td>
<td>7</td>
<td>2.2</td>
<td>3.5</td>
</tr>
<tr>
<td>1.5-3 x 10^6 Isologous mesenteric lymph node cells</td>
<td>0.8</td>
<td>20</td>
<td>75</td>
<td>0.6</td>
<td>4.1</td>
</tr>
</tbody>
</table>
A MUTUAL SPARING EFFECT OF THE X AND Y CHROMOSOMES TO THE
STERILIZING EFFECTS OF X-RAYS

D. L. Lindsley    J. E. Petty    Guthrie T. Pratt

It has long been known that a high proportion of the sons of irradiated males are sterile. From pre-
liminary studies that were designed to assess the contribution of the various chromosomes to this
sterility, it was concluded that the irradiated autosomes make no significant contribution to sterility,
whereas the irradiated X chromosome, and to a considerably greater extend an irradiated Y chromosome,
do cause sterility of sons that carry them. We had expected that sons receiving both an irradiated X and
an irradiated Y chromosome would exhibit an incidence of sterility that was the sum of the X-linked + the
Y-linked sterility that we had measured, and in the first small-scale experiments this seemed to be the
case. Upon repetition, however, the sterility of X/Y*, A/A* (*represents the paternally derived irradiated
elements and A represents one autosomal complement) males show about the same frequency of sterility as
X*/Y*, A/A* males when comparable doses are used.

The present experiments represent an attempt to accurately settle this question. Large numbers of
X/Y*, A/A* males were recovered from a cross of w Q Q X/Y* Q Q that had received a series of doses of
X rays. Similarly X*/Y*, A/A* sons were recovered from XX/O Q Q crossed to X/Y/Y males that had receiv-
ed various doses or radiation. The fertility of these sons was tested by crossing to stock XX, w/Y vir-
gins.

The results of two dose curves, each utilizing over 2,000 males tested, are shown in Figure 7. It is
evident that there is no difference in the amount of induced sterility in X/Y*; A/A* males and X*/Y*, A/A*
males. The replacement of X with X* seems to have no effect, and yet we know that X*/Y; A/A* males ex-
hibit a considerable proportion of sterility. It appears that when both the X chromosome and the Y chro-
mosome are present in the same irradiated spermatozoon, they have some sparing action on each other.

Three types of male sterility were previously described: prespermatogenic, spermatogenic, and post-
spermatogenic. In the present experiments over 600 males were dissected and scored microscopically for

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ORNL-3095, pp 35–38.
THE EFFECT OF GENE DOSAGE ON XANTHINE DEHYDROGENASE ACTIVITY IN DROSOPHILA

E. H. Grell

Introduction. — Two loci are known to control xanthine dehydrogenase activity in Drosophila. Mutations at either the maroon-like (ma-I) locus or the rosy (ry) locus cause an absence of the enzyme. In a previous report, enzyme activity was reported to be constant with different doses of ma-I+ (ref 35). Similar dosage studies with ry+ have been completed.

Results. — A chromosome deficient for the ry locus and a small chromosomal fragment containing the ry+ locus on a centromere were produced with X rays. The heterozygote for the deficient chromosome and a normal chromosome has one dose of ry+. Flies with two normal chromosomes have two doses, and flies with two normal chromosomes and the duplication have three doses of ry+.

Xanthine dehydrogenase activity in males and females with one, two and three doses was measured. The level of xanthine dehydrogenase activity was affected by dose and was roughly proportional to doses of ry+. Males and females gave the same response.

Discussion. — Of the two loci that are known to be involved in the formation of active xanthine dehydrogenase, only the dosage of ry+ is reflected in the level of enzyme. The most straightforward interpretation is that both ry+ and ma-I+ contribute a substance to make active enzyme. Perhaps the enzyme is made of two polypeptide chains like human hemoglobin. The ma-I+ substance is produced in excess and one dose of ma-I+ is sufficient for at least two doses of ry+. The lack of a dose effect at the ma-I+ locus then is only the result of the limiting action of ry+, and not necessarily a property of the ma-I+ gene.

THE ROLE OF DISTRIBUTIVE PAIRING IN SECONDARY NONDISJUNCTION

Rhoda F. Grell

Introduction. — The discovery of highly non-random assortment of nonhomologous chromosomes36 has provided a new tool for the study of meiotic events in the Drosophila female. As the result of such studies, evidence has been obtained indicating that associations between nonhomologues which lead to nonrandom assortment occur subsequent to exchange and involve only chromosomes that have not undergone recombination with their homologues.37 Secondary nondisjunction, i.e., X-nondisjunction which results from XX → Y segregation, resembles nonrandom assortment in that known homology between the X and Y is restricted to a single locus and crossing over does not occur between these chromosomes. Furthermore, secondary exceptions are, like the products of nonhomologous associations, noncrossovers.

36 R. F. Grell, Genetics 42, 374 (1957).
According to the currently accepted view, associations between the X and Y that lead to secondary exceptions occur before exchange and preclude crossing over between the X’s. The consequences required by this hypothesis are for both an increase in no-exchange X-tetrads and a decrease in X-crossing over that are correlated with the frequency of secondary nondisjunction. The process of secondary nondisjunction has been re-examined to determine whether such a correlation exists.

**Method.** – The effect of an unmarked, Canton-S Y chromosome on disjunction and recombination of the X’s was measured for isosequential X’s as well as for a variety of X-inversion heterozygotes. In all cases the test (XXY) and control (XX) females were sisters, thus insuring uniformity of genetic background. Progeny were reared from single females under strictly controlled conditions of temperature and humidity.

**Results.** – The results of the crosses are given in Table 6. The crossover values for XXY females, expected from the classical hypothesis (column 5), are obtained by reducing the observed crossover frequencies in XX females by the percent of nondisjunction in XXY females. With the normal X the observed crossover frequency is seen to be increased rather than decreased by a Y. Regional analysis discloses the increase to be located in the distal portion of the X. The frequency of no-exchange tetrads, as revealed by tetrad analysis, is unchanged by the Y (4.73 and 4.98 for XX and XXY, respectively) but double exchange tetrads are increased at the expense of singles.

Heterozygotes for distally located inversions show a decrease in crossing over with a Y that is, according to "t" tests, significantly less than that predicted. Heterozygotes for medial, proximal or near total inversions show an increase. In the latter cases, the increase is attributable to a distal enhancement in crossing over that was previously noted for the normal X’s. The decrease with distal inversions arises from a marked, intrabrachial increase in crossing over for regions most distant from the inversion breakpoint in the XX female and from the elimination of this increase by the Y.

The effect of the Y on nondisjunction and crossing over may best be gauged by a comparison of columns 3 and 7. The percent of alteration in crossing over by the Y (column 7) should, on the classical model, be negatively correlated with the nondisjunction frequency (column 3). No correlation is observed.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Type of Inversion</th>
<th>Secondary Nondisjunction (%)</th>
<th>Crossing Over</th>
<th>Alteration in Crossing Over (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+(Y)</td>
<td>None</td>
<td>2.20</td>
<td>6.36 ± 1.14</td>
<td>62.36 ± 1.12</td>
</tr>
<tr>
<td>+/+ y^+/(Y)</td>
<td>None</td>
<td>1.82</td>
<td>67.24 ± 0.95</td>
<td>65.98 ± 0.96</td>
</tr>
<tr>
<td>se^7/+/(Y)</td>
<td>Distal</td>
<td>26.31</td>
<td>32.65 ± 1.09</td>
<td>24.06 ± 0.88</td>
</tr>
<tr>
<td>65/+/(Y)</td>
<td>Distal</td>
<td>25.89</td>
<td>21.80 ± 0.83</td>
<td>16.16 ± 0.64</td>
</tr>
<tr>
<td>dl-49/+/(Y)</td>
<td>Distal</td>
<td>50.58</td>
<td>5.49 ± 0.70</td>
<td>2.71 ± 0.35</td>
</tr>
<tr>
<td>AB/+/(Y)</td>
<td>Medial</td>
<td>29.34</td>
<td>18.17 ± 0.92</td>
<td>12.84 ± 0.67</td>
</tr>
<tr>
<td>BM^1/+/(Y)</td>
<td>Proximal</td>
<td>18.53</td>
<td>31.95 ± 0.97</td>
<td>26.03 ± 0.82</td>
</tr>
<tr>
<td>se^8/+/(Y)</td>
<td>Near total</td>
<td>19.16</td>
<td>8.72 ± 0.56</td>
<td>7.04 ± 0.44</td>
</tr>
</tbody>
</table>

^\text{Primary nondisjunction less than 1\% in all cases.}
Summary. — The results indicate that the classical model for secondary nondisjunction is no longer tenable. It is proposed that secondary exceptions are not the consequence of pre-exchange XY (or XXY) pairing but rather, that they arise after exchange from distributive pairing between the Y and no-exchange X-tetrads. These observations constitute strong confirmatory evidence for the "distributive pairing" hypothesis of meiosis.

INTRALOCUS RECOMBINATION WITH A NOTCH DEFICIENCY IN DROSOPHILA

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

Introduction. — Studies at the Notch locus have revealed a pseudoallelic system composed of a minimum of six recessive lethals and four recessive visible mutations. Some of the recessive lethals were known to be cytologically nondeficient, and an equal number were assumed to be nondeficient when recombination was observed at the pseudoallelic level. This report describes the unexpected recombination observed between a recessive lethal associated with an observable cytological deficiency for a single salivary band, and two other separate recessive visible pseudoallelic loci.

Methods and Results. — The following cross was made: Heterozygous females, \( w^c h N^{264-39} \pm/\pm spl rb \), were mated with \( w^a fa^n sp \) males. The genetic distance from white-cherry (\( w^c h \)) to the cytologically deficient mutant Notch (\( N^{264-39} \)) is about 1.5 units and from Notch or split (\( spl \)) to ruby (\( rb \)) it is approximately 4.5, so that one can use \( w^c h \) and \( rb \) to reveal the linear order of \( N \) and \( spl \) in the event that crossing over occurs between these two pseudoalleles. The cross unexpectedly yielded 26 \( \pm \pm \pm \) recombinant chromosomes and one \( w^c h \pm \pm \pm \) nonrecombinant out of an estimated total of 48,000; a recombination value of approximately 0.05% and a position of the deficiency-Notch to the left of its pseudoallele \( spl \).

Since the mutant facet \( (fa) \), also a pseudoallele of \( spl \) and \( N \), lies to the left of \( spl \), the following cross was made: Females \( w^c h N^{264-39} \pm/\pm fa rb \) were mated to \( w^a fa^n rb \) males, and 14 \( w^c h \pm \pm rb \) recombinant plus one \( w^c h \pm \pm \pm \pm \) nonrecombinant chromosomes were obtained out of an approximate total of 47,500 tested, placing \( N^{264-39} \) about 0.03 units to the right of facet. Considering the results of both experiments, \( N^{264-39} \) is localized between its two pseudoalleles \( fa \) and \( spl \).

Conclusions. — It seems unlikely that the cytology of this deficiency-Notch is in error since a deletion of salivary band 3C7 has been observed by two independent investigators. Assuming the cytology to be correct, it follows that the Notch locus cannot be restricted to a single salivary band, since a series of other pseudoalleles of \( N^{264-39} \) are known to be localized at various positions within the 0.07 genetic units to the right of this deficiency and cannot be included in it or recombination would not occur. In other words, the functional unit or cistron called the Notch locus is probably composed of two or more salivary bands only one of which is 3C7.

The nonrecombinant \( w^c h \pm \pm \pm \) chromosomes obtained in the two experiments are valuable for research. Their origin can be explained in two ways: (1) a double crossover occurred between \( w^c h \) and \( rb \) with one of the crossovers being localized between \( N^{264-39} \) and the recessive visibles \( fa \) and \( spl \), and (2) an additional mutation, probably pseudoallelic to Notch, occurred on the chromosome which already carried the deficiency and restored the functional properties of the protein synthesized by the cistron. It is possible to discriminate between the two alternatives since according to (1) the chromosome will possess salivary band 3C7; if (2) is correct, the chromosome will be deficient for the band.
THE TURNOVER OF LABELED PROTEINS OF THE NUCLEUS OF AMOEBA DURING GROWTH AND DIVISION

D. M. Prescott
Romance F. Carrier

Introduction. — Previously\(^1\) we reported on two protein fractions in the amoeba nucleus. The first fraction migrated between the nucleus and cytoplasm but was more concentrated in the nucleus. The second fraction appeared to be retained by the nucleus even through several cell cycles. We have continued studies on the latter protein labeled with tritiated amino acids to determine whether it is conserved in the nucleus over a very long period of growth and division.

Methods. — *Amoeba proteus* was incubated for 24 hr in a mixture of six tritiated amino acids. This incubation period included the very short G1, the S period, and most of G2. After the labeling period, the amoebae were washed and grown on nonradioactive food at 23°C. Every 24 hr, one-half of the cytoplasm was amputated from each amoeba. This operation prevented cell division and allowed the replacement of the original radioactive cytoplasm with newly synthesized, nonradioactive cytoplasm. The operation was repeated up to 30 times, and the changes in labeled proteins of the nucleus were followed by autoradiography.

Results. — As previously reported, a migrating and a "nonmigrating" fraction of protein were found in the nucleus with 12 operations (99.9% replacement of the original cytoplasm). During the next 15 operations the radioactivity of nuclear proteins decreased steadily and by operation 27 a few amoebae no longer

contained labeled protein. After 30 operations, no amoeba contained label in the nucleus. The "nonmigrating" protein fraction was thus shown to be lost from the nucleus over a prolonged period of interphase growth. As a control for this experiment we have labeled amoebae with H\textsuperscript{2}-thymidine and found that no radioactivity is lost from the nucleus during the course of 30 cytoplasmic amputations.

Amoebae labeled with tritiated amino acids and cut 15 times have also been allowed to enter mitosis. During mitosis the "nonmigrating" fraction of protein becomes dispersed uniformly throughout the cytoplasm. There is no indication that the chromosomes or the mitotic spindle contain any radioactive protein. During telophase the labeled protein begins to return to the reconstituting nucleus and by early interphase the "nonmigrating" protein has been completely withdrawn from the cytoplasm and concentrated in the nucleus.

**Discussion and Conclusions.**—The "nonmigrating" protein has been shown to be completely lost from the nucleus during a long period of interphase growth. According to these experiments, no protein fraction in the nucleus is conserved (retains label) in the same sense that DNA is conserved. However, the sensitivity of the method is such that a nuclear protein which is present as less than one ten-millionth of the total nuclear protein would not be detected. Almost all (if not all) of the "nonmigrating" protein leaves the nucleus during mitosis and, therefore, cannot be considered as structural elements of the chromosomes.

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**DEVELOPMENT OF THE MULTINUCLEOLAR CONDITION IN AMPHIBIAN OOCYTES**

O. L. Miller, Jr.

Studies\textsuperscript{2} on the ultrastructure of nucleolar development in amphibian oocytes have been continued. The pachytene stage in early oocytes (20–30 μm diam) of *Rana clamitans* was identified in thin sections by the presence of synaptinemal pairing complexes within the chromatin material. A maximum of two nucleoli, each 2 to 4 μm in diam and apparently associated with chromosomal material, was observed in such cells. Following this stage, the chromatin material becomes diffuse while the number of nucleoli increases, the majority of these now assuming a peripheral position in the nucleus. Phase-contrast observations of oocyte squashes reveal 20 to 50 nucleoli ranging from 5 to 20 μm in diam to be present in 200 μm eggs. The larger of these apparently are formed by fusion of several smaller nucleoli.

In the pachytene and early lambrush stages, basic nucleolar fine structure consists of a fibrous core 2 to 4 μm in diam surrounded by a loose network of granular threads 100 to 150 nm in diam. In later oocytes, these external strands become compressed around the central nucleolar core to form a homogenous cortex of 150- to 200-A diam granules.

In 200μm oocytes of *Triturus viridescens*, on the other hand, several hundred peripheral nucleoli 1 to 4 μm in diam are present. Nucleolar structure previous to this stage consists solely of a fibrous component similar to the core of *Rana* nucleoli or a fibrous component containing small internal areas of granulation. As the oocyte enlarges, external granulation appears until in 300- to 400-μm diam eggs the nucleolar structure in *Triturus* oocytes is comparable to that in *Rana*.

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FINE STRUCTURE OF THE ORAL AREA OF TETRAHYMENA PATULA

O. L. Miller, Jr. G. E. Stone

Introduction. — In axenic culture, the polymorphic ciliate, Tetrahymena patula, consists of two predominant cell types, a small-mouthed form (microstome) and a carnivorous macrostome approximately twice as large as the microstome form. The latter possesses a large cytopharyngeal pouch absent in the microstome and both forms have the tetrahymenal-type buccal apparatus. Experimental manipulation permits synchronous transformation of the microstome to the macrostome form, a process which includes the formation of a new and enlarged mouth.

Observations and Discussion. — With the exception of over-all size, preliminary investigations have revealed only minor differences in the fully developed oral structure of the two cell types.

1. Membranelles. Each of the membranelles which lie on the inner wall of the buccal cavity consists of three long rows of kinetosomes with attached cilia. A series of parallel fibers originating at the base of the inner row of kinetosomes in each of the three membranelles runs toward the adjacent, lower membranelle or, in the case of the third membranelle, toward the junction of the inner and outer walls of the buccal cavity. After a short distance, a portion of each fiber rises and subdivides to form a periodic array of fine fibrils which lie immediately under the pellicle and run parallel with the long axes of the membranelles. The remaining portion of these kinetosomal fibers continues through the cytoplasm to pass underneath and in direct contact with kinetosomes of the next membranelle.

2. Undulating Membrane Complex. The undulating membrane runs around the top of the outer wall of the buccal overture and consists of a single row of cilia. Thin-section observations show, however, that there are actually two rows of kinetosomes in the membrane base. The outer row is ciliated while the inner row of kinetosomes, which are alternately spaced relative to those in the outer row, lie deeper in the cytoplasm and are entirely devoid of cilia. Fibers project straight down from the base of kinetosomes in both rows and join to form a tangled network which underlies the entire length of the membrane.

The surface of the outer buccal wall is covered with so-called oral ribs which have been variously described as stuck cilia, fibers, or pellicular differentiations. These structures begin near the base of the undulating membrane and descend in decreasing number to the cytosome. A 1:1 relation exists between the inner row of kinetosomes and ribs, and there apparently is a fine connection between the fiber system of the undulating membrane and the tips of the oral ribs. The ribs appear in cross-section as raised, thickened ridges of the inner pellicle layer with the outer layer adhering only at the top of the ridges. Immediately underlying nearly the entire area of oral ribs is a relatively thick differentiated layer or mat composed of fine fibers within a ground substance.

Investigations in progress deal with the changes in fine structure during the development of the macrostomal mouth.
EFFECTS OF AMINO ACID DEPRIVATION ON PROTEIN AND DNA SYNTHESIS IN TETRAHYMENA PYRIFORMIS

G. E. Stone

Introduction. — Tetrahymena pyriformis demonstrates three different responses to amino acid deprivation as a function of the interdivision cycle: (1) an early phase during which treatment will inhibit the next division, (2) an intermediate phase during which the result of treatment will be only a delay in division, and (3) a late phase during which amino acid deprivation apparently has no effect on the onset of division.

Experiments were designed to determine how long cells are able to incorporate other tritiated amino acids after being deprived of histidine and tryptophan. This should provide some indication of the relative efficiency of amino acid deprivation to inhibit protein synthesis and also indicate to what extent protein synthesis can be inhibited without preventing the next division. A study of the effects of amino acid deprivation on the DNA synthesis period was also made.

Methods. — Cells which had just completed division in a proteose-peptone-supplemented medium were immediately transferred to complete synthetic medium or to medium lacking histidine and tryptophan. Small samples of cells were then incubated with tritiated amino acids (5 μc/ml H3-leucine, 5 μc/ml H3-proline, 5 μc/ml H3-tyrosine, and 10 μc/ml H3-arginine) for 10 min at known times after division. After the 10-min incubation the cells were washed and dried on slides, extracted with hot TCA, covered with NTB 3 emulsion, and exposed for four days.

To determine the effect of amino acid deprivation on the S period, cells at known times after division were incubated for 30 min in 10 μc/ml H3-thymidine in (a) supplemented medium, (b) complete synthetic medium, and (c) synthetic medium lacking histidine and tryptophan. Microspectrophotometric measurements were made on Feulgen-stained macronuclei of (a) recently divided cells which had been incubated in supplemented medium and (b) cells which had been transferred to medium deficient in histidine and tryptophan at 1 1/2 hr after division.

Results and Discussion. — Preliminary experiments indicate that T. pyriformis incorporates tritiated amino acids in the absence of histidine and tryptophan at a decreasing rate for approximately 2 hr. After this time incorporation has essentially ceased. This means that the cells can continue through at least the last 35% of the interdivision cycle in the absence of protein synthesis, as measured by tritiated amino acid incorporation.

The DNA synthesis period during growth in supplemented medium was found to last 1 1/2 hr, beginning approximately 1 hr after completion of division. DNA synthesis is then completed 45–60 min before initiation of the next division. In complete synthetic medium the S period was found to begin approximately 1 hr after completion of division and continued until the next division, essentially eliminating G2. Cells

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placed in deficient medium immediately after division did not incorporate H\textsuperscript{3}-thymidine over a period of 330 min. However, cells deprived of histidine and tryptophan 1\textsuperscript{1/4} hr after division are able to double their DNA, as determined from microspectrophotometric measurements on the macronuclei.

Other experiments are in progress which will give some indication as to whether DNA synthesis can be initiated in the absence of histidine and tryptophan when the cells are deprived immediately before the S period.

**STUDIES ON MESODERALIZATION OF THE TRITURUS ECTODERM WITH THE BONE MARROW FACTOR**

Tuneo Yamada

**Introduction.** — It has been shown that a 3-hr treatment of a piece of the prospective ectoderm of the early gastrula of *Triturus pyrrhogaster* with a solution of a specific protein fraction isolated from the bone marrow of the guinea pig causes mesodermal differentiation in the ectoderm, when it is cultured subsequently *in vitro* in Holtfreter solution.\textsuperscript{4} A similar piece of untreated ectoderm invariably forms a cluster of epidermal cells under the same culture conditions. Synthesis of macromolecules in the treated and nontreated cells currently is being studied by autoradiography and immunoelectrophoresis. In assessing these data, knowledge of the mode of cell multiplication and its relation to the competence of the ectoderm has been found necessary. The first part of this report concerns an attempt to study cell multiplication during and after treatment. In the second part, experiments are described which define the period of competence toward the mesodermalizing treatment and relate it to cell generation.

1. **Cell Multiplication.** — A study was made to determine whether the treatment affects the pattern of cell multiplication before the first morphological sign of mesodermalization becomes observable (100 hr at 18\textdegree C). The measurement of the mitotic index was not adopted as the method, because of (a) partial synchrony of cell division disturbs interpretation of the data and (b) the possibility of mitotic abnormalities suggested by Sirakami.\textsuperscript{5} Instead, data on the cell size were utilized on the following grounds: First, no measurable change in cell size occurs during any given interphase in the developmental phase in question. Secondly, cell division cleaves the cell into two approximately equal halves. For the cell size measurement the explants were disaggregated into the individual cells with calcium-free Holtfreter solution. Disaggregated cells assume a spherical shape, and the diameter was measured on a random sample of the living cells. The histograms of cell size distribution within each explant and the average cell size data of each explant were prepared for the experimental and control explants cultured for 25 min to 100 hr after the treatment with bone marrow factor. No significant differences in the average cell size and the histogram patterns between the control and experimental explants were found throughout the phase studied. According to the data, cells go through two or three divisions during this same period, and the average generation time during the first half of the period is ca 3 hr at 18\textdegree C. Clear indications were obtained for an increase in the generation time during the period studied.

\textsuperscript{4}T. Yamada, *J. Cellular Comp. Physiol.*, Suppl. (in press).

\textsuperscript{5}K. Sirakami, *Embryologia* 3, 1–22 (1956).
If we assume the absence of cell size change during interphase and an approximately symmetrical division in the experimental and control series, the results imply that the bone marrow factor does not cause detectable shift in the cell multiplication pattern before its morphogenetic effect becomes apparent. This suggests that in the primary mechanism of mesodermalization a shift of cell multiplication is not involved. The results, however, are still compatible with the idea that subsequent to the phase studied the treated cells begin to multiply in a different way from the control cells. A consideration based mainly on the data makes it improbable that the hypothesis of clonal selection applies to the present case.

2. The Period of Competence. — Since in the present system the exposure time to the inducing influence is easily controlled, it is possible to determine the period of competence. In the first series of experiments the prospective ectoderm was isolated from two stages of the blastula and the pieces were treated with fraction ASP at 100 μg/ml in Holtfreter solution (pH 7.3) for 3 hr and then carefully washed and cultured in the standard culture medium. Mesodermalization of the ectoderm was observed in a high percentage of cases. In these experiments the stage of the donor embryo was so adjusted that it did not attain the earliest gastrula stage when the treatment was terminated. The result demonstrates that the competence for mesodermalization is already present during the blastula stage. This was quite unexpected in view of the generally held, but never precisely evidenced, idea that competence of the ectoderm appears only at the early gastrula stage (cf Balinsky). In another series of experiments, the prospective epidermis was isolated from the various stages of gastrulation and studied in the same way. The data indicate a gradual decrease of competence in the course of gastrulation. Altogether the experiments indicate that the competent period of the prospective ectoderm extends from the blastula to the middle gastrula stage, with the highest level of competence at the onset of gastrulation. This period coincides with, or includes, the period of competence for neural differentiation of various types of the same ectoderm. This contradicts the theoretical viewpoint of Flickinger according to which the specificity in morphogenetic effects of various inducing factors is dependent on the differences in the developmental stages at which they act on the reacting cells.

Measurements of cell size during the blastula and gastrula stages reveal that cells undergo an average of more than four divisions during the period of competence. Considering further data, it is probable that at the cellular level competence is not restricted to a single cell generation but distributed over more than two generations.

ELECTRON MICROSCOPIC OBSERVATION OF ECTODERMAL CELLS TREATED WITH BONE MARROW FACTOR

Shuichi Karasaki Tuneo Yamada

Introduction. — This study constitutes one of the analyses of mesodermalization of the Triturus ectoderm under the influence of a specific protein fraction separated from bone marrow of the guinea pig (cf Yamada).

8 T. Yamada, this report.
The successive changes of the fine structure occurring within the treated ectodermal cells were studied in thin sections with the electron microscope and compared with those of the control ectodermal cells.

**Method.** — A piece of the prospective ectoderm was isolated from the early gastrula of *Triturus pyrrohogaster* and put in a medium in which 100 µg/ml of fraction ASP was present. After different periods of immersion, the explant was fixed in a 2% buffered osmium tetroxide solution, dehydrated in ethanol, and embedded in Epon mixture. Such explants form mesodermal tissues in 95% of cases if cultured for more than 1 week. As a control, a comparable piece of ectoderm was cultured in the standard culture medium for the same duration, fixed and processed in the same way. Control ectoderm, on further culture, always forms a cluster of epidermal cells.

**Results and Discussion.** — The following sequences of events were seen.

1. After 15 min treatment with ASP solution, an invagination of the cell membrane accompanied by formation of large number of small vesicles could be observed. This suggests pinocytotic uptake of the protein from the culture medium. In some cases the invagination reached the vicinity of the nucleus. The cytoplasm, which was originally devoid of extensive vesicular components, often became filled with vesicles.

2. After 3 hr or more of treatment, the alignment of cells became irregular, intercellular spaces were enlarged, and cellular protrusions ran within the spaces. This observation deserves attention in view of the importance attached to cell affinity in differentiation phenomena.

3. Within 3 hr of treatment the nuclear envelope developed many irregular invaginations which allowed cytoplasmic strands to extend into the nuclear area. This suggests an involvement of the nucleus in the sequence of events leading to the alteration in the pathway of differentiation.

4. After 6 hr, the yolk platelets began to lose the superficial layer, and after 12 hr 90% of yolk platelets were devoid of this layer. Subsequently, pieces of the disintegrating, crystalline main bodies of the yolk platelets were observed within laminar membranes. In the cells of control ectoderm, similar changes in the yolk platelets occur, but at a much later phase of culture and at a much lower frequency. This observation, together with data on fluorescent antibody staining indicating the presence of bone marrow protein on the yolk platelets of treated cells, suggests a role of yolk platelets in the mechanism of induction.

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**CRYSTALLINE STRUCTURE IN AMPHIBIAN YOLK PLATELETS**

Shuichi Karasaki

**Introduction.** — In a previous report,9 the presence of a crystalline lattice structure in the main body of amphibian yolk platelets was suggested. However, a definite arrangement of the crystalline lattice and its internal units was not proposed. Additional study with ripe eggs and embryos of five different amphibian species now allows a more comprehensive description of the crystalline structure of yolk platelets.

**Results and Discussion.** — Near-focus pictures of 362 yolk platelets showed that 290 contained a main body displaying periodic patterns. Two types of patterns, a dot and a band pattern, were observed. The dot pattern (observed in only 18 cases) consists of a hexagonal array of approximately isometric dots.

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Each dot has a diameter of $45 \pm 5 \text{Å}$. The center-to-center distance between the dots was found to range from 70 to 90 Å with an average of 81 Å. The much more frequent band patterns consist of alternating dense and less dense bands with a spacing of 35 to 100 Å with an average of 71 Å. Moreover, in some sections a secondary system of parallel bands intersects the primary band system at angles ranging from 20 to 90°. Some intersecting bands display cuboid nets with a spacing of about 80 Å and hexagonal nets with a spacing of 70 Å. The prominent types of patterns observed seem to depend entirely upon the angle at which the main body was sectioned rather than upon magnification artifact or real difference between crystalline structures. Consideration of and comparison with various crystalline models suggests that the periodic patterns observed in the platelets would be expected from a simple hexagonal lattice; this model is consistent with birefringence and microscopic lamination\textsuperscript{10} described for yolk platelets. From the electron micrographs one may assume that each crystal is composed of cylindrical rods arranged parallel to their long axis in the simple hexagonal lattice. This assumption is strengthened by the observation that a slight tilt of the section or electron beam causes the dot pattern to transform into a band pattern. On the other hand, in some sections a dot pattern at one edge of the crystal changed into a band pattern in the interior and into a dot pattern again at the opposite edge. The latter type of double pattern is also seen in the electron micrographs of crystals of virus\textsuperscript{11} and ferritin\textsuperscript{12} composed of individual spherical particles. According to biochemical analysis\textsuperscript{13} of amphibian yolk, the crystalline body consists of two moles of phosphoprotein per mole of a lipoprotein. If the hexagonal arrayed dots observed with the electron microscope are assumed to represent spherical macromolecules, then the diameter of such dots ($45 \pm 5 \text{Å}$) fall within the range suggested for the diameter (41 Å) of the phosphoprotein molecules, as calculated from the molecular weight.\textsuperscript{13} The localization of the lipoprotein, however, is uncertain. If one assumes a cylinder with the hemispherical ends for a unit containing two phosphoprotein and one lipoprotein molecules, the axial length and diameter of such a unit can be calculated as 143 and 80 Å, respectively. Recent X-ray diffraction analysis of amphibian yolk\textsuperscript{14} has revealed that the primary lattice constant for both fresh and formalin-fixed samples is 164 Å, which may represent the length of a phosphoprotein and lipoprotein unit. The two phosphoprotein molecules in such a unit would then be about 80 Å apart (center-to-center distance) from one another and also a similar distance apart from phosphoprotein molecules in neighboring units.

EXPOSURE OF BIOLOGICAL SPECIMENS TO HIGH FLUXES OF THERMAL NEUTRONS. II. EFFECTS OF CAPTURE-GAMMA RADIATION

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

Introduction. — It has been shown that the amount of killing of bacteriophage T4 in an exposure chamber near the face of the LITR (Low Intensity Test Reactor) was about 100 times higher than expected from calculated gamma radiation from fission of U\textsuperscript{235} in the reactor core.\textsuperscript{15} The excess of dose was thought to be

\textsuperscript{13} R. A. Wallace, this report.
\textsuperscript{14} R. Honjin, personal communication.
from gamma and beta radiation produced by thermal neutron capture. The excess of dose, which we shall call the gamma equivalent dose as deduced from phage inactivation, was accurately measured; however, the true nature of the active agent was only inferred to be gamma radiation, with possibly some beta radiation effects.

Results. — We can now safely conclude that most of the phage inactivation can be attributed to gamma radiation from thermal neutron capture in the phage environment. This was proved by showing that the killing was correlated directly with the thermal neutron flux independently from the amount of lead or polyethylene shielding added to the initial lead shielding (Fig. 8). Added lead was used to shield phage from fission-gamma radiation and added polyethylene was used as a moderator for fast neutrons and as a control for the added lead part of the experiment. In neither case did the points depart from the exponential curve toward the direction of increased survival as would be expected if fission-gamma shielding had been ineffective or if the fast neutron flux had contributed to the inactivation of the phage.

An attempt was made to estimate the capture gamma dose rate at the sample location. This computation was made assuming that the lead and the aluminum constituting the irradiation facility did not contain any impurities, and, therefore, the calculated dose rate could be slightly underestimated. The results of this computation indicate that a dose rate of approximately $10^5$ roentgen/hr has to be expected at the irradiation site.

Basing computations on the previous experiments of Atwood and Mukai, it appears that at a level of $10^{17}$ thermal neutrons per cm$^2$, the biological effect on phage will become obvious. At this radiation level, the feasibility of the thermal neutron experiment appears to be conditioned by the ratio of thermal neutrons per cm$^2$ to gamma radiation in roentgens. This ratio ($nvt/yt$) in the HB-2 hole of the LITR under conditions of minimal shielding and minimal chemical protection is equal to $(2.31 \times 10^{15}$ thermal neutrons per cm$^2$)$/ (2.16 \times 10^5 \text{ r}) = 1.07 \times 10^9$. This ratio simplifies comparison between different conditions of radiation shielding and between different reactors: It should be kept in mind that the value of this ratio for an efficient experiment involving phage should be in the order of $10^{12}$.

Having shown that most of the inactivation is due to capture gamma, it was of interest to determine the $nvt/yt$ ratio under conditions where capture-gamma radiation is minimized. A simple experiment was performed at the MITR (Massachusetts Institute of Technology Reactor), a deuterium oxide–moderated reactor. Three bismuth plugs, 1 in. wide by 3 in. long, were placed between the phage and the reactor core; these were expected to stop only a fraction of the fission-gamma radiation. Placement of efficient shielding was considered impractical and too expensive to do without a preliminary test. The $nvt/yt$ ratio of this preliminary experiment was $7.6 \times 10^9$, only 30% lower than the HB-2 hole of the LITR. Most of the gamma radiation seemed to be fission gamma as indicated by the known flux within the reactor and by the rapid rate of phage inactivation. The gamma equivalent dose was in the range of $1.5 \times 10^6 \text{ r/hr}$.

Discussion. — In order to perform the biological experiments on phage that we envision, it will be necessary to increase the $nvt/yt$ ratio 50 to 100 times. Appropriate shields producing less capture-gamma

Fig. 8. Dose-Action Curve for Exposure of Bacteriophage T4 in the LITR with Different Amounts of Added Shielding.
radiation must be constructed. Not all of the increase of the \textit{nvt/yt} ratio need come from shielding modification however, since chemical protection can also help to change the ratio. Both shielding design and chemical protective methods are being explored.

\textbf{NUCLEAR REACTIVATION IN THE WASP \textit{HABROBRACON}}

\begin{flushleft}
E.-A. Löbbecke \hspace{2cm} R. C. von Borstel
\end{flushleft}

\textbf{Introduction.} – In the parasitic wasp \textit{Habrobracon} unfertilized eggs develop normally to become haploid males. It has been observed that newly laid eggs exposed to ultraviolet radiation on the surface, where the nucleus lies, are killed at a reduced frequency early in development if the eggs have been fertilized before irradiation.\textsuperscript{17} This study was undertaken to determine: (1) if the phenomenon is indicative of a true repair of ultraviolet radiation-induced damage, some kind of delayed death, or a case of tremendously enhanced induction of recessive lethal frequency, (2) the stage or stages at which death would have occurred if repair had not taken place, and (3) if the fertilization-dependent recoverable portion is identical to the photorecoverable portion of ultraviolet radiation damage.

\textbf{Results.} – Dose-hatchability curves were constructed for eggs from virgin females, eggs from mated females, eggs from virgin females with posttreatment of photoreactivating light, and eggs from mated females with posttreatments of photoreactivating light. Since not all eggs from a mated female are fertilized, the data were analyzed in a way so that the early deaths from the irradiated fertilized eggs could be compared with those of the unfertilized eggs\textsuperscript{18} (Fig.9). It can be seen that nuclear reactivation is largely independent from photoreactivation. It also appears that as the dose increases the sperm and egg nucleus both are damaged; this is manifested by the fertilized egg curve approaching the unfertilized egg curve in both the nonphotoreactivated and photoreactivated experiments.

It was determined that the eggs dying early in development are the embryos susceptible to repair when the sperm are present. That the repair is not merely a delay of time of death to posthatching stages of development is demonstrated in Fig. 10. Here it is seen that as the dose of ultraviolet radiation increases, the sex ratio of the survivors of females to total adults also increases. If the repair were merely covering up recessive lethal damage, then the recessive lethality observed in \textit{F}_{1} females should all be expressed early in development. This was not the case, and besides, not enough recessive lethality was found to account for all the repair.

\textbf{Discussion.} – Nuclear reactivation is a phenomenon that can be observed only in systems where haploids and diploids are both viable, or where cells with more than one haploid nucleus exist in the same cytoplasm such as the conidial spores of \textit{Neurospora}.


Fig. 9. Dose-Hatchability Curves of Fertilized and Unfertilized Eggs With and Without Photoreactivation.

A phenomenon akin to nuclear reactivation has been observed in yeast following X radiation\(^{19}\) and in \textit{Neurospora} following ultraviolet irradiation.\(^{20}\) In \textit{Habrobracon}, nuclear reactivation after exposure to X radiation appears to be very slight.\(^{17}\) In yeast and \textit{Neurospora}, it is not clear whether the dominant lethal component or the recessive lethal component is being reactivated to the normal condition. In \textit{Habrobracon} it seems clear from the early death data that true killing of the nucleus is being countered. The type of


Fig. 10. Dose-Action Curves Showing the Increase in Proportion of Females Among Offspring as a Function of Dose of Ultraviolet Radiation. The right ordinate demonstrates this proportion as a measure of reactivation of gamete damage.

killing by radiation may not be genetic in the sense of chromosome breakage or loss, but the radiation effect is certainly upon nuclear components or centrioles. It would appear that in some fashion the undamaged nucleus is able to assist the damaged nucleus through the early divisions by supplying an essential step (of synthesis?); after one or more divisions, the damaged nucleus recovers.

THE EYE COLOR MUTANTS OF THE WASP HABROBRACON

G. S. Van Pelt

In a previous report on the eye color mutants of the parasitic wasp, Habrobracon juglandis, an allelic series was described at the cantaloup locus, and it was stated that tests were being performed to localize these alleles with respect to other genes on chromosome I (ref 21). Allelism studies with a cantaloup stock, from a source other than this laboratory which gave proper linkage values with adjacent markers, revealed that the six alleles being tested were not at the cantaloup locus but at a new locus, designated the garnet locus.

Before biochemical studies on the pteridine and ommochrome pigments of *Habrobracon* could be continued, it became necessary to check all existing mutant stocks with respect to allelism, linkage, and color. The eye color of each mutant type raised at uniform conditions is being described, using the Color Harmony Manual as a standard color reference. Eye color can vary with rearing temperature, age, and gene dosage; the existence of these effects are being noted for each mutant.

Gene dosage is exhibited by three of the six mutants at the garnet locus, whereas the other three exhibit dosage compensation. This phenomenon is particularly interesting in the case of *Habrobracon*, since normally the males are haploid, coming from unfertilized eggs, and the females are diploid, coming from fertilized eggs. This dosage effect could be the action of one gene as compared to two, since the homozygous females have an eye color lighter than the hemizygous males. Crosses made to test this basis for color difference have so far produced three diploid males having an eye color like the diploid female. No triploid females have yet been obtained.

The normal wild-type eye color of *Habrobracon* is black and all mutants are necessarily lighter. Combinations of two or more mutants in the homozygous condition usually cause the eye to be white. A new mutant at the red locus, barolo (*rd^b*), exhibits a lightening effect on the alleles at the garnet locus but a complete lack of color in combination with mutants at other loci. At the present time, there are 15 eye color mutants representing five loci, and two new mutants still being tested.
Mammalian Genetics and Development

Section Chief - W. L. Russell

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<td>J. R. Inman</td>
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<td>Barbara A. Barbee(^b)</td>
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<td>E. H. Y. Chu</td>
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<tr>
<td>Georgia M. Guinn</td>
<td>Julia A. Joyner(^b)</td>
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<td>Patricia R. Hunsicker</td>
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<td>Martha M. Larsen</td>
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<td>H. M. Thompson, Jr.</td>
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<td>Sandra J. Weaver</td>
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<td>Florence N. Woodiel</td>
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<td>D. Patricia Wright</td>
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</table>

\(^a\) Consultant.
\(^b\) Student Trainee.

Effects of Radiation on Mammalian Gametogenesis

| E. F. Oakberg                       | Evelyn Clark           |
|                                    | Margaret S. Mitchell\(^b\) |
|                                    |                        |

\(^b\) Student Trainee.

MUTATION FREQUENCY AND RADIATION DOSE RATE

W. L. Russell Elizabeth M. Kelly

The mutation frequency obtained to date in a new fractionated dose experiment is already highly significantly greater than any mutation rate found before for spermatogonia. This indicates that a new factor affecting the mutational response is involved. It will be important to explore this factor further, both for an understanding of its mechanism and for its bearing on the problem of radiation hazards.

Method. – Adult male 101 x C3H F1 hybrid mice were exposed to a total dose of 1000 r of x rays (250 kvp, 15 ma, inherent filtration 3 mm Al, HVL 0.4 mm Cu) delivered at approximately 90 r/min in two fractions of 500 r separated by 24 hr. As in our other 1000 r experiments, the anterior two-thirds of the body of each animal was shielded with lead. The males were mated to females of our standard test stock, which is homozygous for seven specific marker genes. Mutation at the seven specific loci were scored in offspring conceived after the males’ temporary sterile period, the median length of which was approximately 2 weeks greater than a single 1000 r exposure.
Results and Conclusion. — The mutation frequency for this fractionation experiment is presented in Table 7 along with results for single, 90 r/min exposures of 300, 600 and 1000 r reported earlier, but augmented, for the 300 r and 1000 r doses, by new data. The control mutation frequency given in Table 7 was obtained by pooling results from a large number of experiments. All these data are plotted in Fig. 11, which shows also a straight line fitted by the least squares method (using Poisson weighting) to the 0, 300 and 600 r points.

As has already been mentioned, the induced (experimental minus control) mutation rate of $49.2 \times 10^{-8}$ per roentgen, per locus, obtained in the fractionated-dose experiment is the highest ever observed in spermatogonia. It is more than five times the rate of $8.5 \times 10^{-8}$ per roentgen, per locus, given by a single dose of 1000 r. The difference is highly significant ($P < 0.00005$). It is also more than twice the average induced mutation rate per roentgen for the single-dose experiments at 300 and 600 r ($P = 0.0002$).

This large and statistically significant increase over the mutation rate that would have been expected on the basis of linear extrapolation from the rates at lower doses shows that a new phenomenon is involved. The results indicate that the spermatogonial population at 24 hr after the initial exposure to 500 r may be especially sensitive to mutation induction and that the cells, therefore, respond with a high mutation rate to the second 500 r exposure. The increased sensitivity may plausibly be attributed to radiation-induced change in the array of cell division stages in the spermatogonial population. Whatever the real explanation is, the length of the time interval between fractions is crucial. In another fractionation experiment, in which two doses of 500 r were given 2 hr apart, the preliminary results show little, if any, elevation of the mutation frequency with this shorter time interval.

The factor causing the fractionation effect reported here may turn out to be of more general importance than the one responsible for the drop in mutation rate when the dose is increased from 600 to 1000 r (single exposures). The latter effect can still be accounted for in terms of cell selection, and the present evidence already indicates that this may be important only at very high doses. The fractionation factor happened to be discovered in a high-dose experiment, but this is no guarantee that it will not also be found


| Dose (r) | Interval Between Fractions (hr) | Number of Offspring | Number of Mutations at 7 Loci | Mean Number of Mutations per Locus per Gamete $\times 10^5$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>531,500</td>
<td>28</td>
<td>0.75</td>
</tr>
<tr>
<td>300</td>
<td></td>
<td>65,548</td>
<td>40</td>
<td>8.72</td>
</tr>
<tr>
<td>600</td>
<td></td>
<td>119,326</td>
<td>111</td>
<td>13.29</td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td>44,649</td>
<td>29</td>
<td>9.28</td>
</tr>
<tr>
<td>500 + 500</td>
<td>24</td>
<td>11,164</td>
<td>39</td>
<td>49.91</td>
</tr>
</tbody>
</table>
to be somewhat effective in the lower-dose experiments now being done. Another important question also being explored is whether any fractionation effect of this sort can be found in oocytes.

SEX CHROMOSOME ANOMALIES FROM IRRADIATION OF MEIOTIC PROPHASE (PRE-DICTYATE) STAGES OF THE MOUSE

Liane B. Russell Clyde Lee Saylors

Introduction. — A program is underway in which various germ-cell stages of the mouse are being compared for sensitivity to the induction of sex-chromosome anomalies. Results have already been published on pronuclear stages of the zygote, on mature spermatozoa, and on spermatocytes in different prophase stages. Of the various germ-cell stages accessible for study, meiosis in the female presents the greatest technical difficulties. The work done so far has been restricted to pre-dictyate stages, and even there

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we have not yet been able to obtain conclusive results. The experiment is being repeated, but since this second run will not be completed for some time, the results obtained to date will be presented here.

In the female, meiotic prophase stages through diplotene are completed during fetal and neonatal life.\(^7\,^8\) For this reason, a succession of two experimental matings is required to study the effect of irradiation on such stages. The procedure followed is outlined in Fig. 12.

Fig. 12. Experimental Matings for Study of Pre-Dictyate Stages.

Method. — Wild-type \((101 \times C3H)F_1\) female mice were mated to \(Ta/Y\) males from various stocks. Matings were timed by the observation of vaginal plugs. Some of the pregnant females were then irradiated in one of several stages of gestation; others were allowed to give birth and their female newborn or day-old offspring were irradiated. In this manner a population of \(Ta/+\) females irradiated at various postconception ages, ranging from day 13\(\frac{1}{2}\) to day 20\(\frac{1}{2}\), inclusive, was obtained. These \(Ta/+\) females were raised to maturity and then mated to \(Blo/Y\) males. The doses chosen were based on our earlier experience with survival of fetally irradiated mice\(^9\) and with fertility of females following fetal and neonatal irradiation.\(^10\,^11\) Doses were 250 r for days 13\(\frac{1}{2}\)–18\(\frac{1}{2}\), inclusive; and 200 r for days 19\(\frac{1}{2}\) and 20\(\frac{1}{2}\). Unfortunately, the yield for certain stages was considerably lower than anticipated, and in the repeat experiment now underway, lower doses at these stages are being used.

Fig. 12 shows that, of various possible exceptional genotypes, only some (circled with solid line) are phenotypically detectable. Provided crossing over does not occur, detectability is 50% (genotypes ringed

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\(^11\) W. L. Russell, unpublished data.
by solid circles) but could be higher in the doubtful case that the Ta/+/Blo type (surrounded by broken circle) is recognizable. Crossovers proximal to Tabby, combined with nondisjunction, would produce XXY types that are indistinguishable from normal segregants; and XXX types probably even less likely to be detected than the XXX formed in the absence of crossing over. XO and YO would, of course, not be affected by crossing over. Detectability may then be summarized as follows: (1) for absence of maternal X, 50%; (2) for presence of two maternal X's, (a) assuming none of the XXX types is detectable, 50% minus half the crossover frequency (Ta-locus to centromere) and (b) assuming that all XXX types are detectable, 100% minus half the crossover frequency. For purposes of approximate calculation in this preliminary experiment, detectability of all exceptional types has been taken as 50%.

**Results.** – The results to date are summarized in Table 8. The only exceptional animals of the types outlined were two Blo/O females which were offspring of mothers irradiated as 17½ or 18½-day fetuses. Since the spontaneous frequency of OX P is extremely low, it appears highly probable that these two cases were induced by the irradiation of prophase oocytes. No exceptional cases were found among an approximately equal number of offspring from females irradiated in earlier fetal stages, or among a considerably smaller number of offspring of females irradiated as newborns or day-olds. Since no XXX or XXY animals have been found so far, there is to date no evidence for induced nondisjunction.

<table>
<thead>
<tr>
<th>Post-Conception Day Irradiated</th>
<th>Number of Animals Classified</th>
<th>Number of Exceptions</th>
<th>Adjusted\textsuperscript{a} Induced Frequency per ( \times 10^{-5} ) of ( X^M ) Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>498</td>
<td>0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>13½-16½</td>
<td>454</td>
<td>0 0 1</td>
<td>0</td>
</tr>
<tr>
<td>17½-18½</td>
<td>475</td>
<td>2 0 0</td>
<td>3.4</td>
</tr>
<tr>
<td>19½, 20½</td>
<td>144</td>
<td>0 0 2</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Calculated on basis of 50% detectability.

It may be mentioned that, in addition to the Blo/O individuals, there were two Ta/O and one +/O. Of these three females, which presumably resulted from loss of a paternal sex chromosome, two and one, respectively, occurred among offspring of the 19½-day and 14½-day irradiated groups. The total incidence is, so far, within the normal spontaneous range and it need, therefore, not be assumed that irradiation of very young oocytes creates conditions which, later, on predispose to loss of the male-contributed chromosomes.

It may be noted that the primary oocytes of 17½- and 18½-day fetuses yielded a similar frequency of X/O as did primary spermatocytes of the "middle" period (i.e., sampled in matings made 28–35 days after irradiation). Whether similar prophase stages are involved has not, to date, been determined. Until XXY animals are obtained, it would be incautious to assume that nondisjunction is induced by irradiation in prophase (pre-dictyate) oocytes.
For purposes of comparison, Table 9 lists the present results together with a figure derived in a similar manner (irradiated minus control frequencies per roentgen) from already published data for the early female pronucleus. The extreme sensitivity of chromosomes in the pronucleus stage of the zygote is as strikingly illustrated here as it was in the case of comparison of male germ cell stages.

Table 9. Irradiation of Female Germ Cell Stages

<table>
<thead>
<tr>
<th>Post-Conception Day Irradiated</th>
<th>Adjusted Induced Frequency per $\alpha_{\text{rad}}$ $(\times 10^{-5})$</th>
<th>$X^M$ Loss</th>
<th>XXY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early $\varphi$ pronucleus in zygote</td>
<td>18.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prophase primary oocyte</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.5, 20.5 days postconception</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17.5, 18.5 days postconception</td>
<td>3.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13.5–16.5 days postconception</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>


THE EFFECTS OF ULTRAVIOLET RADIATION ON MAMMALIAN CHROMOSOMES

E. H. Y. Chu

Introduction. — Previous studies, in which Chinese hamster tissue-culture cells were exposed to monochromatic ultraviolet (uv) radiations, demonstrated that the region near wavelength 2652 $\AA$ was the most effective in inducing chromosome aberrations and suggested a possible photorecovery by visible light. Further experiments confirmed the action spectrum for aberration production previously found, but showed an absence of photoreactivation or photoprotection. Chromosome "demolition," largely induced at 2804 $\AA$, is probably a separate phenomenon from the usual chromosome aberrations resulting from breakage and reunion. Autoradiographic and chemical experiments, combined with radiation, have been conducted to correlate the observed chromosome abnormalities with the organization and duplication of chromosomes.

Results. — The chromosome aberration types induced by uv were qualitatively indistinguishable from those induced by x rays. In contrast to plant materials, a large number of chromatid exchanges can be induced by uv in mammalian cells. Aberration frequencies increased with dose throughout the range

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studies, although the multiple chromosome fragmentations and exchanges found at higher doses severely limited the accuracy of analysis.

Conventional chromosome aberrations reached a peak frequency at 2652 Å. At 2804 Å, however, additional abnormal cells were observed in which chromosomes were totally or mostly demolished, forming condensed, irregular chromatin masses. They were morphologically different from the shattered chromosomes in plant materials treated with uv (2650 Å) or fluorodeoxyuridine. At this wavelength, chromosome demolition began to appear even at a relatively low incident dose of 25 ergs/mm² and continued to increase in frequency with dose; but at 2652 Å, it became detectable only at a much higher dose (200 ergs/mm²).

Four duplicate experiments failed to show evidence of either photoprotection or photoreactivation of the uv-induced chromosome aberrations. The combined results are shown in Table 10.

Table 10. Results of Photoreactivation Studies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Cells Scored</th>
<th>Percentage Normal</th>
<th>Aberrations per Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cd.</td>
</tr>
<tr>
<td>2652 (100)</td>
<td>700</td>
<td>59.6</td>
<td>0.35</td>
</tr>
<tr>
<td>2652 (100) + PR</td>
<td>465</td>
<td>62.2</td>
<td>0.35</td>
</tr>
<tr>
<td>PR + 2652 (100)</td>
<td>100</td>
<td>56</td>
<td>0.32</td>
</tr>
<tr>
<td>2804 (100)</td>
<td>600</td>
<td>68.3</td>
<td>0.28</td>
</tr>
<tr>
<td>2804 (100) + PR</td>
<td>625</td>
<td>66.1</td>
<td>0.29</td>
</tr>
<tr>
<td>PR + 2804 (100)</td>
<td>100</td>
<td>59</td>
<td>0.22</td>
</tr>
<tr>
<td>PR</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>98</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*a uv surface exposure of 100 ergs/mm² at 2652 Å or 2804 Å, respectively.

*b Photoreactivating wavelengths in the range 3300–4000 Å for 20 min at 37°C (estimated incident energy: 120 ergs mm⁻² sec⁻¹).

Autoradiography using tritiated thymidine indicated that the average cell generation time was 24 hr (G1 plus mitosis, 12–14 hr; S, 8 hr; G2, 2–4 hr). Chromosomes were effectively duplicated very early at S. Chromatid aberrations can be induced at both G2 and S periods, probably at a higher rate during S. There is also evidence of temporary reversion to partial DNA synthesis following irradiation.

**Discussion.** — The fact that the action spectrum for chromosome aberration production approximately parallels the uv absorption spectrum of nucleic acids suggests that nucleic acids (probably DNA) are the primary sites of injury, as has been shown in many other organisms. Experiments in progress in which bromodeoxyuridine-treated cells were irradiated with various uv wavelengths should provide more critical evidence. Chromosome breakage and reunion can occur freely at certain periods in cell cycle, during as

well as outside the DNA synthetic phase. The nature of this initial damage and its manifestation as a
final metaphase chromosome aberration are still unclear. The observed chromosome demolition, however,
represents a different phenomenon, which probably involves protein and is triggered by uv, leading to the
disruption of normal organization of chromosomes.

RELATIVE EFFECTIVENESS OF X-RAYS AND 130 Mev PROTONS
AS MEASURED BY SPERMATOGONIAL KILLING

E. F. Oakberg    Evelyn Clark

Our previous experiments on the effectiveness of different radiation qualities on spermatogonial killing
have dealt primarily with Co\textsuperscript{60} gamma rays and neutrons. Both 2.5 and 14.1 Mev neutrons gave RBE's of
about 2 in these comparisons.\textsuperscript{17} In the present experiments, the effectiveness of 130 Mev protons from
the Harvard proton cyclotron and x-rays were compared.

Mice were exposed in plastic tubes to doses of 0, 5, 10, 25, 50, 100, and 200 rads. In order to control
possible effects of stress arising from shipping the mice to Boston and maintaining them in strange animal
quarters, a comparable series of x ray exposures (280 kvp, 19 ma, HVL 1.3 mm Cu, 1 mm Al + 0.25 mm Cu,
70 r/min), were made at the Massachusetts General Hospital.

All mice were killed 72 hr after exposure. Histological techniques and quantitative evaluation of the
slides were the same as described previously.\textsuperscript{18}

Data presented in Table 11 reveal a lower effectiveness of 130 Mev protons for all three cell types.
This result was not expected, since, on the basis of LET, comparable biological effectiveness of 130 Mev

\textsuperscript{17}E. F. Oakberg and E. Clark, \textit{J. Cellular Comp. Physiol.} \textbf{58} (suppl 1), 173–82 (1961).

\begin{table}[h]
\centering
\caption{Experimental/Control Ratios of Normal Cells
Observed 72 hr After Irradiation}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Dose & \multicolumn{3}{|c|}{Type A Spermatogonia\textsuperscript{a}} & \multicolumn{2}{|c|}{Type B Spermatogonia\textsuperscript{b}} & \multicolumn{1}{|c|}{Preleptotene Spermatocytes\textsuperscript{c}} \\
(rad) & \textsuperscript{130 Mev} Protons & X Rays & \textsuperscript{130 Mev} Protons & X Rays & \textsuperscript{130 Mev} Protons & X Rays \\
\hline
5 & 0.85 & 0.89 & 1.013 & 0.93 & 0.99 & 0.93 \\
10 & 0.97 & 0.75 & 0.96 & 0.89 & 1.005 & 0.85 \\
25 & 0.73 & 0.60 & 0.81 & 0.71 & 0.72 & 0.64 \\
50 & 0.50 & 0.34 & 0.43 & 0.17 & 0.33 & 0.13 \\
100 & 0.32 & 0.20 & 0.08 & 0.02 & 0.01 & 0.00 \\
200 & 0.18 & 0.12 & 0.01 & 0.00 & 0.00 & 0.00 \\
\hline
\end{tabular}
\textsuperscript{a}Irradiated and scored as type A spermatogonia.
\textsuperscript{b}Irradiated as type A, scored as type B spermatogonia.
\textsuperscript{c}Irradiated as late type A and early intermediate spermatogonia, scored as preleptotene spermatocytes.
protons and 280 kv x rays should have been observed. This difference in effectiveness cannot be considered established until the effect of the highly pulsed nature of the proton beam can be evaluated.

Female mice also were exposed to both 130 Mev protons and x rays, and oocyte survival will be determined. Should the lower effectiveness of 130 Mev protons observed in the male be established, determination of the relative effectiveness of these radiations in oocytes is of special interest, since both DNA synthesis and cell division are excluded from the oocyte response.
EFFECT OF CULTURAL CONDITIONS ON RADIOSENSITIVITY OF BACTERIA

G. E. Stapleton

Earlier reports\textsuperscript{1,2} have described some characteristics of the development of radiation resistance of \textit{Escherichia coli} in poorly buffered glucose media. One such report\textsuperscript{1} has dealt with the nutritional requirements and the time sequence for the processes involved in the gaining of resistance by such cultures.

Recent experiments indicate that the final resistance measured in late stationary phase is the result of processes that occur in two phases, which are separable on the basis of nutritional requirements and the macromolecular composition of the cell populations.

Phase 1, which follows in time the logarithmic phase, is characterized by a major pH change in the culture and a subsequent increase in cell size and concentration of DNA, RNA, and protein per cell. The survival curves for cells in this phase reflect the multiplicity of the macromolecular components and as compared with the survival curves for sensitive cell populations resemble the classic differences often reported for haploid and diploid cell populations of yeast. To date it has not been possible to have cells reach this phase in the absence of a major pH shift or without a complete complement of amino acids. A number of hypotheses have been presented for explanation of how this phase may be reached including the possibility of inhibition of cell division without attendant inhibition of macromolecules. In any case, it is remarkable that the cell populations are essentially homogeneously doubled in size and have a double complement of the macromolecular components per cell.

Phase 2 progresses with time over the 8 to 10 hr following Phase 1 and proceeds without further cell division and without measurable change in cell size or concentration of DNA per cell, but with a large increase in radioresistance (Fig. 13).

By harvesting cultures that have proceeded to Phase 1 and continuing the incubation of these cell populations in a variety of media and conditions, it has been possible to show that this phase is different in several respects from the earlier phase. First, it can proceed in a simple inorganic salts-glucose medium (M-9). The carbon and nitrogen sources are required, and the processes leading to maximal resistance are strongly pH-dependent, being severely inhibited at a pH of 7.0 or above.

Although the nature of the processes that occur in either phase have not been adequately studied to date, it would seem that the separation of the phases will be helpful in understanding the biochemical nature of this phenomenon.

**GENETIC ANALYSIS OF RADIATION SENSITIVITY IN ESCHERICHIA COLI**

H. I. Adler  
J. C. Copeland

In an earlier report we demonstrated that certain differences in radiation sensitivity of strains of the bacterium *Escherichia coli* can be attributed to the expression of a group of genes. On the basis of conjugation experiments, the genes have been assigned to the Histidine-Proline region of the *E. coli* linkage map. In almost all cases, these genes influenced sensitivity to both ultraviolet (2537 A) and x irradiation.

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The particular difference in sensitivity studied existed for stationary phase cultures of *E. coli* K12 AB312 (resistant) and *E. coli* K12 AB531 (sensitive). The data obtained from crosses indicated that a large group of genes may be involved and that they influence survival after either ultraviolet or x irradiation. It was suggested that these genes might be acting in various recovery processes common to both UV and x-ray damaged cells. More recent experiments have established that these genes, although present in all cells at all stages of the growth cycle, are effective in influencing the survival of stationary phase but not log-phase cultures. The LD$_{90}$ for stationary phase AB312 is 40 kr (kiloroentgen) and for AB531 it is 10 kr, whereas in the case of cells taken from log phase cultures the LD$_{90}$ for both organisms is approximately 15 kr. Although log phase AB312 and AB531 cells are of approximately equal sensitivity, progeny from a mating of these organisms exhibit a range of sensitivities. Apparently, two cultures of the same radiation sensitivity may contain genes which, when combined in certain ways, can alter sensitivity.

It has been suggested that, under certain conditions, single gene mutations may lead to large changes in radiation sensitivity.$^{4,5}$

Most of our recent effort has been directed toward obtaining such mutants in strains of *E. coli* useful in mating experiments. If such a mutant is obtained, it should be relatively easy to locate the gene involved on the linkage map and it may be possible to reveal the gene-product responsible for the change in sensitivity. To date, we have examined the x-ray sensitivity of 1400 clones obtained from cultures subjected to various mutagenic and selective treatments. The mutagens employed have been x irradiation, ultraviolet irradiation (2537 A), hydrogen peroxide, camphor, MnCl$_2$, and ethylmethane sulfonate. These have been used in various combinations and certain of them have proven effective in the production of antibiotic resistant mutants. However, we have not detected any mutants of enhanced radiation resistance or sensitivity.

Recently, we have developed a technique for determining the radiation sensitivity of 128 cultures simultaneously. This increases the efficiency of our mutant screening procedure by a factor of -10. It is hoped that by employing this new technique we will obtain the mutants desired for continuing the study of the genetic control of radiation sensitivity in bacteria.

**THE EFFECT OF ULTRAVIOLET RADIATION (2537 A) ON INDUCED PROTEIN SYNTHESIS AND VIABILITY IN RHODOPSEUDOMONAS SPHEROIDES**

K. A. Fry R. K. Clayton H. I. Adler

Early literature$^6-^8$ on the effects of x rays on protein synthesis and viability of bacteria indicated that the synthesis of protein is more resistant than viability. Clayton$^9,10$ and Clayton and Adler$^{11}$ found

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that the ability of *Rhodopseudomonas spheroides* to perform induced, *de novo* synthesis of catalase was inhibited to the same degree as viability over a considerable range of x-ray doses. The inhibition of induced protein synthesis was observed immediately after irradiation. A study of kinetic features of the inhibition led to the suggestion that the radiation effect might be directly on nucleic-acid structures controlling enzyme synthesis. For this reason, experiments on the effects of ultraviolet irradiation (2537 Å) on induced catalase synthesis in *R. spheroides* have been undertaken. Preliminary results indicate that the induced synthesis of catalase is more resistant to ultraviolet irradiation than is cell viability. The inactivation curves for the two processes differ by at least a factor of 2. Both phenomena are photoreactivable. In addition, there is some indication that enzyme synthesis may be recoverable at elevated temperatures.

**PLATING EFFICIENCY OF LOGARITHMIC PHASE CULTURES OF *ESCHERICHIA COLI* K12**

Helen G. Pilkinton H. I. Adler

Logarithmic phase cells taken from cultures of certain *E. coli* K12 strains growing in a nutrient broth-yeast extract liquid medium are unable to initiate colony formation on a synthetic medium known to be adequate for stationary phase cells of the same strains. This phenomenon is not observed if logarithmic cells are obtained from a synthetic liquid medium and is only occasionally observed if nutrient broth without yeast extract is used. This unusual behavior of certain *E. coli* K12 strains interferes with the interpretation of experiments being done in this laboratory on the genetic control of radiation sensitivity.

Various combinations of the growth medium components have been examined in an effort to provide conditions in which cells taken from logarithmic phase nutrient broth-yeast extract cultures can initiate colony development on synthetic media. These experiments and others in which the effect of various diluents used in the dilution and plating procedure have been evaluated suggest that a material is present in yeast extract that is necessary for the initiation of cell division on synthetic media. This material apparently leaks out of logarithmic but not stationary phase cells during the dilution process. Supplementation of the synthetic plating medium with as little as 0.8 μg of yeast extract per ml of medium allows normal colony development. The nature of this growth promoting agent(s) is not known but it is not methionine or Vitamin B₁. These are requirements of the *E. coli* strain being studied and are always present in adequate supply in the synthetic medium. The incorporation of trace amounts of yeast extract in the synthetic plating medium should make possible a more meaningful interpretation of bacterial conjugation experiments.
Mammalian Recovery

<table>
<thead>
<tr>
<th>Secondary Disease</th>
<th>Recovery in Vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. C. Congdon</td>
<td>L. H. Smith</td>
</tr>
<tr>
<td>R. R. Bigelow</td>
<td>T. W. McKinley, Jr.</td>
</tr>
<tr>
<td>Gino Doria</td>
<td>Hilda A. Griscom</td>
</tr>
<tr>
<td>W. H. McArthur</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hemopoietic and Lymphatic Stem Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joan W. Goodman</td>
</tr>
</tbody>
</table>

a Consultant.
b Former investigator from abroad.
c Student trainee.
d ORINS Fellow.
e Visiting investigator from abroad (International Cooperation Administration).

TRANSPLANTATION OF PERITONEAL CELLS

Joan W. Goodman

Introduction. — The properties and topographical distribution of stem cells of the hemopoietic and lymphatic systems are of major importance to transplantation studies as well as experimental hematology. The proliferative and functional capacities of stem cells in the peripheral blood of mice have recently been reported.1 Similar studies are being made on the properties of nucleated cells harvested from the peritoneal cavity as well as other sites.

Methods and Results. — Serological methods were used to establish the strain of origin of erythrocytes and leukocytes in mice given a lethal dose of irradiation and a single injection of peritoneal cells.

F1 hybrid mice were used as donors, and cells were obtained by washing the peritoneal cavity with heparinized saline. The usual yield was three to five million cells per young adult donor. Cells were pooled from about 100 donors for injection into a small number of irradiated inbred parent strain recipients. Not every experiment resulted in survival of irradiated recipients. In five experiments, transplants from peritoneal cells were established, and a number of chimeras survived for several months after irradiation with circulating blood cells of donor type.

In all experiments, Fe59 uptake was measured within the second week after transplantation. When appreciable uptake was found, reaction of the cells with donor-specific antisera released the label into the lysate, whereas reaction with nonspecific antiserum or nonimmune sera released none of the label. Hosts were irradiated at 900 r. Results from one such experiment are shown in Table 12.

Histologic studies were made on spleen, bone marrow, and lymph nodes obtained at autopsy from transplanted as well as control irradiated mice. Transplanted mice showed microscopic evidence of erythropoiesis and granulopoiesis in spleen red pulp and bone marrow. Lymphatic tissue proliferation was noted in spleen white pulp and cortex of lymph nodes.

Discussion. - These results indicate that stem cells of the hemopoietic and lymphatic systems are normally present in the peritoneal cavity as well as in bone marrow, spleen, and circulating blood of mice. Information available about the presence or absence of stem cells in various anatomical locations in the mouse is shown in Table 13.

Table 12. Identification of Fe$^{59}$-Labeled RBC of Individual Peritoneal Cell Chimeras$^a$

<table>
<thead>
<tr>
<th>Host</th>
<th>Peritoneal Cells</th>
<th>Hours After Fe$^{59}$ Injection</th>
<th>Percentage$^b$ Fe$^{59}$ Count in Lysate from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor Strain</td>
<td>Millions of Cells</td>
<td>B6 Anti D2</td>
</tr>
<tr>
<td>D2</td>
<td>D2</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>D2</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>B6D2F₁</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>B6D2F₁</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>D2</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>B6D2F₁</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>B6D2F₁</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Eight days after transplantation of peritoneal cells.
$^b$Based on distilled water count as 100%.

Table 13. Location of Stem Cells

<table>
<thead>
<tr>
<th>System</th>
<th>Hemopoietic</th>
<th>Lymphopoietic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Spleen</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Blood</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Peritoneal cavity</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Thymus</td>
<td>Absent (?)</td>
<td>Present</td>
</tr>
<tr>
<td>Pleural cavity$^a$</td>
<td>(?)</td>
<td>(?)</td>
</tr>
<tr>
<td>Subcutaneous tissues</td>
<td>(?)</td>
<td>Present</td>
</tr>
<tr>
<td>Other organs and tissues</td>
<td>(?) Absent</td>
<td>(?) May be present in blood in many tissues. Extravascular presence not determined.</td>
</tr>
</tbody>
</table>

$^a$Morphologically contains same cells as peritoneal cavity.
A METHOD FOR STUDYING FEASIBILITY OF POSTIRRADIATION TREATMENT OF MOUSE BONE-MARROW CELLS

L. H. Smith

Introduction. — Treatment of radiation injury to cells has been extensively studied in a variety of organisms, both plant and animal. Specific alterations in the physical and chemical environment influence the severity of observable radiation-induced lesions and the reproductive integrity of cells. Although there are conceptual problems associated with treatment of radiation injury of the cell, it would be advantageous to have a means whereby similar alterations could be tested on a normal mammalian cell population. During the past few years an in vitro system has been developed\(^2\) that allows assessment of the effects of post- as well as preirradiation conditions on the reproductivity of mouse bone-marrow cells. With this system, in vitro effects are reflected in the ability of these injected cells to transplant and proliferate in the lethally irradiated mouse. Proliferation has been assessed in terms of 30-day survival\(^3\) or by other methods that reflect hemopoietic activity in vivo.\(^4\) We are exploring a method whereby this activity is measured in terms of 24-hr Fe\(^{59}\) uptake by the spleen.

Methods and Discussion. — From the results of a preliminary series of experiments, the following procedure is currently used. Male (C\(^3\)H x C\(^5\)7BL)\(_1\) mice are exposed to 950 r of 250-kv whole-body x rays.


\(^3\) Ibid.


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Fig. 14. The Effect of Bone Marrow Cell Dose on Percentage Uptake of Fe\(^{59}\) by Spleen. Each point is the mean ± one standard error. The number to the right of the mean is the number of mice.
Twenty four hours later they are injected intravenously with graded doses of isologous bone marrow cells followed seven days later by 0.2 µc of Fe$^{59}$ intraperitoneally. Spleens are removed 24 hr after injection of Fe$^{59}$ and counted in a well-type crystal scintillator. A linear increase in Fe$^{59}$ uptake, denoting erythropoietic activity of the transplanted bone marrow, is observed over a marrow dose range of 0.05 to 0.4 × $10^6$ cells (Fig. 14). Percentage of Fe$^{59}$ uptake of spleen can then be compared in mice injected with normal, irradiated, and irradiated-treated cells.

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**ELECTRONIC CELL COUNTING OF MOUSE BONE-MARROW CELLS**

Hilda Griscom

**Introduction.** — The automatic particle counter has provided the biologist with the potential means of improving cell counting techniques. Previous to the development of this instrument, visual counting in a fixed volume chamber was the sole and somewhat unreliable means for counting cells. Because of interest in quantitative bone-marrow transplantation in irradiated mice it was desirable to have as accurate a measure of cell numbers as possible. Impetus to utilize the automatic counter was provided by a recent report describing automatic counting of human bone-marrow cells.$^5$

**Methods.** — The instrument used was a Model A Coulter provided with a 100-µ aperture and operated at an aperture current of 6 and at a gain of 4. At these settings, the required plateau was obtained at threshold readings between 18 and 26. Suspensions of bone-marrow cells of the mouse were prepared for automatic counting in 0.9% NaCl containing $1 \times 10^{-2}$% saponin (v/w) as an erythrocyte lysing agent. For comparative visual counts, the standard hemocytometric method was used.

**Results.** — Over the range 10 to $90 \times 10^3$ marrow cells, the instrument was linear, none of the readings falling more than 5% from a straight line fit by eye. Over a period of five weeks, marrow suspensions were prepared and counted using the cell counter and hemocytometer. Table 14 shows a typical set of data that exemplifies the better reproducibility of cell counts obtained from the automatic counter.

**Discussion.** — The most striking feature of the instrument is its reproducibility. Other advantages are that (1) cell counts can be rapidly obtained, and (2) dilute suspensions (at least as low as $20 \times 10^3$ cells/ml) can be counted. As shown in Table 14 (marrow pool number 1), the electronic counts are about 10% higher than chamber counts. With other samples, electronic counts were also usually higher. This difference could be compensated for by altering one of the instrument settings, although debris in the same size range as cells could well account for higher electronic counts.

---

Table 14. A Comparison of the Electronic and Hemocytometric Methods for Counting Suspensions of Mouse Bone-Marrow Cells

<table>
<thead>
<tr>
<th>Marrow Pool Number</th>
<th>Sample Number</th>
<th>Electronic Counter (cells/ml × 10^3)</th>
<th>Hemocytometer(^a) (cells/ml × 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>69.2, 68.9, 68.5</td>
<td>62.5, 61.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>69.2, 67.3, 67.9</td>
<td>58.5, 55.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>67.3, 67.7, 67.1</td>
<td>60.3, 51.5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>70.8, 68.7, 70.5</td>
<td>68.8, 62.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>64.2, 63.8, 64.2</td>
<td>57.5, 57.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>68.5, 68.3, 68.7</td>
<td>67.3, 65.3</td>
</tr>
</tbody>
</table>

\(^a\)Each number obtained from counts on one side of a chamber.

STUDIES ON THE ONTOGENY OF TRANSPLANTATION ANTIGENS IN MICE

Gino Doria

Introduction and Method. – The development of transplantation antigens in mice was investigated by studying the capability of hemopoietic tissues to induce homotransplantation immunity as a function of their age. The method used was based on the fact that lethally irradiated mice are able to reject homologous bone marrow if they have been preimmunized to hemopoietic tissues of the same donor strain. The bone-marrow rejection was estimated by measuring Fe\(^{59}\) uptake in erythrocytes of the irradiated recipients. The hemopoietic tissues studied were: C3H fetal (13\(\frac{1}{2}\) day-old), 15\(\frac{1}{2}\) day-old, 17\(\frac{1}{2}\) day-old) liver; neonatal (19\(\frac{1}{2}\) day-old) liver; and adult (90-day-old) spleen. The method consisted of injecting different cell numbers of either tissue into groups of LAF\(_1\) mice. These and noninjected LAF\(_1\) mice (control group) were given an x-ray total-body dose of 800 r 24 hr later. All the mice were injected with a challenge dose of C3H bone marrow within 2 hr after irradiation, and with a tracer dose of Fe\(^{59}\) nine days after the bone-marrow injection. Radioactivity was measured in a blood sample taken 24 hr after injection of the isotope. The degree of antigenicity of the tissues studied was estimated by the ratio of the Fe\(^{59}\) uptake in the group of mice injected with any dose of the tested cells to the Fe\(^{59}\) uptake in the control group of noninjected mice: the smaller the ratio, the greater the antigenicity.

Results. – The antigenicities of fetal and neonatal livers, compared to that of adult spleen (assigned a value of 100%), were: 0%, 13\(\frac{1}{2}\) days; 5%, 15\(\frac{1}{2}\) days; 8%, 17\(\frac{1}{2}\) days; 16%, 19\(\frac{1}{2}\) days. By use of proper controls it was possible to reject the hypothesis that the measured antigenicity was a result of further development of the injected cells, which could have grown in the recipient during the time of the experiment.
FETAL THYMUS TRANSPLANTATION IN HOMOLOGOUS BONE-MARROW CHIMERAS

C. C. Congdon

Introduction. — Additional results are presented on the effect of fetal thymus transplantation on survival from secondary disease. Preliminary results were given in the last progress report. The studies reported here are an attempt to use recent evidence that the fetal thymus disburses cells near or after the time of birth to other lymphatic tissues where they develop into an immune system unique to the individual animal. Since the fetal thymus cells lack immunological competence, it was thought that they might supplant, by being present in greater numbers, foreign immunologically competent cells, and that they themselves would regenerate a tolerant immune mechanism aborting the secondary disease syndrome.

Results. — The findings obtained so far (Table 15) do not demonstrate significant increase in survival from secondary disease, although in Experiment 1 animals receiving fetal homologous (ACA) thymus had a greater 90-day survival rate than any other group. A similar group in Experiment 2, with a different homologous (1C3F1) fetal thymus, did not show a beneficial effect of the thymus injection. Neither did subcutaneous transplantation of the thymus help the situation (Experiment 2). When the fetal thymus donor was a third strain (ASW), quite different from the bone-marrow donor (BDF1) or the irradiated recipient (1C3F1) as reported in Experiment 3, there was no appreciable difference in survival from the control groups.

The immunological competence of adult thymus cells in contrast to the fetal cells is further demonstrated in Experiment 1, where adult homologous thymus (ACA) caused accelerated death times in the foreign marrow-treated recipients.

Discussion. — Two other variables need to be explored in deciding about the potential use of this approach in circumventing secondary disease. One is to increase many fold the number of fetal thymus cells injected, so that if there is any competition for transplantation sites with the foreign marrow cells, the thymocytes will be at a great advantage. The other variable would be to inject the thymus cells several days before the marrow to permit a head start in regenerating a new immune mechanism. A genetic variable also needs to be eventually considered in view of probable strain differences in maturation of their immunologic function. In addition, the experiment can be approached in an entirely different way using parent and F1 hybrid mice, as Goodman has done to identify stem cells of hemopoietic and lymphatic tissues. In this method the direction of immune reaction of each transplanted tissue can be controlled. The cells present in each tissue can usually be identified by serologic means when this approach is undertaken.

Finally, it may be that any foreign immunologically competent cell can insert itself into the circulation of stem cells, settle out in lymphatic tissues, and proliferate in response to host antigens regardless of the presence of tolerant cells of the same anatomical type. In this case, control of secondary disease

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6 In collaboration with J. W. Goodman and J. W. Ferrebee (Cooperstown, N.Y.).
9 J. W. Goodman, this report.
Table 15. Fetal Thymus Transplantation

<table>
<thead>
<tr>
<th>Bone Marrow Donor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Thymus Donor&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Recipient&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Number of Mice</th>
<th>Percent Survival at 90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>LAF&lt;sub&gt;1&lt;/sub&gt; males</td>
<td>87</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>LAF&lt;sub&gt;1&lt;/sub&gt; females</td>
<td>100</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>Adult LAF&lt;sub&gt;1&lt;/sub&gt;</td>
<td>45</td>
<td>0</td>
<td>all dead in 17 days</td>
</tr>
<tr>
<td>ACA</td>
<td>Fetal LAF&lt;sub&gt;1&lt;/sub&gt;</td>
<td>50</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>Fetal LAF&lt;sub&gt;1&lt;/sub&gt;</td>
<td>34</td>
<td>17.6</td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>Fetal ACA</td>
<td>27</td>
<td>37.0</td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>Fetal ACA</td>
<td>45</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>Adult ACA</td>
<td>65</td>
<td>1.5</td>
<td>accelerated death</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1C3F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>LAF&lt;sub&gt;1&lt;/sub&gt; males</td>
<td>150</td>
<td>34.6</td>
<td></td>
</tr>
<tr>
<td>1C3F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>LAF&lt;sub&gt;1&lt;/sub&gt; females</td>
<td>59</td>
<td>72.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>209</td>
<td>45.4 combined male-female</td>
</tr>
<tr>
<td>1C3F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Fetal 1C3F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>103</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>1C3F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Fetal 1C3F&lt;sub&gt;1&lt;/sub&gt; (sc)</td>
<td>LAF&lt;sub&gt;1&lt;/sub&gt; males</td>
<td>56</td>
<td>17.8</td>
</tr>
<tr>
<td>1C3F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Fetal 1C3F&lt;sub&gt;1&lt;/sub&gt; (sc)</td>
<td>LAF&lt;sub&gt;1&lt;/sub&gt; females</td>
<td>41</td>
<td>73.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>97</td>
<td>41.2 combined male-female</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDF&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1C3F&lt;sub&gt;1&lt;/sub&gt; males</td>
<td>64</td>
<td>31.2 (5-22-62 start)</td>
<td></td>
</tr>
<tr>
<td>BDF&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1C3F&lt;sub&gt;1&lt;/sub&gt; females</td>
<td>41</td>
<td>26.8 (5-1-62 start)</td>
<td></td>
</tr>
<tr>
<td>BDF&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Adult ASW</td>
<td>44</td>
<td>20.4 (5-1-62 start)</td>
<td></td>
</tr>
<tr>
<td>BDF&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Fetal ASW</td>
<td>36</td>
<td>27.7 (5-22-62 start)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>10 x 10<sup>6</sup> cells intravenously, day 0.
<sup>b</sup>5-17 x 10<sup>6</sup> cells intravenously, day 0 or 1, or 1 thymus subcutaneously (sc).
<sup>c</sup>900 r.
in radiation chimeras must still depend upon understanding the developmental basis for the rapid transformation to a tolerant state of foreign immune system stem cells when fetal liver is injected or the genetic relation between donor and host is close. In the more distant donor-host genetic relationships, the development of tolerance is usually a rare and delayed event, unlikely to be capable of experimental study unless the metabolic consequences of the immune reaction in secondary disease provide new leads to the somatic cell events that determine the state of tolerance in adult animals.\(^{10}\)

### STUDY OF NITROGEN BALANCE IN IRRADIATED MICE GIVEN FOREIGN BONE MARROW

**W. H. McArthur**

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

Very little is known about the altered metabolism of animals with secondary disease. It has been suggested that animals treated with the foreign bone marrow die of a "metabolic starvation."\(^{11}\) It is conceivable that metabolic changes may not be specific for a "homologous disease syndrome," but may instead be the result of debility induced by reaction between grafted foreign cells and host in some rather nonspecific way. On the other hand, if metabolic changes are specific, then these experiments may serve as models for study of immune diseases in general, since it is rather generally felt that the basic underlying mechanism of homologous disease is immunological interaction between grafted cells and host.

This is a partial description of the metabolism of homologous animals compared to normal mice and irradiated mice given isologous bone-marrow cells. The questions are raised as to which changes, if any, are specifically involved in the "homologous disease" syndrome, and can they be worked out? This investigation attempts to measure a rather general metabolic parameter — that of determination of nitrogen balance during recovery in treated animals and determination of any influence of the homologous disease development.

No reports have appeared in the literature concerning this metabolic parameter in connection with homologous disease. It is felt that such a study will aid in designing more specifically mechanistic studies and, in particular, will help to pinpoint the beginning stages of homologous disease.

**Method.** — These experiments consisted of a total of 35 animals: 8 normals, 8 x-irradiated, 9 isologous, and 10 homologous. These data cover the 30- and 60-day periods; 90-day experiments are in progress. Thirty-five animals were used in the 30-day period, whereas only six animals were used in the 60-day study which was a continuation of the 30-day period.

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\(^{10}\) A. L. Kretchmar and W. A. McArthur, this report.

LAF₁/Cum mice were x-irradiated recipients; homologous bone-marrow donors were 1C3F₁/Cum mice. Recipients received 900 r and were injected intravenously with 40 × 10⁶ isologous or homologous bone-marrow cells.

Urine and feces were collected every 48 hr from specially designed metabolic chambers. Nitrogen determinations were made by means of the automatic nitrogen analyzer.

**Results and Discussion.** - The results are summarized in Table 16. X-irradiated mice not given bone marrow show progressively negative nitrogen balance until death, in contrast to normal animals which maintained a positive balance during the experiment. Irradiated animals given either isologous or homologous marrow were able to maintain a positive nitrogen balance which, on the average, was greater than normal. The most interesting observation was that for the first 30 days, animals treated with homologous marrow maintained as positive a nitrogen balance as mice given isologous marrow.

The data for 31–60 days are inconclusive, since studies have been completed on only 2 animals. It, however, suggests that irradiated marrow-treated animals are able to maintain positive nitrogen balance for 60 days after treatment. The nitrogen balance of mice given homologous marrow was similar to that of isologous-treated animals during this later interval as well.

**Table 16. Nitrogen Balance in Normal and Irradiated Animals Given Bone Marrow**

<table>
<thead>
<tr>
<th>Days After Treatment</th>
<th>Normal (8)ᵇ</th>
<th>X Ray Only (8)ᵇ</th>
<th>IBM (9)ᵇ</th>
<th>HBM (10)ᵇ</th>
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</thead>
<tbody>
<tr>
<td>0–6</td>
<td>+0.5</td>
<td>+0.2</td>
<td>+0.5</td>
<td>+10.4</td>
</tr>
<tr>
<td>7–12</td>
<td>+10.0</td>
<td>−1.5</td>
<td>+13.9</td>
<td>+15.7</td>
</tr>
<tr>
<td>13–18</td>
<td>+10.1</td>
<td>−13.5</td>
<td>+15.9</td>
<td>+12.2</td>
</tr>
<tr>
<td>19–24</td>
<td>+10.8</td>
<td>All dead</td>
<td>+6.5</td>
<td>+10.6</td>
</tr>
<tr>
<td>25–30</td>
<td>+13.0</td>
<td></td>
<td>+12.0</td>
<td>+21.8</td>
</tr>
<tr>
<td>31–36ᶜ</td>
<td>+2.0</td>
<td></td>
<td>+17.9</td>
<td>+5.5</td>
</tr>
<tr>
<td>37–42</td>
<td>+0.8</td>
<td></td>
<td>−7.5</td>
<td>+11.4</td>
</tr>
<tr>
<td>43–48</td>
<td>−2.2</td>
<td></td>
<td>+21.2</td>
<td></td>
</tr>
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<td>49–54</td>
<td>+2.3</td>
<td></td>
<td>+25.0</td>
<td>+19.8</td>
</tr>
<tr>
<td>55–60</td>
<td>+13.2</td>
<td></td>
<td>+24.5</td>
<td>+24.2</td>
</tr>
</tbody>
</table>

ᵃChange from original value of baseline.
ᵇNumber of animals in group.
ᶜThe data from 30–60 days are based on only two animals in each group.

It is paradoxical that mice given homologous marrow, while maintaining a normal nitrogen balance, may lose weight. We speculate that this occurs because nitrogen is retained in certain compartments within the animals given homologous marrow; these compartments are either not present or are smaller in the animals treated with isologous marrow. Antigen-antibody complexes, bacterial foci, or parasitic organisms might constitute such abnormal compartments. Since protein in such forms is more concentrated
than in animal tissues which contain about 70% water, the same amount of nitrogen could be retained in
them with little change in body weight. Furthermore, if nitrogen were shifted from normal tissue compart-
ments to abnormal ones, it is possible that the animal could lose weight and retain nitrogen.

Data on food wasted by normal, x-irradiated, isologous and homologous animals were compared during
30-, 60-, and 90-day periods. Normal animals wasted food on the average of 0.450 g/day during the first
30-day period. The x-irradiated controls wasted food on the average of 0.475 g/day during the first 12
days. Isologous animals wasted food on the average of 0.375 g/day for the first 30-day period, while ho-
omologous animals wasted an average of 0.585 g.

During the second 30-day period the average amount wasted in g/day was as follows: normals, 0.490;
isologous, 0.425; and homologous, 0.540.

During the third 30-day period the average amount in g wasted/day was as follows: normals, 0.810;
isologous, 0.660; and homologous, 0.890.

The homologous animals seem to have wasted slightly more food than the normal animals during each
of the three periods, while the x-irradiated controls wasted slightly more than the normals during the first
12 days, and then died. The isologous animals seemed to have wasted the least amount of food in each of
the three periods.

CONCENTRATION OF FREE AMINO ACIDS IN TISSUES OF BONE-MARROW CHIMERAS

A. L. Kretchmar

Introduction. — The pool sizes of three amino acids are notably altered in tissues of homologous bone-
marrow chimeras. These are: aspartic acid, which is elevated in liver; serine, which is decreased in
liver, plasma, and muscle; and glycine, which is also decreased in liver, plasma, and muscle. At present,
the following tentative explanation is considered for these findings. Grafted donor Ab-producing cells
proliferate in the irradiated host and disrupt the balance of aspartic acid synthesis and utilization in liver
cells as early as 3 days after treatment. This may occur by circulating immunologic cell attack on the
liver parenchyma, and is not necessarily accompanied by histologic lesions. The general metabolism of
the host is not at first affected, and the chimera regenerates its hemopoietic tissues and recovers nearly
all the body weight lost after irradiation. A more fully developed and generalized immunologic reaction
reaches peak activity at about 10–14 days after establishment of chimerism. This leads to progressive
injury and alteration of host metabolism manifested by weight loss, reduction in serine and glycine pools,
and less specific effect on glutamine and glutathione levels, which, in general, follow the body weight
changes. Some animals are able to compensate for the metabolic alteration with liver enlargement, but
some ("exceptional animals") cannot, and die with a body weight of less than 15 g and liver weight less
than 1 g. One can speculate that an enlarged liver is essential to the chimera’s ability to regenerate its
hemopoietic system and compensate the metabolic alteration of secondary disease.

12Medical Division, Oak Ridge Institute of Nuclear Studies.
13C. C Congdon and A. L. Kretchmar, J. Exptl. and Molecular Pathol. (in press).
Results. — Aspartic Acid. — Mice in various combinations of donor and recipient strains\textsuperscript{11,13} were irradiated and, on the day of irradiation, injected with \((40 \times 10^6)\) bone-marrow cells. At ten-day intervals (shown in the figures), animals were killed and tissues from five mice in each of the sham, isologous, and homologous marrow-treated groups pooled. This pooled tissue was then homogenized with the 1\% picric acid to extract the free amino acids.\textsuperscript{14} Analysis of the extracts was done with an amino acid analyzer.\textsuperscript{15}

Figure 15 shows that aspartic acid levels in liver of homologous chimeras are elevated to nearly twice the level of isologous marrow-treated or sham-irradiated control mice. The increase may occur as early as three days after treatment, and persists for at least 35 days. Even at the early intervals (3–7 days), the aspartic acid levels in liver of isologous marrow-treated animals is within the range of values among control mice. There is, therefore, an early and specific effect on the pathways of aspartic acid metabolism in liver that is related to treatment of irradiated mice with homologous bone-marrow cells.

Serine and Glycine. — The experimental materials and methods of analysis were the same as for aspartic acid.

Figures 16 and 17 summarize the changes that were found in the levels of serine and glycine. The decrease in serine of liver begins after 7 days of chimerism and reaches minimum levels 21 days after marrow treatment.

Levels in plasma and muscle are affected less rapidly, and not until 35 days after treatment show a definite decrease below levels in sham-irradiated control mice. Similar changes were found in glycine levels which are decreased in liver, plasma, and muscle 21 and 35 days after treatment with homologous bone-marrow cells.

Fig. 16. Decrease in Serine Concentration of Irradiated Mice Treated with Homologous Bone Marrow Cells. Symbols same as for Fig. 15.

Fig. 17. Decrease in Glycine Concentration of Irradiated Mice Treated with Homologous Bone Marrow Cells. Symbols same as for Fig. 15.
Radiation Immunology

T. Makinodan  
J. F. Albright  
E. H. Perkins  
W. E. Bennett  
E. E. Capalbo  
R. D. Owen  
Paul Urso  

Rachel R. Carter  
Martha R. Leonard  
W. J. Peterson  
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aResearch participant.  
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cConsultant.  
dStudent trainee.

RADIOSENSITIVITY OF ANTIBODY-FORMING SPLEEN CELLS AFTER ANTIGENIC STIMULATION

T. Makinodan  
W. J. Peterson

Introduction. — Our previous studies\(^1\) showed that the antibody-forming capacity of spleen cells decreases logarithmically with increase in x-ray dose when these cells are exposed to radiation before antigenic stimulation. Data of this nature, together with those based on kinetic and immunohistochemical studies, permitted us to conclude that cellular division is somehow intimately involved early in the differentiation process of antibody-forming cells.\(^2\) It follows then that there could exist late in the differentiation process a stage(s) in which cells are capable of synthesizing antibody proteins but incapable of undergoing cellular division. To test this hypothesis, studies were carried out to determine the relative radiosensitivity of spleen cells 2 and 3 days after antigenic stimulation (late lag and early log phases, respectively).

Results. — The in vivo culture method\(^3\) was used to determine the six-day secondary antisheep RBC response of spleen cells from C31F1 mice. Our preliminary findings are summarized in Fig. 18.

Discussion. — These complex curves strongly suggest that there exist two populations of cells: a radiosensitive type and a radioresistant type. More elaborate studies are now being carried out to substantiate this finding. Its implication with regard to DNA-, RNA-, and protein-synthetic mechanisms is obvious.


INDEPENDENCE OF RATES OF ANTIBODY PRODUCTION DURING SIMULTANEOUS
SECONDARY RESPONSES TO TWO NON-CROSS-REACTING ANTIGENS

J. F. Albright  Rachel R. Carter

Introduction. — In a previous report we presented studies on the kinetics of secondary antibody production by spleen cells cultured in vivo. It was shown that the initial 12-day activity profile is a typical "growth" curve and is characterized by lag, logarithmic, and stationary phases. The study reported here was performed to determine whether or not the response to one antigen, during each of the three phases (lag, log, and stationary), influences the activity of the same population of cells in responding to a second non-cross-reacting antigen.

Methods and Results. — C31F₁ (C3H/Anf × 101/Cum ♂) mice were preimmunized at 8 weeks with 1 ml each of a 1.0% suspension of sheep and rat erythrocytes. They were used as donors of spleen cells 4–5 weeks later, at which time 12 x 10⁶ cells were transferred to isologous, irradiated (800-r) recipients. All recipients were immediately injected with 1 ml of 1.0% rat RBC. At intervals thereafter, viz 2, 5, and 8 days, groups of recipients received an additional injection of 1 ml of 0.1% sheep RBC. Serum

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was collected at daily intervals thereafter and titrated for antibodies to sheep and rat RBC. In other experiments the response of $12 \times 10^6$ spleen cells taken from donors separately preimmunized to rat and sheep RBC was determined at intervals after isologous transfer.

The results are presented in Fig. 19 (A,B). On the left, curve 1 shows the typical rate of antibody production by spleen cells taken from donors preimmunized with sheep RBC only and stimulated at transfer with 0.1% sheep RBC. Curves 2–4 show the responses by $12 \times 10^6$ cells taken from donors preimmunized against both sheep and rat RBC, stimulated at transfer with 1.0% rat RBC, and with 0.1% sheep RBC on day 2 after transfer (curve 2), day 5 (curve 3), and day 8 (curve 4). The simultaneous production of antibody to rat RBC by the doubly preimmunized cells is shown on the right (curve 1) and compared with the typical response by cells taken from donors immunized only against rat RBC (curve 2). Thus, curve 2 (part A) represents antibody produced against sheep RBC administered during the lag phase of secondary response to rat RBC (curve 1, part B). Curves 3 and 4 (part A) represent anti-sheep production when secondary antigen was given during the logarithmic and stationary phases, respectively, of the secondary anti-rat response (curve 1, part B).

Conclusions. — The curves in part A are similar; there are no significant differences between them in induction periods or slopes of the logarithmic phases. We conclude, therefore, that the simultaneous response to the rat antigen has no effect on the rate or magnitude of production of antibody to sheep cells, and vice versa. Nor does there appear to be any competition between the cells responsible for the two types of antibody for residential space within the recipient, culture animal. It should be noted that there

![Figure 19](image-url)

**Fig. 19.** Effect of Response to One Antigen upon Rate of Antibody Production to a Second Non-Cross-Reacting Antigen. **A,** secondary response of $12 \times 10^6$ cells from mice immunized against sheep RBC only, stimulated with 0.1% sheep RBC (curve 1). Secondary response by $12 \times 10^6$ cells from mice doubly preimmunized against sheep and rat RBC, exposed to secondary rat RBC at transfer and secondary sheep RBC on second day after transfer (curve 2), fifth day after transfer (curve 3), and eighth day after transfer (curve 4). **B,** secondary response of $12 \times 10^6$ spleen cells from mice doubly preimmunized against sheep and rat RBC exposed to rat RBC at transfer and to sheep RBC at 2, 5, or 8 days thereafter (curve 1). Secondary response of $12 \times 10^6$ spleen cells from mice preimmunized only against rat RBC and exposed to rat RBC at transfer (curve 2).
appears to be no net gain or loss of cells which respond to sheep antigen during the 8 days prior to stimulation (compare 4-day titers for the four curves). It may also be noted that the induction period for 0.1% sheep RBC response (about $24 \times 10^6$ RBC) is $2 \frac{1}{2}$ days, whereas that for 1.0% rat RBC (about $160 \times 10^6$ RBC) is $3 \frac{1}{2}$ days.

RE-EVALUATION OF THE IMMUNOLOGICAL BASIS OF SPLENIC ENLARGEMENT IN CHICK EMBRYOS IN RESPONSE TO FOREIGN GRAFTS

J. F. Albright  
Rachel R. Carter  
Sharon Ott

Introduction. — It is generally believed that the embryo is incapable of immune response either to defined soluble and particulate antigens or to foreign tissues. We present evidence herein which is contrary to this view and which is best interpreted as indicating an active immune response by the chick embryo to grafts of certain foreign tissues. The phenomenon of splenomegaly in the chick embryo following grafts of certain immunologically competent tissues to the chorioallantoic membrane (CAM) is interpreted as resulting from transfer of competent cells from the graft to the embryonic spleen. The consequent proliferation of transferred cells during their reaction against the host is believed to account for the resulting splenic enlargement. It is assumed that the embryo is immunologically incompetent and becomes tolerant of the graft.

Methods and Results. — Fertile eggs were incubated for 9-10 days. At that time an opening was made through the shell, and a graft, consisting of a piece of donor spleen (~3-4 mm$^3$), was placed on the CAM. The shell opening was covered with cellophane tape, and the eggs were returned to the incubator. At intervals thereafter, grafted and control eggs were opened, the embryo body weights were estimated, and the spleens and bursae of Fabricius were dissected out and weighed. Donors were adult chickens, turkeys, ducks, guinea fowls, and mice. In some experiments donor mice and chickens were injected with $\text{H}^3$-thymidine in order to label cells of their spleens which were then used as grafts.

The results are given in Fig. 20 (A-C) which shows relative splenic enlargement,

$$\frac{(\text{spleen wt}_{\text{exp.}})(\text{body wt}_{\text{cont.}})}{(\text{spleen wt}_{\text{cont.}})(\text{body wt}_{\text{exp.}})}$$

at each interval of measurement after grafting. The ratio (spleen wt/body wt) for the ungrafted controls are arbitrarily assigned a value of 1. It is clear that grafts of adult chicken spleen are highly effective in inducing embryonic spleen enlargement; duck and turkey grafts are moderately active, while guinea fowl and mouse grafts are least effective. This trend reflects the phylogenetic relations of donors to the chick embryo. If, as is commonly believed, splenic enlargement results from transfer of graft cells to embryonic spleen and an ensuing reaction against the host, it is difficult to explain why splenic enlargement is not greater with increasing genetic disparity. If, on the other hand, splenomegaly is an indication

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of active response by the embryo, these data suggest that the reaction to grafts more distantly related to the embryo is more intense and involves mobilization of active embryo cells to the graft site.

Grafts of tritium-labeled spleens of adult chickens and mice reveal little or no transfer of cells from graft to embryo spleen or bursa. Furthermore, the labeled cells of the grafts decrease sharply with time of residence on the CAM. Thus graft cells are probably not proliferating but are being killed or diluted by invasion of cells from the embryo.
Conclusions. — These data strongly suggest that the chicken embryo is competent to react against grafts of foreign tissues. Recent reports\textsuperscript{6-8} strengthen the belief that the embryo is immunologically competent. Further studies are in progress to determine at what time the embryo becomes competent and the significance of the phenomenon of embryonic tolerance.

SURVIVAL OF LETHALLY IRRADIATED MICE GIVEN HOMOLOGOUS BONE-MARROW CELLS

E. H. Perkins

Introduction. — Mortality of radiation chimeras is generally attributed to severe secondary disease resulting from an \textit{in vivo} antigen-antibody reaction. However, the primary mechanism of this reaction remains controversial (graft-vs-host, or host-vs-graft reactions). To obtain a better insight to this problem, we have used strain combinations such that the \textit{H}-2 antigens will exist solely in either the host or donor cells.

Results. — Lethally irradiated (900 r) (C3H × 101)\textit{F}_1 mice were injected with 5 × 10\textsuperscript{6} homologous (C57BL × C3H)\textit{F}_1 bone-marrow cells, and the mortality was recorded for 210 days. The reciprocal combination of injecting 5 × 10\textsuperscript{6} (C3H × 101)\textit{F}_1 bone-marrow cells into lethally irradiated (800 r) (C57BL × C3H)\textit{F}_1 was also carried out in paired experiments. Figure 21 summarizes the results of four paired experiments.

Discussion. — Mortality during the first 30 days after x-ray and bone-marrow cell treatment was high when (C57BL × C3H)\textit{F}_1 donor cells were injected into (C3H × 101)\textit{F}_1 recipients. One explanation for this high 30-day mortality is that an insufficient dose of marrow cells was administered. If this were so, one must then conclude that in this donor-host combination a 20-fold increase in the homologous bone-marrow cell dose over that of an optimal dose of isologous bone-marrow cells is still suboptimal, for we observed in our control experiments that a dose of 0.25 × 10\textsuperscript{6} isologous bone-marrow cells is sufficient to protect 100\% of the lethally irradiated recipients. An alternative explanation is that a host-vs-graft reaction is taking place during this period after treatment, for strong \textit{H}-2 antigens exist in the donor marrow cells. In the reciprocal combination, where strong \textit{H}-2 antigens exist in the host cells, the mortality was low. No significant difference in the mortality pattern was observed between these two reciprocal combinations 30 to 210 days posttreatment. These results suggest that there exist strong non-\textit{H}-2 histoincompatible antigens in these strain combinations and/or both host-vs-graft and graft-vs-host reactions are taking place.

RECOVERY OF ANTIBODY-PRODUCING CAPACITY OF SPLEEN CELLS FROM X IRRADIATION

E. E. Capalbo    Martha R. Leonard

Introduction. — It has been shown that the antibody-forming capacity of sublethally irradiated animals recovers in time. Other work has been concerned with the extent to which antibody production is reduced immediately following x irradiation. These investigations have not revealed the extent to which recovery of antibody production from radiation depends upon proliferation of new cells or repair of reversible injury of damaged cells. An estimate of the degree to which each process contributes to recovery of immune capacity can be obtained by (1) quantitatively relating antibody-forming potential to cell number at intervals after radiation, and (2) determining the time and rate of cellular proliferation after radiation treatment. This report deals with such studies.

Methods and Results. — The isologous, in vivo transfer method was used to determine the antibody-forming capacity of a constant number of spleen cells taken from donors at intervals following irradiation.

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Footnotes:
C31F₁ (C3H/Anf Cum 9 x 101/Cum 9) mice were exposed to 500 r of x rays 6 weeks after immunization with sheep RBC. Immediately, and at intervals up to 4 weeks thereafter, 12 x 10⁶ spleen cells were transferred to irradiated isologous recipients where they were secondarily stimulated with sheep RBC. Serum samples were collected and titrated for antibody on the fifth day after cell transfer. Simultaneous determinations were made of secondary antibody production by 12 x 10⁶ normal, unirradiated cells. Antibody-producing ability rose sharply to a peak on the fifth day following irradiation. Relative to the unirradiated control cells, the functional capacity of irradiated cells was only about 10% on the fifth day.

![Diagram](https://example.com/diagram.png)

**Fig. 22.** A, Relative Antibody-Forming Activity of 12 x 10⁶ Spleen Cells at Intervals After 500 r if (a) Proliferation of Cells Began Immediately (Hypothetical) or (b) at the Beginning of the Third Day (Observed). B, proportion of total 5-day antibody which can be accounted for by proliferation of new cells and recovery of injured cells.
The return of proliferative ability and the rate of proliferation of spleen cells of the preimmunized mice exposed to 500 r were estimated by injecting H\(^3\)-thymidine immediately or at 2 to 4 days after irradiation. No evidence of proliferation was found immediately after nor on the second day after irradiation. However, cells were dividing at a normal rate on the fourth day, and the mean generation time was 24 hr.

**Discussion.** From these data we can estimate the extent to which provision of new cells by division accounts for the antibody-producing activity found at 5 days after 500 r (Fig. 22). We have previously found that 500 r inactivates 99.7% of normal secondary capacity of spleen cells. Therefore, of the 12 x 10\(^6\) cells transferred to recipients at time zero, only about 3 x 10\(^4\) are unaffected. Cellular proliferation does not return before the beginning of the third day. We assume that it does begin at this time and that the mean generation time of lymphoidal cells is 24 hr. Thus, it is readily seen that, from the 3 x 10\(^4\) unaffected cells at the beginning of the third day, 2.4 x 10\(^5\) will have been derived by the end of the fifth day. The latter cell number corresponds to an antibody titer of 3.3. However, the observed antibody titer was 5.6, which corresponds to 1.2 x 10\(^6\) cells. Therefore, of the antibody produced by cells on the fifth day after 500 r, only 20% could have been produced by cells derived through divisions; the remaining 80% must be the product of cells, initially damaged by radiation, which have recovered.
### Pathology and Physiology

**Section Chief — A. C. Upton**

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<tr>
<th>Pathological Effects of Neutrons and High-Energy Radiation in Mammals</th>
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<td>J. H. Oldham&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>R. L. Tyndall&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Nancy S. LaPrade&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup>Consultant.  
<sup>b</sup>Research participant.  
<sup>c</sup>Student trainee.  
<sup>d</sup>Research associate.

### Late Somatic Effects of X Radiation in Mice Treated with AET and Isologous Bone Marrow

G. E. Cosgrove | K. W. Christenberry | C. C. Congdon | D. G. Doherty<sup>1</sup>

**Introduction.** — Radioprotective chemicals (AET) or isologous bone marrow (IBM) have been used successfully to alter the course of the acute radiation syndrome. In the present experiments, animals which survive acute doses of radiation with such treatment have been followed until death to note changes in survival, disease incidence, graying of fur, and cataract formation.

**Methods and Results.** — Young adult female (101/Cum x C3H/Anf Cum)<sup>F<sub>1</sub></sup> mice were exposed to 350–1800 r of 250-kvp whole-body x rays. Some of the exposure groups were treated immediately prior to irradiation with 8.8 mg of AET per mouse, while others received 10 x 10<sup>6</sup> isologous femoral bone-marrow (IBM) cells after irradiation; additional groups received both treatments. Selected mice were checked periodically for graying of fur and lens opacities, and an autopsy was performed on each mouse. The major findings in the various groups are indicated in Table 17. Radiation shortened the

<sup>1</sup>Chemical Protection and Enzyme Catalysis Group.
Table 17. Long-Term Survival and Pathologic Changes in Irradiated (101 X C3H)F₁ Female Mice as Influenced by AET and Bone-Marrow Treatment

<table>
<thead>
<tr>
<th>X-Ray Dose (r)</th>
<th>Treatment</th>
<th>Number of Mice</th>
<th>Mean Age at Death (days)</th>
<th>Neoplasm Incidence (%)</th>
<th>Incidence of Glomerulosclerosis (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thymic Lymphoma</td>
<td>Other Leukemias</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>198</td>
<td>866</td>
<td>0.3</td>
<td>4</td>
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<tr>
<td>350</td>
<td></td>
<td>89</td>
<td>649</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>500</td>
<td>AET</td>
<td>107</td>
<td>633</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>500</td>
<td>IBM</td>
<td>83</td>
<td>631</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>500</td>
<td>AET and IBM</td>
<td>90</td>
<td>618</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>700-800</td>
<td></td>
<td>186</td>
<td>549</td>
<td>4</td>
<td>12</td>
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<tr>
<td>700</td>
<td>AET</td>
<td>84</td>
<td>608</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>700</td>
<td>IBM</td>
<td>89</td>
<td>584</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>700</td>
<td>AET and IBM</td>
<td>87</td>
<td>549</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1100-1200</td>
<td>IBM</td>
<td>113</td>
<td>500</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>1200-1400</td>
<td>AET</td>
<td>126</td>
<td>460</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>1600-1800</td>
<td>AET and IBM</td>
<td>110</td>
<td>455</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Including only 30-day survivors.*
life-span in all groups, but the degree of life-shortening per roentgen was less at the higher dose levels especially in mice given AET and/or IBM; that is, the mean survival time of the heavily irradiated, treated groups (1100–1800 r) was only a little shorter than that of the LD₅₀ radiation controls (700–800 r). The incidence of neoplasms was lower in heavily irradiated, treated animals than in mice given smaller doses of radiation with or without protective treatment; however, tumors of the ovary were numerous after radiation and neither AET nor IBM appeared to protect against their induction. The incidence of thymic lymphoma was increased by radiation, and its induction was inhibited by either AET or IBM treatment. Reticulum cell lymphomas were not induced by radiation. Graying of fur in irradiated mice was reduced by pretreatment with AET. Late kidney damage (glomerulosclerosis) was common in survivors of heavy radiation doses and not consistently reduced by AET or IBM treatment. The induction of lens opacities was not altered by AET or IBM.

Discussion. – The effects of AET and IBM on the induction of late somatic radiation effects appear complex. Although induction of thymic lymphomas and graying of the fur were inhibited by AET and/or IBM, other protective effects were not conclusively demonstrated. Even the lesser life-shortening effectiveness of radiation in the most heavily irradiated, treated mice cannot be confidently ascribed to protection by AET or IBM in the absence of similar protective effects at lower dose levels. Final interpretation of the long-term protection afforded by AET and IBM will, therefore, require further analysis of dose-response relationships, as influenced by intercurrent mortality from specific radiation-induced diseases.

DIFFERENTIAL RADIOPROTECTION OF HEMOPOIETIC AND IMMUNE CELLS IN THE MOUSE SPLEEN BY AET

Gustavo Cudkowicz  Audrey Eisenstadt

Introduction. – There is evidence that AET, a radioprotective agent, does not protect hemopoietic and immunologic cells equally when given in vivo. The present study was undertaken to establish whether a differential radioprotective action of AET could also be detected in cells irradiated in vitro in the presence of AET.

Results. – Spleen cells of C3H/Anf donor mice were exposed in plastic Petri dishes at room temperature to 200 r of 300-kvp x rays with and without AET dissolved in the suspending medium (1 mg AET/ml). The cell suspension was then injected intravenously into two types of recipients: (1) lethally irradiated, isologous recipients to test its ability to prevent acute radiation death, and (2) sublethally irradiated (C57BL x C3H/Anf)F₁ hybrids, to test its ability to induce wasting disease. The results are presented in Tables 18 and 19.

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2 AET: S,β-aminoethylisothiuronium·Br·HBr.
5 G. Cudkowicz, Transplant Bull. 29, 19 (1962).
Irradiation of spleen-cell suspensions reduced their ability to protect lethally irradiated recipients against early death and to induce wasting disease in F₁ hybrid recipients. When the spleen-cell suspensions were irradiated in the presence of AET, their ability to protect isologous recipients from acute death was largely retained, but not their ability to induce lethal wasting disease.

Discussion. — The results reconfirm that although AET is a radioprotective agent for hemopoietic elements in mouse-spleen-cell suspensions, it affords little protection to the cells responsible for the wasting syndrome. Hence, the results suggest a method by which cells of the immune system may be selectively destroyed in prospective grafts by radiation in vitro.
RELATIVE INABILITY OF C57BL BONE-MARROW CELLS TO PROLIFERATE IN F₁ HYBRID MICE

Gustavo Cudkowicz  Audrey Eisenstadt

**Introduction.** — Lymphoid leukemia cells⁶ and antibody-forming spleen cells⁷ of C57BL mice do not grow as well in F₁ hybrid mice as in isogenic recipients, or as well as cells of other parental strains into their F₁ hybrids. The relative inability of the C57BL cells to proliferate in the hybrid mice has been ascribed to "allergic death" of such cells owing to the presence of excessive amounts of antigen, or to an exhaustive graft-versus-host reaction. The present study was undertaken to explore whether nonantibody-forming C57BL cells (bone marrow) would also grow only poorly in the hybrids. The quantitative relationship between dose of injected parental cells and growth, both in isogenic and F₁ hybrid recipients, was assessed. Another objective was to determine the part played by immunogenetic mechanisms in determining the fate of C57BL cells transplanted into F₁ hybrids.

**Results.** — Parental mice of the C57BL/10 ScSn. (BL.10) strain were mated with C3HfB and with co-isogenic partners differing by a single allele at the H-1, H-2, or H-3 histocompatibility locus. The F₁ hybrid offspring were x irradiated (600 r) and 1 to 10 x 10⁶ nucleated BL.10 marrow cells were injected intravenously immediately thereafter. Five days later proliferation of the injected cells was evaluated by determining the uptake in the recipient's spleen of I¹³¹ labeled iododeoxyuridine (IUdR), a specific DNA precursor. The results (Table 20) indicate that when the parents of the F₁ hybrid differed at the H-1 or H-3 locus the BL.10 marrow cells grew as well in the hybrid as in an isogenic host. However, when the parents differed at the H-2 locus alone or at the H-2 and other loci, proliferation of the BL.10 cells was considerably reduced.

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**Table 20. Iodine-131 UdR Uptake in the Spleen of Recipient Mice Five Days After Exposure to 600 r of X Rays and BL.10 Bone-Marrow Cell Infusion**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Recipient Genotype</th>
<th>IUdR Uptake (% x 10²)ᵇ</th>
<th>1 x 10⁶ Cells Injected</th>
<th>10 x 10⁶ Cells Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL.10</td>
<td>H-1ᵇᵇ H-2ᵇᵇ H-3ᵃᵃ</td>
<td>21.2 ± 4.8 (3)ᶜ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(BL.10 x BL.10.BY)F₁</td>
<td>H-1ᶜᵈ</td>
<td>29.1 ± 3.0 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(BL.10 x BL.10.14M)F₁</td>
<td>H-3ᵃᶜ</td>
<td>22.1 ± 6.9 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(BL.10 x BL.10.D2)F₁</td>
<td>H-2ᵇᵈ</td>
<td>0 (7)</td>
<td>5.6 ± 2.1 (7)ᶜ</td>
<td></td>
</tr>
<tr>
<td>(BL.10 x CeH(c)B)F₁</td>
<td>H-1ᶜᵃ H-2ᵇᵏ H-3ᵃᵇ</td>
<td>0 (7)</td>
<td>23.7 ± 8.8 (5)</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ(% x 10²) of I¹³¹ UdR injected into the recipient mouse ± one standard deviation.
ᵇOnly heterozygosity at known H loci is listed.
ᶜNumber of mice in parentheses.
Discussion. — A number of bone-marrow cells of C57BL genotype sufficient to provide optimal growth in isogenic recipients failed to grow in F₁ hybrids when the parent mice differed by a histocompatibility allele at the strong H-2 locus, but proliferated well when the parents possessed the same allele at the H-2 locus, suggesting an immunogenetic mechanism.

EFFECTS OF NITROGEN MUSTARD (HN2) ON NERVOUS TISSUES

N. S. Wolf

Introduction. — McDonald and Asano⁸ have shown that intravenously administered nitrogen mustard (HN2) produces hemorrhage and cell death in the brain of the mouse in doses of LD₅₀ and above. The present studies deal with the nature of the damage to the nervous tissues, the conditions under which they are produced, and their importance in the cause of death following lethal doses of HN2.

Methods. — Young adult RF/Up and hybrid B6D2F₁ mice of both sexes received doses of HN2 varying from LD₈₀ to LD₁₀₀ in saline, in 50% bovine serum, or in 2.5% (w/v) bovine serum albumin (BSA). Survival following variations in the preparation of the HN2 and injection of potentially protective agents was observed. Histologic sections, stained with hematoxylin and eosin and with special stains for nervous tissue and enzyme content, were examined.

Results and Discussion. — Since two kinds of overt effects, the "waltzing effect" and death, could be independently altered by altering the experimental procedures, it appears that HN2 may cause two types or areas of nervous tissue damage. The "waltzing effect" consists of ataxia, head palsy, and partial loss of the self-righting reflex. This effect can be avoided by intraperitoneal injection of the HN2 or by dividing the intravenous dose into three or more injections separated by a time interval of a few minutes. It is also absent if the injected HN2 is phosphate buffered to pH 7.4 or if 50% serum is used as diluent. On the other hand, division of the intravenous dosage has no effect on the mortality rate, and intraperitoneal doses equivalent in killing effect to the intravenous ones do not cause "waltzing." Also, HN2 injected at pH 7.4 and above is more lethal than when injected at low pH (see also White⁹).

When HN2 is adjusted to a pH of 7.5 with phosphate buffer, it has a soporific effect on mice. This effect is greatly enhanced by diluting the HN2 with 2.5% BSA in saline at pH 7.5, and death follows within 12 to 36 hr, instead of the usual median time of 5 to 6 days that is observed after intravenous injections of HN2 in normal saline. Nitrogen mustard (HN2) dissolved in BSA-saline at pH 5.3 is only slightly more toxic than HN2 in normal saline. It appears likely that HN2 and BSA combine in some manner since injection of BSA just before or 30 min after HN2 has little or no effect on morbidity or survival.

Gross and histologic examination of tissues of mice treated with HN2 reveal extensive damage both to gut epithelium and to hemopoietic tissues. However, by varying dose and route of administration of HN2 it may be demonstrated that death most often occurs when these tissues are already in an advanced

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stage of repair. Histologic study of tissues and limited bacteriological survey does not incriminate infection as the major cause of death. Brain damage, consisting of proliferation of capillary endothelium, hemorrhage, and death of some neurons, is most commonly seen after the seventh postinjection day. Few of the mice injected i.v. with an LD₉₀ dose of HN2 survive seven days whereas most i.p. injected with equivalent mortality rate survive for 8–11 days. In addition, preliminary studies indicate that widespread demyelination may occur, not only in the CNS, but in peripheral nerves as well. This condition can be demonstrated as early as the fifth day post-HN2 injection by means of special staining technics. It may be that this type of damage is correlated with the signs (consisting of excitement or depression, spontaneous convulsions, tonic or clonic spasms, and contractural or atonic paralysis) displayed by HN2-injected mice from the third postinjection day onward.

Several reaction products of HN2 are formed in aqueous solution. If a nervous tissue-damaging product is present in the solution, elimination or protection against the product may allow injection of sufficiently large doses to bring about a more permanent depression of hemopoietic tissues in experimental animals, and thus allow truly comparative studies with ionizing radiation.

**DELAYED EFFECTS STUDIES IN RF MICE WITH 14 Mev FAST NEUTRONS**

E. B. Darden, Jr.  C. E. Cosgrove  K. W. Christenberry  M. Kastenbaum

M. L. Davis  J. L. Conklin

**Introduction.** — Relatively few studies of the delayed somatic effects of fast neutrons have been made to date. Studies with accelerator-produced neutrons of essentially a single energy and substantially free of contaminating gamma radiation have been carried out here and elsewhere, but the dose levels used and the number of animals involved have been limited. The present study was set up to obtain information on life-shortening and delayed pathological effects, particularly leukemia, over a wide range of single sublethal doses of 14 Mev neutrons. The irradiation phase has been completed, with a 30-day survival of 99%.

**Material and Method.** — A total of 1600 animals were set up with two endpoints particularly in mind, survival and incidence of myeloid leukemia as a function of dose. Female RF mice, ten weeks old, were exposed in groups of about 50 per week to 50, 100, 200, and 400 rads. Mice were irradiated in individual cylindrical nylon containers located on a 20 cm radius around the target. Uniformity of the whole-body exposure was improved by slowly revolving the array, each container being pivoted, ferris-wheel fashion, so as to be free to turn on its own axis. Thirty mice could be irradiated simultaneously at an average dose rate of about 85 rads/hour. Control mice were similarly revolved with the accelerator off.

**Results and Discussion.** — The limited information available six months postirradiation can be summarized briefly. There was no appreciable difference in body weight between control and irradiated groups after the first 30 days following irradiation. Peripheral white- and red-blood-cell counts showed dose-dependent depression after exposure but returned to normal by about the fifth month. The incidence

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10Biometrics Group.
of leukemia, particularly thymic lymphoma, is elevated in irradiated mice. Lens opacities developed in all but the control group by three months postirradiation are in general agreement with results of previous study with 14 Mev neutrons.11

THIRD ALLELE AT THE SOL LOCUS AFFECTING THE CHARACTERISTICS OF MOUSE HEMOGLOBINS

R. A. Popp

Introduction. — At least two loci are now known to affect the physicochemical properties of mouse hemoglobins.12,13 The Hb locus governs the variant electrophoretic patterns of mouse hemoglobins; the other locus, Sol, governs solubility and crystalline properties of mouse carboxymonoxyhemoglobins. Strain NB mice have a hemoglobin whose properties cannot be explained on the basis of previously described alleles at Hb and Sol. Evidence for a previously unknown third allele at the Sol locus is presented here.

Methods and Results. — Parental strains (BALB/c Hb2/Hb2;Sol2/Sol2 and NB), which may be presumed to be Hb1/Hb1;Sol3/Sol3, were mated to obtain F1 progeny; the latter were subsequently mated

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| Hemoglobin Type | Electrophoretic Pattern | Solubility (OD, 575 m)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1/Hb1;Sol3/Sol3</td>
<td>Single</td>
<td>0.06</td>
</tr>
<tr>
<td>Hb1/Hb1;Sol2/Sol2</td>
<td>Single</td>
<td>0.02</td>
</tr>
<tr>
<td>Hb1/Hb1;Sol2/Sol2</td>
<td>Single</td>
<td>0.01</td>
</tr>
<tr>
<td>Hb1/Hb2;Sol3/Sol3</td>
<td>Diffuse</td>
<td>0.52</td>
</tr>
<tr>
<td>Hb1/Hb2;Sol2/Sol2</td>
<td>Diffuse</td>
<td>0.42</td>
</tr>
<tr>
<td>Hb2/Hb2;Sol3/Sol3</td>
<td>Diffuse</td>
<td>0.12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Characteristics of Precipitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb2/Hb2;Sol2/Sol2</td>
<td>Amorphous precipitate</td>
</tr>
</tbody>
</table>

Table 21. Hemoglobin Types in (BALB/c × NB)F2 Mice

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These classes can be distinguished from one another by progeny testing.
to produce F₂ progeny. The hemoglobins of (BALB/c × NB)F₂ mice were analyzed by starch gel electrophoresis, and the solubility and crystalline properties of their HbCO were studied by the use of previously described procedures.¹³ The F₂ progeny were also classified according to sex, short-ear, and coat-color markers. The observations on the hemoglobins of the (BALB/c × NB)F₂ mice were then compared with those reported earlier on (C57BL × SEC)F₂ (ref 12) and (C57BL × BALB/c)F₂ mice.¹³

A total of nine classes of mice differing in hemoglobin type were found among the F₂ progeny (Table 21). Six of the classes were identified on the basis of electrophoretic pattern, solubility, and crystalline properties alone, and the remaining three classes of mice were classified by test crossing. A total of 200 F₂ progeny have been classified (Table 22). The numbers obtained for the respective classes agree well with those expected for a dihybrid cross (probability 0.2).

Differences in coat-color markers at the albino locus of NB (c⁹b/c⁹b) and BALB/c (c/c) mice also permitted us to determine that the recombination frequencies of c with Hb¹ and of c⁹b with Hb² were approximately 5%, which agrees well with the value of 4.6% reported earlier.¹⁴ The recombination frequency of c with Sol³ and of c⁹b with Sol² was approximately 50%, and Sol segregated independently with other loci examined, that is, p, a, d, se, and sex.

Discussion. — The hemoglobins of (BALB/c × NB)F₂ mice have been characterized in this study and compared with those of (C57BL × BALB/c)F₂ mice. If the alleles at Hb were identical in NB and C57BL mice, and differed at the Sol locus, one would predict that three hemoglobin types found among

Table 22. (BALB/c × NB)F₂ Progeny Classified for Hb and Sol

<table>
<thead>
<tr>
<th>Hemoglobin Type of Progeny</th>
<th>Mating (BALB/c × NB)F₁ × (BALB/c × NB)F₁</th>
<th>Observed</th>
<th>Experimental</th>
<th>d²/Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb¹/Hb¹;Sol²/Sol³</td>
<td></td>
<td>11</td>
<td>12.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Hb¹/Hb¹;Sol²/Sol³</td>
<td></td>
<td>28</td>
<td>25.0</td>
<td>0.36</td>
</tr>
<tr>
<td>Hb¹/Hb²;Sol²/Sol³</td>
<td></td>
<td>21</td>
<td>12.5</td>
<td>5.78</td>
</tr>
<tr>
<td>Hb¹/Hb²;Sol²/Sol³</td>
<td></td>
<td>21</td>
<td>25.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Hb¹/Hb²;Sol²/Sol³</td>
<td></td>
<td>48</td>
<td>50.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Hb²/Hb²;Sol²/Sol³</td>
<td></td>
<td>25</td>
<td>25.0</td>
<td>0.00</td>
</tr>
<tr>
<td>Hb²/Hb²;Sol²/Sol³</td>
<td></td>
<td>46</td>
<td>50.0</td>
<td>0.32</td>
</tr>
</tbody>
</table>

χ² = 7.36
P = 0.2


Results have been combined since the three classes cannot be distinguished without subsequent test cross analysis.
(BALB/c × NB)F₂ progeny would be identical with three types of hemoglobins found among (C57BL × BALB/c)F₂ mice. However, such identity would not be expected if NB and C57BL mice have different Hb alleles. A comparison of the data shows that Hb₁/Hb₁;Sol²/Sol², Hb₁/Hb²;Sol²/Sol², and Hb²/Hb²;Sol²/Sol² hemoglobins were found in both studies, which indicates that the Hb alleles of NB and C57BL mice are alike and that the differences between the hemoglobins of strain NB and C57BL mice are controlled by another locus, presumably Sol.

DIFFERENCES AMONG TRYPTIC PEPTIDES OF THE α-CHAIN OF MOUSE HEMOGLOBIN GOVERNED BY ALLELES AT THE SOL LOCUS

R. A. Popp J. H. Oldham

Introduction. — It has been shown previously that the molecular composition of the β-chain of mouse hemoglobin is controlled by the Hb locus. Although the mice used also possessed different Sol alleles, the peptide patterns of the α-chains were indistinguishable. Thus, the locus that governs the molecular composition of the α-chain of mouse hemoglobin had not been established. A variant peptide has been found in hemoglobin of strain NB mice; the peptide lies in the α-chain and results of genetic tests and peptide analyses indicate that the α-chain is governed by the Sol locus.

Methods and Results. — The hemoglobin types of (BALB/c × NB)F₂ mice were identified by their electrophoretic, solubility, and crystallographic properties. Mice that were homozygous for Sol² and Sol³ alleles were selected from the F₂ progeny (Sol²/Sol² and Sol³/Sol³ segregants). Procedures for preparing globin, separating the α- and β-chains by column chromatography, tryptic digestion, fingerprinting, and staining of chromatograms have been described.

The peptide patterns of the α-chains of Sol³/Sol³ segregant and NB globins versus Sol²/Sol² segregant and BALB/c globins differ by single peptides, 31 and 25. These peptides have similar chromatographic and electrophoretic properties, but differ in amino acid composition. Peptide-31 reacted with specific stains used to identify arginine, histidine, and tyrosine, whereas peptide-25 did not.

Discussion. — Peptide patterns of tryptic hydrolysates of the globins of NB, Sol³/Sol³ segregant, BALB/c, and Sol²/Sol² segregant mice indicated that peptide-31 of NB hemoglobin was associated with the Sol³ locus but not with the Sol²/Sol² genotype of F₂ progeny. Chromatograms of the tryptic peptides of individual α- and β-chains of NB and Sol³/Sol³ segregant globins showed that peptide-31 was present in the α-chain. Results of this and a previous study suggest that the molecular compositions of the α- and β-chains of mouse hemoglobin are controlled by two loci that segregate independently, the primary

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structure of the $\alpha$- and $\beta$-chains being controlled by $\text{Sol}$ and $\text{Hb}$, respectively. Thus, the mechanisms of inheritance of hemoglobins in mouse and man appear to be similar since the synthesis of human hemoglobin is also controlled by two loci which segregate independently.  

RNA ANALYSIS OF COXSACKIE B3 VIRUS INFECTED HELA CELL CULTURES

R. L. Tyndall  
K. B. Jacobson

Introduction. — A recent report\textsuperscript{23} has revealed no alteration in the isotope distribution among the bases of $\text{P}^{32}$-labeled HeLa cell RNA upon poliovirus infection, although a previous study\textsuperscript{24} with unlabeled cells failed to detect any alteration in base ratios of poliovirus infected HeLa cells. The present experiments were undertaken to analyze the RNA of $\text{P}^{32}$-labeled HeLa S3 cell cultures infected with Coxsackie B3 virus.

Methods. — Three-day cultures of HeLa S3 cells were overlaid with 35 ml of medium 199 containing 2.0 mc of $\text{P}^{32}$. The test cultures were infected with Coxsackie B3 virus at a multiplicity $> 2.0$ one hr after addition of the $\text{P}^{32}$ medium. Both uninfected control cultures and infected test cultures were harvested 5 hr after addition of $\text{P}^{32}$.

Three fractions of RNA were isolated from phenol extracts of the harvested cultures, an aqueous RNA, an interface RNA, and RNA associated with the DNA that was concomitantly extracted. The isotope distribution among the bases of these three RNA fractions was determined by the method of Jacobson.\textsuperscript{25} Preliminary analyses of the patterns or "fingertips" of these various RNA fractions have been made by two dimensional chromatography of pancreatic RNAase digests of each of the three fractions from both control and virus-infected cultures.

Results and Discussion. — The aqueous and interface RNA of uninfected cells exhibited similar isotope distribution among their bases. Moreover, the isotope distribution among the bases of both of these fractions was similarly altered upon Coxsackie B3 virus infection. The DNA-associated RNA revealed an isotope distribution among its bases markedly different from that of aqueous and interface RNA, and the distribution was not altered upon Coxsackie B3 virus infection. Preliminary results of the RNA fingerprints indicated some differences both between the three individual RNA fractions and also between control and virus-infected samples of the same fraction.

\textsuperscript{22}Enzymology Group.
\textsuperscript{25}K. B. Jacobson, \textit{Science}, in press.
ULTRARAPID CENTRIFUGAL FREEZING OF SUSPENSIONS OF WHOLE CELLS

N. G. Anderson  J. G. Green

**Introduction.** — Although the effects of rapid freezing of cells have been extensively investigated, few methods have been devised which afford sufficiently rapid cooling rates to prevent crystalline ice formation. However, droplet freezing which has been studied by Rinfret¹ appears to be suitable for the development of techniques affording very rapid cooling rates. This paper reports the utilization of a centrifugal method for the ultrarapid freezing of fine droplets of cell suspensions.

**Experimental.** — The freezing was performed in a rotor consisting of an outer brass shell supporting a removable inner machined-Lucite cup with an inward projecting rim. In use, the rotor, driven by an

¹A. P. Rinfret, personal communication.
International PR-2 refrigerated centrifuge, was cooled by repeated filling with liquid nitrogen and was finally filled with the coolant immediately preceding freezing of the cell suspension. At operational speeds, the liquid nitrogen would stand in the rotor limited by the rim of the Lucite cup. Observation of the performance of the rotor and injection of the cell suspensions were facilitated by a Lucite cover fitted to the centrifuge.

The material to be frozen was injected into the rotor through a 23-gage cannula from a hypodermic syringe inclined to the axis of rotation and placed to direct a stream into the coolant. Experiments were conducted with water to assess droplet formation, with versenated whole rat blood to evaluate droplet formation of a cell suspension and with suspensions of washed yeast cells (Saccharomyces cerevisiae) to determine treatment effects on cell viability.

In the experiments conducted with water or blood, the frozen droplets were photographed for evaluation of bead size and character. The frozen droplets of yeast cell suspensions recovered in liquid nitrogen were warmed to room temperature by either slow or rapid warming techniques. In the slow warming procedure, the suspension was permitted to warm to room temperature at a slow speed in the opened centrifuge, a process requiring 90 min. In the rapid warming procedure, the frozen droplets of suspension in liquid nitrogen were transferred from the rotor to a shallow cup fabricated from aluminum foil and precooled in the liquid nitrogen. The aluminum cup was placed in a 1.5-liter beaker, and when the liquid nitrogen was nearly evaporated, 1 liter of 0.06 M potassium phosphate plating diluent was poured over the beads. Viability of the frozen-thawed cells was assessed by a suitable culture technique and evaluated from control samples treated similarly, but not frozen.

Evaluation of the photographs obtained from the experiments performed with water and with whole rat blood indicate that as the speed of the rotor containing liquid nitrogen was increased, droplet size became progressively finer and more regular in appearance compared with droplets formed by expelling a stream into a stationary rotor. At the chosen operational speed of 5000 rpm, the frozen droplets were desirably small and relatively uniform; however, surface cracking was noted in the larger beads.

In the experiments conducted at the operational speed, no yeast cells survived the ultrarapid freezing and slow warming procedure. In two experiments conducted with the ultrarapid freezing and rapid warming procedure, viability of the treated cells was 10.89 and 9.96% of control values.

Discussion. — Although a more detailed treatment of the mechanics of droplet formation and freezing will be presented elsewhere, a brief summary of apparent behavior is pertinent. Upon striking the surface of the liquid nitrogen, the droplets expelled from the syringe appeared to be broken into many small droplets before freezing occurred. The small droplets in contact with the coolant rapidly reached rotational equilibrium with the surrounding liquid and then moved centrifugally to the cup wall. The droplets appeared to be in contact with liquid nitrogen at all times during the freezing process, and their spherical shape indicated that freezing progressed substantially during the period of approximately $1.7 \times 10^{-5}$ sec theoretically required to reach the cup wall.

Although the survival figures for the yeast cells appear to be relatively low, they assume considerable significance when compared with survival figures of 0.006% obtained with cell suspensions rapidly shell frozen in a dry ice-ethanol bath. Since survival of the cells was intimately related to the warming rate, further investigation of this aspect of the experimentation appears to be warranted.
NUCLEOTIDE ANALYTICAL SYSTEM

C. L. Burger

Introduction. — The development of a high-resolution system for chromatographing nucleotides, nucleosides, and bases has raised afresh the problems of the nature of the differences between different batches of ion exchange resin having the same cross linkage. Arrangements have been made to test batches of Dowex 1 x 8 resin as they are manufactured.

Experimental. — The chief differences noted to date are in the separation of some of the bases from their corresponding ribonucleosides. Mixtures of the bases (cyto, ura, ade, and gua) or of nucleosides (cyd, urd, ado, or guo) are readily resolved however.

Experiments with resins of various cross linkages showed that the differences observed could not be related to differences in cross linkage.

To eliminate the possibility that the resin might be inadvertent mixtures of several different types, staining methods were devised which readily and completely distinguished the anionic and cationic resin beads used in this laboratory. Attempts to distinguish between different cross linkages by using dyes of different molecular weight revealed that all the dyes used up to a molecular weight of 1519 permeated the resin beads completely.

Discussion — The results suggest that ion exchange beads are more permeable than was previously thought, and it also provides a method for observing small differences between resin beads.

A DENSITY GRADIENT FOR THE ISOLATION OF METABOLICALLY ACTIVE THYMUS NUCLEI

W. D. Fisher G. B. Cline

Introduction. — Thymus nuclei and nuclear ribonucleoprotein (RNP) particles prepared in a sucrose density gradient are not usable for certain metabolic studies. A brief exposure to hypertonic sucrose solutions abolishes the ability of the nucleus and of RNP particles to incorporate amino acids into protein and RNA precursors into RNA. Preliminary to other studies, an attempt has been made to devise a density gradient which will yield metabolically active nuclei and nuclear RNP particles. The ease and resolution of isolation procedures would be considerably enhanced if such a gradient were available.

Results. — The ability of isolated calf-thymus nuclei to incorporate 1-C\textsuperscript{14}-alanine was measured after exposure of nuclei originally isolated in 0.25 M sucrose–0.003 M CaCl\textsubscript{2} to various concentrations of clinical dextran, polyvinylpyrrollidone (PVP), and polyethylene glycol (Carbowax, mol. wt 20.000). Some incorporation occurred in the nuclei exposed to these solutions provided that the


osmotic pressure (as determined by freezing-point depression measurements) was the same as that of 0.25 M sucrose. Exposure of the nuclei to hypertonic solutions of any of the test substances or to hypertonic solutions consisting of the test substances and sucrose inactivated them.

**Discussion.** - Of the substances tested only dextran appears practical for preparing a density gradient since the large freezing point depressions, (much larger than expected on basis of molecular weight) of Carbowax and PVP solutions limit the useful concentrations to narrow ranges of density.

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**STUDIES ON SYNCHRONIZED CELLS**

**T. W. James**

**Introduction.** - Cultures of protozoans with induced division synchrony are systems which to some degree approximate the growth and division behavior of single cells. Previous work has shown that repetitive temperature cycles which are adjusted to the cell cycle of various unicellular organisms will bring mass cultures of them into division synchrony. Furthermore, a continuous culture maintained on a 24-hr temperature cycle can be set up so that all the cells divide each cycle and the divisions are confined to a period which is small relative to the cycle length. Under optimal conditions we have reduced this time to 2 hr.

**Methods.** - The organism chosen for the present investigation is the flagellate *Astasia longa*. It has a simple nutrition (acetate, vitamins B₁ and B₁₂, and mineral salts), can be grown in mass suspension cultures, and has a distinct cytology. Studies on the biochemical changes over the synchronized cell cycle can thus be undertaken with confidence that the changes observed can be assigned to specific times in the synchronized cycle. The validity of such results relative to the real cell cycle can be ascertained by use of various time markers in the cytology of the organism. Previous work has shown the time of synthesis of major cellular constituents such as total protein, RNA, and DNA relative to cell division. Present investigations are aimed at determining the time of appearance or changes in the nucleotide and amino acid pools since these are synthesized *de novo* by this organism. By estimating the amounts of nucleotides available in these organisms the size of the culture system necessary to provide sufficient material for analysis has been determined.

To accomplish these ends a culture synchronizer is being constructed in the Cell Physiology Group. It will be provided with an improved temperature programming system as well as a means of monitoring cell number over the synchronized cycle.

The device will consist of a continuous culture apparatus with control elements for monitoring and changing micronutrients, pH, and temperature. Oxygen and CO₂ tension may also be monitored with appropriate electrodes (Severinghouse for O₂ and Clarke electrodes for CO₂). A Coulter counter and cell size plotter is being modified to continuously monitor cell number and cell size over the synchronized cycle.

**Discussion.** - General plans have been made for the production of synchronized populations of *Astasia longa*. Specific experiments will be undertaken with the completion of the basic equipment.
AUTOMATED DETERMINATION OF PROTEIN IN THE PRESENCE OF SUCROSE WITH THE FOLIN-CIOCALTEU REAGENT

H. Schuel N. G. Anderson

Introduction. — The new zonal ultracentrifuge has made it possible to achieve high resolution mass-fractionations of many kinds of biological material. In order to exploit this instrument as a quantitative research tool for cellular physiology, it is necessary to measure accurately and automatically the protein content of the isolated fractions. We have used the Technicon Autoanalyzer in conjunction with the Lowry modification of the Folin-phenol reaction to measure total protein, but the sucrose, which is used to set up the density gradient, interferes with the color development. This report describes our attempts to overcome the interference by modifying the analytical procedure.

Experimental. — The concentrated sucrose solutions used for the gradient had a very high viscosity (up to 182 times that of water for 66% sucrose at 20°C), and it appeared that the interference might be the result of incomplete mixing of the sample and reagents in the lines. A power mixer composed of a solenoid and magnetic fleas within the line was developed to ensure complete initial mixing and dilution. However, although this improved the situation considerably, it failed to completely abolish the sucrose effect.

The final color developed by the Lowry procedure is a result of the Biuret reaction of protein with copper ions in alkali, and the reduction of the phosphomolybdic-phosphotungstic (Folin-Ciocalteu) reagent by the tyrosine and tryptophan residues in the protein sample. As can be seen in Fig. 23, the data suggest that sucrose reduces the Biuret contribution to the color development by means of some kind of reaction with the copper ions. Sucrose did not reduce the amount of color produced by bovine plasma proteins in the presence of the Folin reagent and buffer; that is, without copper. In the presence of 1 × copper solution (concentration, 0.04% CuSO₄·5H₂O in 0.08% Na-K-tartrate) and also in 2.5 × copper solution plus the Folin reagent and buffer, sucrose caused a sharp reduction in the amount of color produced. The sucrose interference was almost completely abolished when a 10 × copper solution was used. It was not possible to prepare more concentrated copper reagents because the metal would not remain in solution. Alkali could be added to the copper-tartrate solution to produce an extremely stable standard Biuret reagent, but this kind of preparation failed to overcome the sucrose interference.

Additional evidence in support of a reaction between ionic copper in alkali and sucrose can be obtained by examination of the UV absorption spectra. Copper and sucrose produce a very intense absorption in the vicinity of 270 μν. This has made it impossible to take advantage of the intense absorption produced by Biuret and protein in the same region when the analysis must be made in the presence of sucrose.

Discussion. — It is hoped that additional experiments will lead to a satisfactory method for the
determination of total protein in the presence of sucrose.

AUTOMATIC RECORDING OF CYTOCHROME OXIDASE ACTIVITY IN RAT LIVER
BREI FRACTIONS SEPARATED IN THE ZONAL ULTRACENTRIFUGE

S. R. Tipton      H. Schuel      N. G. Anderson

Introduction. — The development of the zonal ultracentrifuge has made it possible to isolate during
one centrifugation the major components of rat liver brei (i.e., soluble phase, ribosomes, microsomes,
mitochondria, membranous fragments, and nuclei). In order to exploit this fractionation system for
the study of cellular physiology it is necessary to have reliable enzymatic markers for the various
species of subcellular organelles. Furthermore, it is desirable to preserve the high resolution
achieved in the centrifuge by making the enzyme assays continuously and automatically on the gra­
dient as it is recovered from the rotor. This report describes the development of an automated assay
for cytochrome oxidase to serve as the marker for the mitochondria.

Experimental Method and Discussion. — The method utilizes reduced cytochrome C as the sub­
strate by following the loss of its characteristic absorption band at 550 μm as it is being oxidized
by active cytochrome oxidase. The analytical system employed modules from the Technicon Auto­
 analyzer (proportioning pump, transmission lines, and incubation coils) and a recording Beckman DB
spectrophotometer equipped with 1-cm flow cells. The system was constructed so that aliquots of


the same tissue sample passed through both flow cells at exactly the same time. The sample aliquot passing through the reference side of the spectrophotometer had been incubated with the substrate at room temperature for 120 sec, while the aliquot passing through the standard side had been incubated for only 20 sec. Oxidase activity thus registered as a positive deflection on the recorder, and represented the slope of the reaction rate between 20 and 120 sec, a direct function of the first order velocity constant for the oxidation. A switching system made it possible to introduce potassium ferricyanide only into the reference line in order to measure the $\Delta O D$ for complete oxidation of the cytochrome C. This assay system was extremely sensitive, and also provided a continuous blank for aut-oxidation of the substrate and the turbidity of the brei fractions.

The distribution of cytochrome oxidase activity (measured by an earlier version of the assay system) in rat liver subjected to 10,000 rpm for 60 min can be seen in Fig. 24. Cytochrome oxidase was associated only with the mitochondria, which were sedimented under these conditions to their density-equilibrium point, 43% sucrose. No activity was detected in the microsomal and nuclear fractions.

Fig. 24. Distribution of Cytochrome Oxidase in Rat Liver Brei Fractions Isolated in the Zonal Ultracentrifuge.
The high sucrose concentrations used to make the density gradient mixed poorly with the buffer and substrate solutions in the lines, resulting in a smearing of activity. A power mixer composed of a solenoid and a magnetic flea within the line has been developed to ensure complete initial mixing and dilution. As can be seen in Fig. 24, the oxidase appeared to smear slightly on the nuclear side of the mitochondrial peak. Tests are now in progress using the power mixer in the analytical system to determine whether the shape of the curve is an artifact or represents a heterogenous family of mitochondria.

AN ANALYSIS OF THE AMPHIBIAN YOLK PLATELET

R. A. Wallace

Introduction. — An electron microscopic analysis of amphibian yolk platelets has revealed two basic components — an inner crystalline main body and an outer superficial layer. Dense bodies, approximately 45 Å in diameter, appear to be arranged in a simple hexagonal lattice within the main body and have a center-to-center distance of 81 Å. The superficial layer, however, is without ordered structure and evidence has been obtained that its main component is a polysaccharide.

Results and Discussion. — Recently it has been possible to isolate intact yolk platelets from the amphibian egg by employing a sucrose-PVP gradient with centrifugation. Analysis of the “wet pellet” thus obtained (Table 23) indicates that weight loss by subsequent dialysis can be entirely attributed to sucrose originally present, and that the intact platelet is approximately 30% H₂O. Analysis of

Table 23. Analysis of the Wet Pellet Weight of Isolated Yolk Platelets

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Percent of Wet-Pellet Weight</th>
<th>Average Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight</td>
<td>46, 47, 47, 49, 50</td>
<td>48</td>
</tr>
<tr>
<td>Dialyzed dry weight</td>
<td>28, 29, 31, 32</td>
<td>30</td>
</tr>
<tr>
<td>Sucrose</td>
<td>18, 18, 19</td>
<td>18</td>
</tr>
<tr>
<td>PVP</td>
<td>2.7, 2.7, 2.9</td>
<td>3</td>
</tr>
<tr>
<td>Medium H₂O</td>
<td>39, 39, 42</td>
<td>40</td>
</tr>
<tr>
<td>Platelet solids</td>
<td>27, 28, 29</td>
<td>28</td>
</tr>
</tbody>
</table>

Based on sucrose analysis and a determination that the suspension medium contains sucrose, PVP, and H₂O in the weight ratios of 30.3, 4.5, and 65.2, respectively.
dialyzed platelets (Table 24) indicates that \( \text{H}_2\text{O} \) extraction of the platelets (which removes the superficial layer\(^{11}\)) recovers all the carbohydrate and PVP originally present. Methyl alcohol (80%) will also remove all carbohydrate from dialyzed platelets, indicating the sugar has a low molecular weight. Gel filtration of the \( \text{H}_2\text{O} \) extractable fraction on Sephadex G-50 resulted in all the carbohydrate coming off as sucrose. This sugar from the isolation medium was apparently trapped by the platelets and not removed by long periods (24 to 48 hr) of dialysis. No evidence for polysaccharide material in the superficial layer (or main body) was therefore obtained. The amino acid composition of the \( \text{H}_2\text{O} \)-extracted protein was analyzed and found to be similar to that of the main body protein. The presence of histone-like material in the yolk platelet as suggested by Horn\(^{12}\) has therefore also not been substantiated by this analysis, but a specific extraction of yolk platelets for histone will still be necessary before a final conclusion may be made.

### Table 24. Analysis of Isolated and Dialyzed Yolk Platelets

<table>
<thead>
<tr>
<th>Fraction</th>
<th>g per 100 g Nitrogen (%)</th>
<th>Phosphorus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialyzed yolk platelets</td>
<td>100</td>
<td>13.0</td>
</tr>
<tr>
<td>(1) Extractable with ( \text{H}_2\text{O} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) PVP</td>
<td>16–19</td>
<td>8.6</td>
</tr>
<tr>
<td>(b) Carbohydrate</td>
<td>9–10</td>
<td>12.6</td>
</tr>
<tr>
<td>(c) Protein</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(2) Nonextractable with ( \text{H}_2\text{O} )</td>
<td>81–84</td>
<td>14.1</td>
</tr>
<tr>
<td>(a) Phosphoprotein</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>(b) Lipoprotein</td>
<td>71–74</td>
<td></td>
</tr>
<tr>
<td>(c) Lipid</td>
<td>12.5</td>
<td></td>
</tr>
</tbody>
</table>

The components of the main body (nonextractable with \( \text{H}_2\text{O} \)) have been resolved as two proteins—a phosphoprotein and lipoprotein. The latter component readily and irreversibly oxidizes (air, \( \text{H}_2\text{O}_2 \)) to form a dimer and other polymeric units and such a phenomenon may explain the varying amounts of a third, "fast" ultracentrifugal component as reported by others.\(^{13-15}\) The two proteins may be separated from one another by a careful ammonium sulfate fractionation, and physical measurements on the isolated products are given in Table 25. The properties of the two proteins are quite analogous to the phosvitin and lipovitellin isolated from chicken yolk;\(^{16}\) this terminology will henceforth be used. An amino acid analysis of the phosvitin (phosphoprotein) has shown that one of every three amino acids

---


\(^{13}\)O. Schjeide *et al.*, *Growth* 19, 297 (1955).


is serine, thus exactly providing the necessary esterification sites for the associated phosphate. The lipovitellin (lipoprotein) further has the interesting properties of being maximally insoluble in isotonic salt solutions and of readily forming strands upon manipulation. This may prove to have a relation to the observed rapid proliferation of membranous structures around yolk platelets during dissolution in \textit{vitro}.\textsuperscript{17}

| Table 25. Summary of Physical Measurements on Yolk Proteins in the Main Body |
|-----------------------------|-------------|-------------|
| Property                    | Lipoprotein | Phosphoprotein |
| $S_{20,w}$ (Svedbergs)      | 11.9        | 3.07        |
| $[\eta]$ (cc/gm)            | 5.5         | 8.5         |
| $\bar{v}$ (ml/gm)           | 0.77        | 0.60        |
| $M$ (from $S$ and $[\eta]$) | $4.1 \times 10^5$ | $3.5 \times 10^4$ |
| $M$ (Archibald)             | $3.8 \times 10^5$ | $3.1 \times 10^4$ |
| Isoelectric pH              | 8.9         | <3.0        |
| Lipid (%)                   | 18          | 0           |
| Phosphorus (%)              | 0.5         | 10          |
| $E_{1}^{1%}$ 280 m\textmu | 7.59        | 1.96        |

The data from Table 25 may be further calculated to show that in the main body of yolk platelets there are two moles of phosvitin for every mole of lipovitellin. A unit comprised of these elements along with a determined value of 15 wt \% associated water will occupy a volume of $687 \times 10^{-21}$ cc. A unit of this volume in the form of a rod with perfectly rounded edges and having a diameter of 81 A will have a length of 162 A. This would fit well with the 80-A hexagonal packing of elements observed microscopically by Karasaki\textsuperscript{9} and the 164-A periodicity observed with x-ray diffraction on fresh platelets.\textsuperscript{18} The dense 45-A-diameter particles, as observed by Karasaki,\textsuperscript{9} would thus represent the phosvitin. The two molecules of this protein within each 164-A-long unit must also be 81-A apart from one another in order to explain the observed periodicities. A model for the packing of elements within the main body of yolk platelets (which arbitrarily assumes a symmetrical relationship between the phosvitin and lipovitellin) has thus been constructed in collaboration with S. Karasaki and is presented in Fig. 25. This preliminary model will serve as a working hypothesis for further investigations.


Introduction. — A number of experimental factors affect the survival of cells at subzero temperatures. Thus, high percentages survive with certain suspending media but not with others, with certain cooling and warming rates but not with others; and in certain species but not in others. In the case of yeast and certain other microorganisms, low-temperature injury can be accounted for in somewhat more mechanistic terms: It is associated with the formation of intracellular ice.¹⁹–²² Conversely, the ability

to survive low-temperature exposure indicates either that little or no intracellular ice has formed or that it has formed without being lethal.

**Method and Discussion.** One factor affecting survival is the cooling rate. Low cooling velocities usually produce higher survivals than higher rates, with 1°C/min often considered the "optimum." There also seem to be qualitative inverse correlations between water content and survival and between cell size and survival in some organisms. Thus, survival is often enhanced by partially dehydrating cells in hypertonic media, or by short-term vacuum desiccation. These facts suggest that the likelihood of cells freezing internally depends on their water content at various temperatures, and their water content is partially dependent on their permeability to water and the ratio of cell surface to volume. The purpose of the following discussion is to develop a quantitative formulation of these possible interrelations. Two basic assumptions underlie the analysis: One is that the cell membrane is much more permeable to water than to solutes, and the second is that the cytoplasm in intact cells obeys Raoult's law, the fundamental physical-chemical equation describing the activity of ideal dilute solutions. Space restrictions require that the argument be given in outline form.

If a cell remains unfrozen as the temperature drops below 0°C, the vapor pressure of the supercooled intracellular water falls less rapidly with decreasing temperature than that of external ice. The intracellular vapor pressure at a given temperature will be by Raoult's law:

\[ p_i = p^0 x_i \]

where \( p^0 \) is the vapor pressure of pure water, and \( x_i \) the mole fraction of water. Taking logarithms and differentiating with respect to temperature we obtain

\[ \frac{d \ln p_i}{dT} = \frac{d \ln p^0}{dT} + \frac{d \ln x_i}{dT} \]

But by the Clausius-Clapyron equation\(^2^3\)

\[ \frac{d \ln p^0}{dT} = \frac{L_v}{RT^2} \]

where \( L_v \) is the latent heat of vaporization; hence,

\[ \frac{d \ln p_i}{dT} = \frac{L_v}{RT^2} + \frac{d \ln x_i}{dT} \]

The relation between the vapor pressure of ice outside the cell and temperature is

\[ \frac{d \ln p_e}{dT} = \frac{L_s}{RT^2} \]

where \( L_s \) is the latent heat of sublimation.

Subtracting (4) from (5) we obtain an expression for the difference between the intracellular and extracellular vapor pressure as a function of temperature (when \( L_g \) is the latent heat of fusion); namely,

\[
\frac{d \ln p_e/p_i}{dT} = \frac{L_f}{RT^2} - \frac{d \ln x_i}{dT}.
\]  

(6)

Since \( x_i = n_i/(n_1 + n_2) \) (where \( n_1 \) and \( n_2 \) are number of moles of solvent and solute) and since \( n_1 v_1^0 = V \) (where \( V \) and \( v_1^0 \) are the total volume of intracellular water and the molar volume of water), one can express \( x_i \) in terms of \( V, n_2, \) and \( v_1^0, \) and thus rewrite (6) as

\[
\frac{d \ln p_e/p_i}{dT} = \frac{L_f}{RT^2} - \frac{n_2 v_1^0}{V + n_2 v_1^0} \frac{1}{V} \frac{dV}{dT}.
\]  

(7)

The increasing difference between the internal and external vapor pressure will cause water to flow out of the cell at a rate given by

\[
\frac{dV}{dt} = \frac{ARKT}{v_1^0} \ln \frac{p_e}{p_i},
\]  

(8)

where \( A \) is the area of the cell membrane through which water diffuses, \( K \) is the permeability constant for water, and \( t \) is time.24

Let us assume that the cells are cooling at constant velocity; then,

\[
\frac{dT}{dt} = B,
\]  

(9)

where \( B \) is the cooling velocity. Finally, the permeability constant is itself an exponential function of temperature:25

\[
K = K_g e^{2.303b(T - T_g)},
\]  

(10)

where \( K_g \) is the value of the constant at temperature \( T_g \) and \( b \) the slope.

The processes described by Eqs. (7), (8), (9), and (10) occur simultaneously as the supercooled cells continue cooling. The equations can be combined to eliminate \( \ln p_e/p_i \) and \( t, \) and thus to give the volume of water in a cell, \( V, \) as a function of temperature, \( T, \) and the several constants:

\[
Te^{2.303b(T_g - T)} \frac{d^2V}{dT^2} - \left[ (2.303bT + 1)e^{2.303b(T_g - T)} - \frac{ARKn_2}{2(V + n_2 v_1^0)} \frac{T^2}{V} \right] \frac{dV}{dT} = \frac{L_f AK}{Bv_1^0}.
\]  

(11)

The Mathematics Panel of ORNL has obtained a numerical solution to this second order differential equation and is in the process of solving it for various values of \( K_g, A, n_2, \) and \( B. \) When the data are available, they will provide calculated answers to the following type of questions: How much water remains in a cell at various temperatures? How is this quantity affected by the cooling velocity, by


the volume of water originally present in the cell, by the surface volume ratio, and by the permeability
of the cell to water? How much more water is in the cell than would be present if the internal and ex-
ternal vapor pressures were equal?

This is the same as asking how the various parameters affect the difference between the tempera-
ture of a cell and its true freezing point, and this difference, in turn, is a measure of the degree to
which the cell would be supercooled, and is, therefore, a measure of the likelihood of freezing.

PRELIMINARY DEVELOPMENT OF AN AUTOMATED CARBOHYDRATE ANALYZER

J. G. Green N. G. Anderson

Introduction. - To facilitate the characterization of a carbohydrate compound found in the super-
natant obtained from frozen and thawed yeast as well as the carbohydrate components of cellular ma-
terials, activity directed toward the development of an automated carbohydrate analyzer using the basic
principles of the chromatographic procedure of Khym and Zill was initiated. A workable analyzer,
the result of preliminary studies, is reported here.

Experimental. - Separation of sugars as borate complexes is accomplished on sized analytical
grade Dowex 1 x 8 anion exchange resin obtained from Bio-Rad. Preliminary studies were performed
using a column 30 cm in length by 2.0 cm in diameter, and when encouraging separations were obtained,
subsequent studies were performed with a 150 cm by 0.9 cm jacketed column. The use of a jacketed
column permits varying the temperature at which the separation is attempted; however, all experiments
to date have been conducted at room temperature or at 55°C where better resolution has been obtained.
To check the resolving capability of the column, a test mixture containing four pentoses, four hexoses,
four disaccharides, and one trisaccharide of common occurrence has been used.

Three elution systems in linear gradient have been employed. When an attempt to use the borate-
bicarbonate system utilized by Hallén failed to yield desirable resolution, an effort was made to
use potassium tetraborate by adjusting the pH of the solution to values between 8.0 and 9.2 with per-
chloric acid with subsequent removal of the potassium perchlorate precipitate. Although satisfactory
resolution was obtained with this system, trace residues of perchloric acid adversely affected the resin.
The elution system currently being used successfully is a solution containing 0.15 M sodium tetraborate
with sufficient boric acid added to adjust the pH to the desired value, and the cumulative molarity of the
system varies from 0.3 M to 0.35 M depending upon the amount of boric acid required. Some experiments
have been performed using a system containing 0.18 M sodium tetraborate adjusted to pH as before. Values
of pH ranging from 8.0 to 9.2 have been employed, but best results have been obtained in the region of
8.65 to 8.80 measured at 35°C. Various linear gradients in total quantity of 1 liter varying in concentra-
tion ratio from start to finish of 6:1 to 10:1 diluted with distilled water have been assayed.

The column eluant is assayed colorimetrically by the phenol-sulfuric acid method, since this method offers high specificity and sensitivity for carbohydrates and evidences greater reaction uniformity than the commonly employed anthrone method. Concentrated sulfuric acid delivered by a Lapp model S-20 pump and 80% phenol delivered by a Beckman model 746 solution metering pump are metered and mixed in the ratio of 60:1. At a Technicon bubble cactus the mixed reagent is introduced into the eluant stream delivered through the column at 1.71 ml/min by a Milton Roy Minipump. The combined stream consisting of seven parts of mixed reagent to three parts of eluant is segmented with air introduced from a regulated laboratory supply, and the segmented stream is passed through an insulated mixing-reaction coil where the exothermic development reaction goes to completion. Both the bubble cactus and the terminal end of the coil are cooled by air jets.

The stream is passed through a Technicon flow cell with a light path of 1 cm adapted to a Beckman DU spectrophotometer, and the transmittance of the stream at 490 μ is recorded by a Brown single-pen potentiometric recorder.

A typical assay of the test standard is depicted in Fig. 26. The position of the various sugars may be altered differentially by varying the pH, ionic strength, and gradient ratio of the eluant as well as the temperature of the column. In practice, a set of parameters may be derived for a specific separation problem, and for a given separation, resolution may be checked by altering one or more parameters on alternate trials.

Discussion. — Although the analyzer is usable in its present form, additional developmental activity is planned. Further refinement of the colorimetric assay system is advisable including better control of the reaction system and incorporation of leakage safeguards. To distinguish carbon chain length as well as unit chain length, a system monitoring at two or more wavelengths is contemplated. Greater versatility

![Fig. 26. Chromatogram of Test Standard Containing 13 Sugars Performed on Analytical Grade Dowex 1-X8 Resin at a pH of 8.7 and at 55°C with the Sodium Borate Elution System with a 10:1 Linear Gradient.](image-url)
will be incorporated into the analyzer by the inclusion of molecular sieve or cellulose adsorption procedures for oligosaccharides and ion exchange techniques for phosphorylated derivatives.

**ENZYMATIC PROPERTIES OF POLYPEPTIDYL RIBONUCLEASES**

Fumio Sawada R. R. Becker

**Introduction.** – In attempts to gain some insight into the changes in enzymatic activity noted in several polypeptidyl ribonuclease derivatives, \(^{28-30}\) two additional modified enzymes have been prepared and their activities toward cytidine-2',3'-cyclic phosphate, uridine-2',3'-cyclic phosphate, and yeast RNA determined.

**Results and Discussion.** – The increased rate of hydrolysis of cytidine-2',3'-cyclic phosphate by the polyvalyl ribonuclease derivatives when compared to the corresponding uridine derivative prompted us to determine whether or not selective cleavage occurs when RNA was used as a substrate. It was found by end group analysis that after a 5-min hydrolysis, the ratio of cytidine end groups to uridine end groups was 1.4, a change in specificity of enzyme too small to be used advantageously in RNA degradation.

Table 26 summarizes the pertinent data for a series of ribonuclease derivatives using the two cyclic phosphates as substrates. The slight differences noted in the constants for the polyvalyl ribonuclease derivative and the native enzyme from those reported earlier\(^{20}\) have resulted from fractionation of both the crystalline ribonuclease used and the polyvalyl derivative prepared from

\[^{28}\text{R. R. Becker, } Polyamino Acids, Polypeptides and Proteins, University of Wisconsin Press, Madison, 1962.}\n
\[^{30}\text{C. B. Anfinsen, M. Sela, and J. P. Cooke, } J. Biol. Chem. 237, 1825 (1962).}\n
Table 26. Comparison of Enzymatic Activities of RNase and Some Derivatives

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Moles Amino Acid Residue Added per Mole</th>
<th>Optimal pH (^{d})</th>
<th>Enzymatic Activity (^{b})</th>
<th>(K_m) (10^{-3}) M (^{c})</th>
<th>(K_s) (sec (^{-1})) (^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cl (^d)</td>
<td>U! (^d)</td>
<td>Cl!</td>
<td>U!</td>
</tr>
<tr>
<td>RNase (native)</td>
<td>0</td>
<td>6.8</td>
<td>6.6</td>
<td>1.07</td>
<td>0.28</td>
</tr>
<tr>
<td>Polyglycyl-</td>
<td>30</td>
<td>6.7</td>
<td>6.6</td>
<td>0.78</td>
<td>0.17</td>
</tr>
<tr>
<td>Polyglycyl-</td>
<td>49</td>
<td>6.6</td>
<td>6.4-6.6</td>
<td>0.57</td>
<td>0.11</td>
</tr>
<tr>
<td>Poly-L-valyl-</td>
<td>20</td>
<td>7.1</td>
<td>6.6</td>
<td>1.92</td>
<td>0.18</td>
</tr>
<tr>
<td>Poly-DL-phenylalanyl-</td>
<td>8</td>
<td>6.85</td>
<td>6.6</td>
<td>1.18</td>
<td>0.25</td>
</tr>
</tbody>
</table>

\(^{a}\)S, 0.8 x 10^-4 M; \(E_0\), 2 x 10^-6 M.

\(^{b}\)Moles substrate mole \(^{-1}\) enzyme sec \(^{-1}\).

\(^{c}\)\(E_0\), 2 x 10^-6 M.

\(^{d}\)U! = uridine-2',3'-cyclic phosphate; Cl! = cytidine-2',3'-cyclic phosphate.
the chromatographed RNase. The shifts in pH optimum noted are small and observed in the polyvalyl case for only the cytidine derivative as substrate. Similar shifts which are not readily explained have been noted in the polyvalyl-chymotrypsin-acetyltyrosinhydrazide system. In all cases, the maximal velocities are less for the RNase derivatives than for the native protein. In both cases in which the enzyme has been modified by the addition of nonpolar amino acid residues, an increased rate is observed which is reflected in the $K_m$ for the cytidine type substrate. For the uridine-type substrate, the decreased rate is reflected in the maximal velocity. The influence of the average chain length is apparent by comparing the two polyglycyl derivatives. The derivative containing 49 residues of added glycine contains seven chains of average length of seven compared to chains with an average chain length of about four for the other polyglycyl derivative. The change in activity produced by the added residues is not large, but in the direction expected by increased substitution of the molecule. It is not possible to draw conclusions with regard to the mechanism of the changes produced by these modifications, but they are clearly related to the nature of the residue added. Obviously, similar experiments using substrate analogs of a more nonpolar nature should be carried out.

**SEPARATION OF ORGANIC AND INORGANIC PARTICULATE MATERIAL FROM NATURAL WATER**

W. T. Lammers

Introduction. — The problems of fractionating and analyzing subcellular components are similar in theory and general method to those of fractionating and analyzing natural water. During the last decade the incidence of contaminants introduced into the biosphere has increased manyfold, and it is of vital interest to be able to elucidate their uptake by and distribution among the various fractions that are found in natural water. At present the uptake and distribution of radionuclides introduced into the Clinch River from ORNL is being investigated, but the theories and methods being developed could be applied equally well to investigations of insecticides, herbicides, or other similar problem contaminants commonly found in water that will be used ultimately for human consumption.

Experimental. — Twenty-seven-liter water samples, collected by proportional flow techniques on an 8-day schedule from regular sites on the Clinch River, were initially separated into two fractions by a continuous flow centrifugation: (1) noncolloidal particulate material, and (2) colloids and solutes. The noncolloidal particulate material was separated into organic and inorganic fractions by density gradient centrifugation in either sucrose or 2-bromoethanol gradients, isolated, and prepared for radionuclide analyses. One liter aliquots of the original raw water sample and of the supernatant water from the continuous flow centrifugation (colloids and solutes) were reduced to a 10 ml volume under vacuum at 35°C and prepared for radionuclide analyses. All fractions were subjected to radionuclide analyses and the results used to approximate the total amount in the river of the various radionuclides in each isolated fraction moving past the sampling site at the time of sampling.

Discussion. — Thus far the raw water samples may be quantitatively separated and isolated into four fractions: (1) noncolloidal organic, (2) noncolloidal inorganic, (3) colloids and solutes, and (4) pure water. Sampling has been conducted for the past two summers and enough samples have been
collected for statistical analyses. The methods have been standardized and some meaningful interpretations should be available at the end of this summer. The next steps are: to continue to improve the qualitative and quantitative degree of separation of organic from inorganic material, and to investigate means of isolating the various homogenious groups of colloids and solutes in the supernatant water from the continuous flow centrifugation. In a few years, it is hoped, a mature, quantitatively reproducible system of fractionation and analyses of the raw water will be worked out from the present preliminary methods. With such a system it should be possible to elucidate any contaminant's uptake by and distribution among the various components of natural water by a modification of the final analytical methods to suit the particular contaminant under investigation. In addition, the basic theory and practice should be applicable to many similar problems of uptake and distribution within multicomponent systems of various types.
Pseudouridine Metabolism

The experiments reported previously\(^1\) established (1) that the pyrimidine requirements of *Tetrahymena pyriformis* can be satisfied by uracil, uridine, or pseudouridine; (2) that the pyrimidine portion of these nucleosides is incorporated directly and without dilution into RNA-uridine and pseudouridine, but; (3) that there is almost total replacement of the 5' and 6’ protons of uridine (or uracil) upon incorporation into RNA-pseudouridine (or into DNA-thymine). It was also observed (4) that the ribose portion of pseudouridine is transferred as such to the purines and, presumably, to pyrimidine as well. The latter would constitute an isomerization and raises the question of the source of the ribose for pseudouridine synthesis when uridine is the precursor, and vice versa.

A repetition of the last experiment, with pseudouridine apparently indiscriminately labeled with tritium in both pyrimidine and ribose portions, gave RNA-CMP, -UMP, and -ΨMP of almost identical specific activities (at about one-fourth the starting specific activity). Degradation of the CMP and UMP yielded cytosine and uracil of specific activities about 20% lower. Thus, the activity of the ribosyl portion of RNA-UMP and RNA-CMP was lower than that of the pyrimidine portion, but it was about three times the activity found in the ribosyl portions of the purine nucleotides. But the millimolarities of glucose, acetate, and purine nucleotides in the medium, at the start, were 5.6, 12.5, and 0.14; that of the pseudouridine was 0.01. Thus the small activity in the RNA-UMP and RNA-CMP indicates a considerably preference for ribosyl, including pseudouridine ribosyl, over glucose as a precursor of the ribosyl of the RNA-pyrimidine nucleotides.

From the fact that RNA-ΨMP has the same specific activity as RNA-UMP and that it is considerably below the starting material, as well as from other experiments, it appears that pseudouridine is not directly phosphorylated to the nucleotide (RNA-precursor) level but rather that uracil combines with a precursor, such as PPRP, to give the mononucleotide precursor of RNA-ΨMP, even when pseudouridine is

feder. Thus it appears that the pseudouridine is split to uracil and a ribosyl derivative and that these two are recombined as needed to give the precursors of RNA-UMP and RNA-\textsuperscript{VMP}. Utilization of the pseudouridine as such would have yielded a higher specific activity in RNA-\textsuperscript{VMP} than in RNA-UMP, which was not observed.

When unlabeled pseudouridine and labeled glucose were fed, all RNA nucleotides became radioactive, the purine ones more (10–20\%) than the pyrimidines, which were all equally labeled. The preference of the pyrimidines for the pyrimidine (pseudouridine) ribosyl moiety is still in evidence; the small difference in specific activities between purine and pyrimidine nucleotides is ascribed to the small amount of pyrimidine nucleoside fed in comparison to the glucose. It is clear, however, that conservation is quite incomplete; if it were complete, the RNA-pyrimidine nucleotides would not have become labeled at all.

With unlabeled uridine and labeled glucose, there is considerably less labeling in the RNA-pyrimidine nucleotides than in the purine ones. Conservation of uridine ribose for pseudouridine synthesis is clearly greater than the reverse, although, again, much ribose is derived from glucose.

With randomly labeled uridine, as with the randomly labeled pseudouridine, a small but definite transfer of ribose to purine nucleotides is observed. RNA-UMP is more active than RNA-\textsuperscript{VMP} and RNA-CMP, which are equal to each other, indicating (1) direct utilization of some uridine and (2) as much conservation of ribose for pseudouridine synthesis as for cytidine synthesis. Degradation of the RNA-CMP and RNA-UMP yields cytosine and uracil of specific activity 40\% less than that of the RNA-\textsuperscript{VMP}; hence, the ribose of the \textsuperscript{VMP} is labeled, indicating ribosyl transfer from—hence isomerization of—the starting uridine.

These experiments indicate that an isomerization of pseudouridine and uridine is possible and does occur, but not to the exclusion of other routes of adding uracil and ribose to each other. Experiments to confirm and assess these reactions more exactly are in progress.

**PURIFICATION OF CALF THYMUS POLYMERASE**

F. J. Bollum G. E. Houts Patricia A. Williams

Fractionation of the soluble protein fraction (about 1 kg protein) from 30 kg of calf thymus gland for DNA polymerase yields about 5 g of a 40-fold purified enzyme designated Fraction D. Chromatography of Fraction D on hydroxylapatite produces 0.5 g of a 200-fold purified DNA-polymerase designated Fraction E. Rechromatography of Fraction E on phosphocellulose results in further removal of inactive protein and a two- to fourfold increase in specific activity (Fxn F).\textsuperscript{2} Conditions for chromatography on DEAE cellulose are currently being reexplored since it is known\textsuperscript{3} that a useful purification can be obtained on this exchanger.


Analyses of Fraction E for DNase and substrate-degrading enzyme contaminants are negative. A trace contamination with phosphodiesterase and end-addition activity remains. Fxn E catalyzes the exact doubling of single-stranded primers, such as \( \phi X-174 \) and heated calf thymus DNA.

The improved preparations of calf thymus DNA-polymerase (Fractions E and F) are currently being used for studies on the enzymic synthesis of biologically active DNA, the mechanics of radiation-induced primer damage, and the action of alkylating agents (e.g., nitrogen mustard) on primer activity. An evaluation of the kinetic constants of the enzyme system and investigations on the mechanism of primer action are also in progress.

ENZYMATIC SYNTHESIS OF BIOLOGICALLY ACTIVE DNA

F. J. Bollum       Jane K. Setlow

A preparation of DNase-free DNA-polymerase (Fraction E) that does not inactivate Hemophilus influenzae transforming DNA has been used in attempts to synthesize genetic material. In preliminary experiments with heat-denatured \( H. influenzae \) DNA primer, there was no increase in biological activity, whereas a substrate-dependent decrease in residual activity remaining after heating was observed instead. It seemed possible that the loss in biological activity might have arisen from end-addition reactions on the denatured primer, resulting in an error in transcription of the genetic code. To avoid this possibility and to attempt complete elimination of residual biological activity in the denatured primer, the polymerase products were chromatographed on methylated albumin columns. With the chromatographic conditions used, no elution of denatured DNA primer (and end-addition products) from the column was expected. Native DNA is found to elute in the middle of the gradient at about 0.60 M NaCl.

The results of the column experiments are shown in Fig. 27, and may be summarized as follows:

1. The DNA-polymerase product from denatured primer does elute in the region expected for native DNA.
2. A two- to fourfold increase in biological activity is observed in the peak eluting in the region of native DNA.
3. About 20% of the residual biological activity of the heated primer also elutes in the region of native DNA.

Since no net increase in biological activity is observed in the reaction mixture and the increase in activity seen in the “native” peak from the columns is only about 2% of that calculated from the amount of DNA synthesized, these results are encouraging but rather confusing. It is hoped that a systematic evaluation of methods for primer denaturation and use of the more highly purified enzyme fractions will circumvent our current difficulties.

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5 Eleanor Groeniger and F. J. Bollum, this report.
Fig. 27. Chromatography of Calf Thymus DNA-Polymerase Products on Methylated Albumin Columns. A, non-incubated sample, denatured *H. influenzae* DNA primer, $^{32}P$ substrates. B, same as A, after 60 min incubation.
SYNTHESIS OF THE THEOPHYLLINE ANALOG OF AET

B. C. Pal

Synthesis of the theophylline analog of 5,2-aminoethylisothiuronium bromide (VI) as a possible radiation protection agent was accomplished according to the following scheme starting from theophylline (V). Yields were consistently good.

\[
\text{V} \quad \xrightarrow{\text{Br CH}_2\text{CH}_2\text{Br, NaOH}} \quad \text{VI}
\]

DEGRADATION OF 3-METHYLADENINE, 3-METHYLHYPOXANTHINE, AND 3-METHYL-6-DIMETHYLAMINOPURINE IN ALKALI

B. C. Pal

To establish definitely the position of the methyl group in 3-methyl-6-dimethylaminopurine (the major product of methylation of 6-dimethylaminopurine with dimethyl sulfate\(^6\)), its degradation in alkali was

studied. Both 3-methyladenine (I) and 3-methyl-6-dimethylaminopurine (II) are converted into 3-methylhypoxanthine (III), which was found to be further degraded into, possibly, 4-methylaminoimidazole-5-carboxamide (IV).

![Chemical structures](image)

**REACTION OF FORMALDEHYDE WITH ADENINE**

B. C. Pal

Although in recent years the reaction of formaldehyde with DNA has assumed importance in studying the secondary structure of the latter, no product of reaction of formaldehyde with the DNA or its constituents has yet been characterized chemically. It is not known definitely, as pointed out by Grossman et al., whether the amino group on the purine and pyrimidine ring or the ring N atoms are the sites of reaction. To decide between the two possibilities, the reaction of formaldehyde with adenine and 6-dimethylaminopurine was studied spectroscopically following the procedure of Fraenkel-Conrat. If the ring N atoms are the sites of reaction, then 6-dimethylaminopurine should show positive evidence of reaction; whereas if the amino group is the site of reaction, 6-dimethylaminopurine should be unreactive toward formaldehyde. No change in the UV spectrum of 6-dimethylaminopurine could be detected on treatment with formaldehyde. It is concluded, therefore, that in adenine the site of reaction with formaldehyde is the amino group.

OLIGODEOXYRIBONUCLEOTIDE SYNTHESIS

Eleanor Groeniger  F. J. Bollum

An "end-addition" enzyme utilizing oligodeoxyribonucleotide primers in calf thymus DNA-polymerase preparations has been investigated to differentiate it from the polymerase. A study of the competitive inhibition of DNA synthesis by pentathymidylic acid gave no evidence of competition. Three different reaction parameters were then studied. The end-addition polymerization reaction had the same pH and magnesium ion requirements as replicative synthesis, although manganous ion at low concentrations can be substituted for magnesium in oligodeoxyribonucleotide synthesis. Polymerization was inhibited by KCl, but to a smaller degree than had previously been found for DNA synthesis. 10

The overall result suggested that the end-addition activity might be different, and therefore separable, from the polymerase enzyme. Direct separation of the end-addition enzyme and the DNA polymerase then was attempted with phosphocellulose, DEAE, and hydroxylapatite columns. Neither phosphocellulose nor hydroxylapatite gave any indication of resolution whereas two definite activity peaks were seen on the DEAE column. The possibility that these peaks represent different types of synthetic activity will be investigated further.

Nucleic Acid Enzymology

Elliot Volkin  M. Helen Jones
F. J. Finamore  Katherine H. Stephenson
J. X. Khym
C. G. Mead\textsuperscript{a}
Anna Ruffilli\textsuperscript{b}

\textsuperscript{a}USPHS Fellow.
\textsuperscript{b}Foreign investigator from abroad.

Dissociation of Specific RNA, Protein and DNA Syntheses
In T2-Infected E. coli B

Elliot Volkin  Anna Ruffilli  M. Helen Jones

Introduction. – T2-infected coli B has long been exploited as a favorite model system for demonstrating RNA, DNA, and protein interrelationships. It has been shown by Hershey that T2 DNA accumulated during chloramphenicol inhibition could be later very efficiently utilized for phage synthesis. Our experiments with the B94 mutant (requiring adenine and arginine) strongly suggest that T2-specific RNA can be accumulated then subsequently function in T2 production. The present experiments are designed to determine whether T2 protein can be accumulated and then later utilized for phage production upon subsequent DNA synthesis. This condition is somewhat more demanding than the other two, since a number of different kinds of protein make up the final T2 virus and they must be assembled in just the right way. Our results demonstrate that T2-specific RNA, T2 protein, and T2 DNA syntheses can indeed be isolated from each other, and if carried out in that order, some yield of infectious T2 particles can be obtained.

Results and Discussion. – These results are brought about by using the DNA inhibitor, 5-fluorodeoxyuridine (FUdR), together with T2-infected coli B or T2-infected coli B94. FUdR is known to prevent DNA synthesis by blocking the action of thymidylate synthetase in the formation of thymidylic acid by methylation of deoxyuridylic acid. Of the two sources of T2 DNA synthesis (exogenous materials and preformed bacterial DNA), it might be assumed that the transformation of bacterial DNA to T2 DNA could proceed in the presence of FUdR but further DNA synthesis would be prevented, thereby reducing yields to a maximal level of about 45 T2 particles per bacterium. However, yields of T2 phage in the presence of FUdR are much lower, generally reaching a maximum of about 2 T2 particles per bacterium. Table 27 demonstrates some typical results. The yields are unaffected by the presence of uridine or whether FUdR is added a few minutes before or after the addition of the infecting T2.
The results are surprising in two respects: (1) yields are so low as to indicate a very inefficient utilization of host DNA, and (2) yields are not zero but generally reach a maximum of about 2. In the case of infection with T7 bacteriophage, the yield is unaffected by the presence of FUdR. It should be recalled that T7 DNA contains no HMC, and, in addition, the source of progeny DNA is exclusively the preformed host DNA. These results led us to think that the FUdR may also prevent the hydroxymethylation of deoxycytidylic acid arising from the breakdown of host DNA and thereby negate bacterial DNA as a source of T2 DNA synthesis. It seemed quite possible, since we use multiplicities of infection of about 8, that the yield of about 2 T2/B may be exclusively a result of utilizing the parental phage DNA as a source for the progeny DNA. We therefore asked whether the yield was indeed a function of the multiplicity of infection, or, in other words, the amount of available parental DNA. Table 28 demonstrates that this is indeed the case. We can interpret these data to mean that under conditions of FUdR inhibition, bacteria that are singly infected (first two columns) will not produce phage, but that the DNA from two or more infecting phage particles must be present and cooperate to produce the completed DNA molecule that is present in the progeny phage. (The yield for multiplicity 2.9, where about three-fourths of the bacteria are multiply infected, reasonably confirm the other data.) It should be stated that where one uses multiplicities between 5 and 15, ultimate phage yields generally vary from 1-3 and do not significantly increase after 1 hr.

<table>
<thead>
<tr>
<th>Table 27. Effect of Varying Concentrations of FUdR on Phage Yield</th>
<th>Table 28. Effect of Multiplicity of Infection on Phage Yield (FUdR Inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>FUdR (μg/ml)</td>
</tr>
<tr>
<td></td>
<td>270</td>
</tr>
<tr>
<td>30</td>
<td>0.05</td>
</tr>
<tr>
<td>60</td>
<td>1.3</td>
</tr>
<tr>
<td>90</td>
<td>1.5</td>
</tr>
<tr>
<td>60</td>
<td>0.02</td>
</tr>
<tr>
<td>90</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Since it had been observed that FUdR does not markedly inhibit RNA and protein synthesis in uninfected bacteria, we carried out some preliminary experiments to determine whether FUdR affects the synthesis of RNA and protein in T2 infected bacteria. In the T2 system, phage infection itself blocks the net synthesis of RNA, so the presence of the so-called T2-specific RNA was looked for by isotope incorporation. Table 29 shows that \( p^{32}O_4 \) was incorporated into RNA where FUdR is present to the same extent as the infected control.

Protein synthesis was tested for by the extent of incorporation of leucine, not only into total protein, but also into the protein precipitating with specific T2 antiserum. Table 30 shows that the same extent of leucine incorporation into total protein takes place as the control, while T2-specific protein
synthesized under conditions of FUdR inhibition reaches a level of about 60 to 70% of that of the uninhibited culture. We conclude then that the extent of T2-specific RNA and protein proceeds at a reasonable rate in cultures where T2 DNA synthesis is inhibited by FUdR.

We now ask whether this accumulated protein can be subsequently utilized if DNA synthesis is permitted. One clue to this can be obtained by comparing the rate and extent of T2 production upon reversal of the FUdR inhibition with that which occurs in the same medium in an uninhibited, or control, infected culture. For good reversal of FUdR inhibition we have found that the best conditions are to wash the infected bacteria free of FUdR and add thymidine and a 1/10 concentration of broth to the washed cells. Table 31 compares the phage yield after reversal of FUdR inhibition with that of the control culture, the control infection being carried out in the same "reversal" medium. It can be seen that T2 production is markedly enhanced by preincubation in FUdR. However, such a result could be obtained even if preformed T2 protein itself were not utilized, but only the specific RNA and/or the newly induced T2-specific enzymes were synthesized in FUdR medium, these being immediately put to use upon removal of FUdR. Thus a higher rate of T2 production would be expected upon allowing T2 DNA synthesis to proceed. To test for the utilizability of the preformed T2 structural protein itself, we resorted to two techniques. First, by placing cells washed free of FUdR into synthetic medium containing chloramphenicol, further protein synthesis is prevented and only nucleic-acid synthesis is permitted. Ordinarily, the addition of chloramphenicol to T2-infected cells inhibits DNA synthesis. With T2-infected cells inhibited with FUdR, removal of FUdR and the addition of chloramphenicol permits the formation of T2 progeny to the extent of about ten T2 particles per infected bacterium. This result shows that some T2 structural protein can be later utilized when DNA synthesis proceeds. However, the yield is much lower than expected if completely efficient utilization of the T2-specific protein took place. It is of interest that the ultimate yield obtained is already attained in 15 min or less after incubation in chloramphenicol. These conclusions can be confirmed by carrying out similar experiments with the B94 mutant, where for this purpose the use of chloramphenicol becomes unnecessary. The coli B94 mutant requires adenine and arginine for growth and T2 production. When both metabolites are present, but FUdR is additionally present after phage infection, T2 yields of less than 2 are obtained. Upon removal of FUdR and replacement of the cells into a medium containing deoxyadenosine alone, yields of about 10 T2/B are again obtainable. These yields of about 10 (actually they may vary between 5 and 15) cannot be increased by initial incubations in FUdR longer than 30 or 40 min, whereas smaller yields are obtained if the FUdR incubation is reduced to less than 30 min.

By the use of the B94 mutant together with FUdR inhibition, it is possible to demonstrate that T2-specific RNA synthesis, T2-specific protein synthesis, and T2 DNA synthesis can be dissociated from each other, and that same yield of infectious phage will be produced by this three-step process (Table 32).

Thus, if the host B94 is infected in the presence of adenine alone to allow RNA synthesis alone (where FUdR is added to ensure the absence of DNA synthesis), no progeny phage are produced. If after 60 min the cells are washed free of adenine and given arginine to allow protein synthesis (again in the
presence of FUdR to prevent DNA synthesis), yields of around one phage per infected bacterium are obtained in 60 min. This result is expected, being similar to that obtained where RNA and protein synthesis (but not DNA synthesis) occur concurrently under FUdR inhibition. If now the cells are washed free of arginine and FUdR, and deoxyadenosine is added to allow only DNA synthesis, yields of phage of 10 to 15 per infected bacterium are obtained. These phage are produced within 10 to 15 min after the introduction of deoxyadenosine to the medium. It is noteworthy that instead of deoxyadenosine alone,

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Lysate in Ab Pellet (counts per sec per 2 ml)</th>
<th>Lysate in Total Protein (counts per sec per 2 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FUdR</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FUdR</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>87</td>
</tr>
<tr>
<td>60</td>
<td>71</td>
<td>118</td>
</tr>
<tr>
<td>90</td>
<td>87</td>
<td>135</td>
</tr>
</tbody>
</table>

Table 31. Effect of Preincubation in FUdR on Phage Yield Upon Reversal

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>FUdR 60 min, Reversed</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.1</td>
<td>12.5</td>
</tr>
<tr>
<td>20</td>
<td>5.7</td>
<td>25.5</td>
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<td>30</td>
<td>30</td>
<td>48</td>
</tr>
<tr>
<td>45</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>135</td>
<td>165</td>
</tr>
</tbody>
</table>

Table 29. Incorporation of P32a into RNA After 90 Min

<table>
<thead>
<tr>
<th>FUdR (30 μg/ml)</th>
<th>Added 3 Min Before T2</th>
<th>Added 3 Min with T2 (+ uracil)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>26</td>
<td>24</td>
<td>28</td>
</tr>
</tbody>
</table>

Table 30. Incorporation of C14-Leucine into Protein

Table 32. T2-Infected B94 Three-Stage Experiment

<table>
<thead>
<tr>
<th>Synthetic Medium, Plusa</th>
<th>Yield (T2/B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ade + FUdR (60)</td>
<td>0.01</td>
</tr>
<tr>
<td>2. Arg + FUdR (60)</td>
<td>0.70</td>
</tr>
<tr>
<td>3a. dAdo + Thym (30)</td>
<td>14.5</td>
</tr>
<tr>
<td>3b. dAdo + Thym + Arg (30)</td>
<td>14.0</td>
</tr>
</tbody>
</table>

aMinutes in parenthesis.
deoxyadenosine and arginine are added to the medium in the final stage, no increase in phage yield is obtained. Thus, in the absence of further T2-specific RNA synthesis, although the required amino acid is available to the bacteria, no further T2 protein is produced. These data confirm our idea, proposed in a previous publication on T2 infection with the host B94, that the T2-specific RNA functions in protein synthesis in a manner unlike a stable catalyst.

Summary. — In T2-infected bacteria the analog, 5-fluorodeoxyuridine, not only prevents de novo DNA synthesis, but also inhibits the conversion of host DNA to viral DNA. Some 1 to 2 phage per bacterium can be produced under multiple infection conditions, apparently by the utilization of T2 parental DNA. T2-specific RNA and protein synthesis proceeds in the presence of FUdR, and a small but definite fraction of the structural protein accumulated can be utilized for the formation of intact T2 particles, when DNA synthesis is subsequently permitted. With the use of the host B94, which requires both adenine and arginine for T2 synthesis, it is possible to dissociate T2-specific RNA, protein, and DNA syntheses and demonstrate that this order of events will produce viable phage.

BIOCHEMICAL STUDIES ON UNDEVELOPED EGGS

F. J. Finamore Katherine H. Stephenson

Occurrence of P₁P₄ Diguanosine 5'-Tetraphosphate in Brine Shrimp Eggs. — Undeveloped eggs of the brine shrimp, Artemia salina, contain large quantities of a symmetrical pyrophosphate ester, P₁P₄ diguanosine 5'-tetraphosphate. The compound is readily isolated from sodium chloride extracts of eggs by virtue of its solubility characteristics and may be purified by ion exchange chromatography. It is soluble in acid and water, soluble with difficulty in alkali, and quite insoluble in ethanol. Complete hydrolysis of the compound with snake venom phosphodiesterase yields guanosine 5'-phosphate and inorganic pyrophosphate exclusively but the first products formed are guanosine 5'-phosphate and guanosine 5'-triphosphate in equimolar amounts.

Attempts are being made currently to isolate specific enzymes from brine shrimp eggs that hydrolyze the pyrophosphate ester.

Protein Synthesis in Amphibian Eggs. — Crude 15,000g supernatant fractions from ovarian eggs of Rana pipiens incorporate amino acids into acid-insoluble proteins. This incorporation shows a striking dependence upon magnesium concentration although there appears to be no absolute magnesium requirement. Amino acid incorporation is inhibited by ribonuclease, glutathione, and para-chloromercuribenzoate but is relatively insensitive to chloramphenicol and heat denaturation.

That the observed amino acid incorporation is directly related to protein synthesis is shown by the close association of C¹⁴ activity with peptides derived from the proteins of amphibian eggs.

Structures morphologically and chemically identical to amphibian egg nucleoli are capable of activating amino acids and incorporating labeled amino acids into proteins. Nucleolar amino acid activating enzymes function similarly to cytoplasmic and nuclear activating enzymes. Optimal activity depends
upon amino acid concentration and the presence of Mg\textsuperscript{2+} and ATP. Leucine and methionine activating enzymes are apparently concentrated in these structures although cysteine, arginine, and aspartic acid activating enzymes appear to be present. Since C\textsuperscript{14} amino acid incorporation does not require additional enzyme fractions, Mg\textsuperscript{2+}, ATP, or GTP, the nucleoli appear to be complete protein synthesizing units within themselves.

USE OF AMINES IN LIQUID-LIQUID EXTRACTIONS OF NUCLEIC ACID AND RELATED COMPOUNDS

J. X. Khym Katherine H. Stephenson Elliot Volkin

Introduction. — Liquid anion exchange has been initiated here to test the feasibility of this technique for use as a method for investigations of nucleic acids and lower-molecular-weight components that are associated with them. Thus far it appears that practical application of this technique can be carried out when the compounds of interest are dissolved in aqueous formate or acetate solutions and extractions made with high-molecular-weight amine formate or acetate salts dissolved in solvents such as amyl acetate or butyl ether.

Results and Discussion. — Many amines have been tested for their use as extractants by employing them as their acetate or formate salts in a manner previously described.\textsuperscript{1} The following observations have been noted.

1. Thus far only primary amines have been found useful as extractants.
2. Formate or acetate salts of long straight chain amines (e.g., C\textsubscript{16}, C\textsubscript{18}) showed insufficient solubility in practical solvents.
3. Solubility difficulties with the amines are minimized when the primary amine is branched considerably.
4. Amines of molecular weight as low as 185 can remove RNA from dilute buffer solution, but this is about the lowest limit that is practical due to losses of the amine salt to the aqueous phase when low molecular weight amines are used as extractants.
5. In order to prevent emulsions and to encourage a reasonably fast phase separation, the minimum concentration of aqueous buffer should be at least 0.1 M.
6. As the buffer concentration is increased from 0.1 M to 1.0 M, a series of decreasing extraction coefficients, with a fixed concentration of amine in the organic phase, can be obtained for nucleoside di-, tri-, and tetraphosphates, intermediate molecular weight oligonucleotides, and RNA.
7. Differences in the extraction coefficients of a particular class of compounds at a given salt concentration indicate that the extraction technique can be used for separation purposes.

Some initial data typical of that now being collected are shown in Table 33.

Compounds

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<th>Mononucleotides</th>
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<td>0.5 M, pH 4.7</td>
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<td>0.5 M, pH 7.5</td>
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<td>0.1 M, pH 8.5</td>
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Molecular weight of 315.

TWO METABOLICALLY DISTINCT TYPES OF RNA ASSOCIATED WITH THE DNA OF DROSOPHILA

C. G. Mead

Previous work has demonstrated the existence of an RNA fraction which is associated with the DNA derived from adults of Drosophila melanogaster. This RNA exhibits a molar nucleotide ratio which is similar to that of the DNA (equating U with T) but different from that of the microsomal RNA or the 105,000 g soluble RNA. Evidence of physical association between this RNA and the DNA results from studies which utilized ultracentrifugation, ECTEOLA cellulose column chromatography and melting-point determinations on untreated and nuclease-treated preparations.

An investigation of the metabolic turnover properties of this DNA associated RNA has been started. Third instar larvae were utilized in these experiments because of their feeding habits which are more suitable for pulse labeling experiments than those of adults. Preliminary experiments demonstrated that the RNA associated with the DNA became sufficiently labeled with $^32$P after larvae had been in contact with medium containing $^32$P orthophosphate for 10 min. Larvae were placed on a $^32$P orthophosphate containing medium for 10 min after which they were washed and transferred to a medium containing $^31$P orthophosphate. Aliquots of larvae were taken thereafter, and the nucleic acids were isolated and purified. The specific radioactivities of the nucleic-acid fractions are presented graphically in Fig. 28 with respect to time after the larvae were introduced to $^31$P orthophosphate containing medium. There appear to be two metabolically distinct types of RNA associated with the DNA which are distinguished by their relative rates of turnover. Both of these RNA species exhibit a turnover rate which is much greater than that of the micromosomal RNA. Attempts at physically characterizing these two types of RNA and at determining their distribution cytologically are presently being pursued.
Fig. 28. Turnover Rate of Larval Nucleic Acids.
Chemical Protection and Enzyme Catalysis

Chemical Protection

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

Introduction. — A previous report\(^1\) established that glutathione (GSH), when administered intraperitoneally in conjunction with 2-aminoethylisothiourea (AET) or 2-mercaptoethylamine (MEA), decreased their chemical toxicity and gave increased radiation protection at the higher radiation dose levels. This investigation has extended the work to include other radiation protective compounds as well as other routes of administration. In addition, the mixtures with GSH have been examined by chromatographic means to determine whether specific complex formation was responsible for the increased biological effect.

Results and Discussion. — The above effects were only found when AET and GSH were mixed together and injected intraperitoneally. The reduction of toxicity was not obtained when AET was administered orally and GSH injected intraperitoneally; in fact, less AET (400 mg/kg vs 640 mg/kg when given alone) could be given and the protective activity was decreased. However, the intraperitoneal injection of a mixture of 3-aminopropylisothiourea (APT) and GSH gave results similar to the AET-GSH mixture in that more APT could be given (360 mg/kg vs 240 mg/kg), and the radiation LD\(_{50}\) was increased from 1500 to 1650 r. This technique failed to give either decreased toxicity or increased protective activity with two substituted isothioureas: D-2-aminobutylisothiourea and 3-amino-propyl-N'-methyl isothiourea.

All the mixtures used previously (MEG-GSH, oxidized glutathione and MEG, and di-2-guanidoethyl disulfide and GSH) were examined by paper chromatography, and whenever a disulfide was used, the mixed disulfide of GSH and MEG was formed in appreciable amounts. It was not present in the active mixture of MEG-GSH, although MEG was complexed in another fashion (perhaps salt linkage) with GSH. Quantitative \(-SH\) determinations verified this finding in that the total \(-SH\) content of the mixture was equal to the sum of the two components. In addition, an MEG-GSH mixture, oxidized until no \(-SH\) remained, containing at least one-third mixed disulfide, failed to provide radiation protection above 1200 r when given intraperitoneally to the mice.

These findings apparently rule out the mixed disulfide as the active agent in providing increased protection.

**ENZYME CATALYSIS**

D. G. Doherty J. A. Duke

**Introduction.** — Although the action of pepsin on various proteins is well known, its hydrolysis of synthetic substrates has been less extensively investigated than the proteolytic enzymes, trypsin and chymotrypsin. As a result, little kinetic and structural evidence exists on which to base speculation on its possible mechanism of action on peptide bonds. However, it is clear from the meager evidence available that its mechanism of action is markedly different from that of chymotrypsin, trypsin, and papain. In this study we hope to develop such information about the essential requirements for binding to the enzyme, and thus gain greater insight as to the groups functioning as the active center of the pepsin molecule.

**Results and Discussion.** — In order to establish a baseline for comparison with other substrates, the kinetics of hydrolysis of carbobenzoxy-L-glutamyl-L-tyrosine at pH 4.0 have been determined, and found to be in essential agreement with those of Casey and Laidler,\(^2\) \(K_m\) being \(2.4 \times 10^{-3}\) and \(k_3\) being \(0.92 \times 10^{-3}\). As additional model compounds, acetyl-L-phenylalanyl-L-tyrosine and acetyl-L-phenylalanyl-L-phenylalanine have been prepared and are being subjected to a kinetic study. Phenylpropionyl-L-phenylalanine and phenyl propionyl-L-tyrosine were synthesized and found not to be split by the enzyme at pHs 2 to 4. Carbobenzoxy-L-tyrosine is also not hydrolyzed by the enzyme. Thus, the acylamino group, in contrast to chymotrypsin, is essential for enzyme activity. In addition, the position of the carboxyl group of acidic type substrates is critical for enzyme activity.

Enzymology

G. D. Novelli

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\^aConsultant.
\^bVisiting investigator from abroad.
\^cUSPHS fellow.
\^dLoanee.
\^eNational Foundation fellow.
\^fLeave of absence, Division of Biology and Medicine, AEC, Washington.
\^gResearch participant.
\^hStudent trainee.

MESSENGER RNA AND THE ROLE OF THE INDUCER IN THE SYNTHESIS OF \( \beta \)-GALACTOSIDASE

J. M. Eisenstadt  G. D. Novelli

Introduction. — Previous studies have established that genetically specific DNA is required for the cell-free synthesis of \( \beta \)-galactosidase by extracts prepared from *E. coli*.\(^1\) Evidence has also been presented which demonstrates that the RNA synthesized by RNA polymerase in the presence of preinduced DNA contains information for the synthesis of \( \beta \)-galactosidase.\(^1\) These studies have suggested that genetic information in DNA is transmitted to a specific RNA species (messenger RNA) which combines in some as yet unknown manner with the ribosomes to form the enzyme-synthesizing unit. The separation of the synthesis of \( \beta \)-galactosidase into discrete steps, (1) the RNA synthetic step and (2) the protein synthesizing step, affords us the opportunity of examining the role of the inducer of enzyme formation in vitro.

To further this end, the RNA synthesized by RNA polymerase in the presence of induced DNA was isolated, and the effect of its addition to the protein-synthesizing system was studied.

**Results and Discussion.** – Initially, experiments were performed with purified RNA polymerase incubated with the ribonucleoside triphosphates, induced DNA, in either the presence or absence of the inducer, TMG. After incubation, the samples were added to the β-galactosidase-synthesizing system and enzyme synthesis was measured. The results of such an experiment proved that the RNA synthesized did contain information and that this information could be transmitted to the protein-synthesizing system. These experiments also demonstrated a requirement for the inducer during the period that the messenger RNA was being synthesized.

It was of interest to determine if there were some other point during the enzyme synthetic process where the inducer acts. RNA was synthesized by RNA polymerase in the presence of inducer and isolated by phenol extraction. This RNA was added to the protein synthesizing system in which the supernatant had been previously treated with protamine sulfate to remove all nucleic acids. The isolated messenger RNA was able to stimulate enzyme formation in either the presence or absence of inducer. The presence of inducer during enzyme synthesis led to about 25% greater enzyme synthesis than in its absence. Such results would suggest that the inducer is required during the time in which the information regulating the amino acid sequence of the enzyme is transmitted from DNA to messenger RNA.

The fact that somewhat more enzyme is made when inducer is also present during the protein synthesizing stage suggests that the inducer may play a stabilizing role with messenger RNA.

**REPRESSOR RNA AND THE CONTROL OF THE SYNTHESIS OF β-GALACTOSIDASE**

J. M. Eisenstadt  
G. D. Novelli

**Introduction.** – Wild-type cells of *E. coli* do not synthesize β-galactosidase unless an inducer is present. This seems to be a general case for most induced enzymes. Since it is known that the information for enzyme synthesis is contained in DNA and can be transmitted to a specific RNA, it would seem that DNA is unable to act except in the presence of inducer. Jacob and Monod,2 largely on the basis of genetic experiments, have developed a detailed theory that attempts to explain the operation of the inducible systems. The essence of this theory is that there are two classes of genes, structural and regulatory. The structural genes contain information for the amino acid sequence of a protein and transmit this information to a "messenger RNA" that functions in protein synthesis at the ribosomal level. The regulator genes are postulated to produce a cytoplasmic repressor which combines with the *operator* and thereby blocks the transcription of the sequence information from DNA to messenger RNA. The inducer is postulated to combine with the repressor and inactivate it. This permits the transcription of the information from the structural gene to messenger RNA. These postulates state that the mechanism of induction is primarily negative, that is, operates by inhibiting protein synthesis, rather than provoking specific protein

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synthesis. The cell-free system we have developed that catalyzes the synthesis of β-galactosidase is admirably suitable to test the details of this theory.³

Results and Discussion. — Using our cell-free system, we have been able to prove that RNA polymerase catalyzes the transcription of structural information of β-galactosidase to a specific RNA.⁴ This RNA can associate with ribosomes, in the absence of DNA, and bring about the synthesis of the enzyme. Thus this RNA can be correctly called "messenger RNA" since it has been established that this RNA does indeed contain information obtained from the gene. A clue to the mechanism of inducer action was obtained when we observed that messenger RNA synthesized in the absence of inducer was incapable of catalyzing enzyme synthesis, but nevertheless it was able to stimulate incorporation of radioactive leucine into general protein. These findings suggested that the inducer was functioning at the level of DNA to either stimulate the synthesis of a specific messenger RNA or inhibit the formation of a specific repressor RNA. A test of these two possibilities was carried out by mixing messenger RNA synthesized in the presence of inducer with RNA synthesized in the absence of inducer and adding the mixture to the ribosomal enzyme synthesizing system. The RNA made in the absence of inducer inhibited enzyme synthesis that is catalyzed by RNA made in presence of inducer, showing clearly that the RNA product of the i⁺ gene is a repressor RNA that inhibits enzyme synthesis at the ribosomal level. The role of the inducer, then, is to inhibit the functioning of the i⁺ gene. Thus, in the wild-type cell the i⁺ gene and the z⁺ gene are both making gene product RNA. The RNA product of the i⁺ gene inhibits the functioning of the RNA product of the z⁺ gene. When inducer enters the system, it inhibits the formation of i⁺ RNA product, and the z⁺ RNA product is allowed to act. These findings require a modification of the Jacob-Monod hypothesis to account for repressor action at the ribosomal level rather than at the level of the gene. A detailed analysis of the mechanism whereby the inducer inhibits the action of the i⁺ gene is under investigation.

EFFECT OF S-RNA ON THE INCORPORATION OF C¹⁴-LEUCINE INTO PROTEIN BY A CELL-FREE PREPARATION FROM E. COLI

A. N. Best S. Nishimura G. D. Novelli

Introduction. — It was previously reported⁵ that a preparation of S-RNA stimulated the cell-free incorporation of C¹⁴-leucine into TCA precipitable protein with a supernatant plus ribosomal system from E. coli. According to present theory, this is not an unexpected finding, since S-RNA is believed to function in the transport of activated amino acids between the soluble activating enzymes and some component of the ribosome. Since the charging of S-RNA with amino acids can be measured independently of the overall incorporation reaction, it became of interest to compare a number of S-RNA preparations for both properties in order to attempt to obtain direct evidence for amino acyl S-RNA as an intermediate in the incorporation reaction.

Results and Discussion. — S-RNA was prepared from the 100,000 g supernatant of E. coli extracts by the detergent procedure of Ofengand et al.⁶ and was further purified by DEAE column chromatography or

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⁴ J. M. Eisenstadt and G. D. Novelli, this report.
by chromatography over methylated albumin columns. The ability to accept C\textsuperscript{14}-leucine was measured by incubating S-RNA with C\textsuperscript{14}-leucine and a purified fraction of activating enzyme prepared by treating the 100,000 g supernatant with streptomycin sulfate and fractionation with ammonium sulfate. The active fraction was precipitated between 0.5 and 0.65 saturation with ammonium sulfate. This same enzyme fraction catalyzed the transfer of amino acyl S-RNA into ribosomal protein as well as the overall incorporation reaction. Using these three assay techniques with S-RNA's of varying ability to accept C\textsuperscript{14}-leucine, no direct correlation was found between the stimulation of incorporation and the ability to transfer or to accept amino acids. Purification of S-RNA by column chromatography increased its ability to accept C\textsuperscript{14}-leucine by sixfold and at the same time decreased its relative stimulation of the incorporation process fourfold. Whatever the stimulatory component is, it is susceptible to pancreatic RNase and to periodate oxidation. We are attempting to isolate and identify this stimulatory factor.

**EFFECT OF DIFFERENT RIBONUCLEASES ON THE INCORPORATION OF C\textsuperscript{14}-LEUCINE IN PROTEIN IN AN E. COLI CELL-FREE SYSTEM**

Susumu Nishimura G. D. Novelli

**Introduction.** — The incorporation of amino acids into protein in a number of cell-free systems is remarkably inhibited by very small amounts of pancreatic ribonuclease. As little as 0.1 \( \mu \text{g/ml} \) gives 80–100\% inhibition of incorporation. Because the ribonuclease isolated from Bacillus subtilis by Nishimura\textsuperscript{7,8} has a specificity for purine phosphodiester linkages rather than the pyrimidine specificity of pancreatic RNase, it was of interest to compare the inhibitory effect of these two of RNase's in the E. coli system.

**Results and Discussion.** — When the effect of the two RNase's was studied in a leucine incorporating system, we found that 0.1 \( \mu \text{g/ml} \) of pancreatic RNase inhibited incorporation by 80\%, and 0.5 \( \mu \text{g/ml} \) inhibited incorporation by 99\%. With the B. subtilis RNase, leucine incorporation was inhibited 55\% by 0.2 \( \mu \text{g/ml} \) and only by 65\% at a concentration of 5 \( \mu \text{g/ml} \). These data suggested that some kind of RNA involved in leucine incorporation is resistant to attack by B. subtilis RNase. Therefore, the effect of these RNases on the incorporation of other amino acids was measured. In these experiments, the supernatant was incubated with RNase for 20 min at 37\(^\circ\)C, and an aliquot was added to ribosomes for measurement of incorporation. The final concentration of both nucleases was 0.25 \( \mu \text{g/ml} \). The B. subtilis enzyme inhibited the incorporation of leucine 50\%, phenylalanine 69\%, isoleucine 97\%, and valine 85\%. The comparable inhibitions by the pancreatic enzyme were: leucine 90\%, phenylalanine 80\%, isoleucine 93\%, and valine 91\%.

Since RNase can be removed by passing a reaction mixture over carboxymethyl cellulose, it was of interest to determine which of the two components, supernatant or ribosomes, was sensitive to nuclease action. Accordingly, supernatants and ribosomes were treated in separate experiments with either B. subtilis RNase (25 \( \mu \text{g/ml} \)) or pancreatic RNase (2.5 \( \mu \text{g/ml} \)), and the nucleases were removed by passing the reaction mixtures over carboxymethyl cellulose. The treated fractions were tested with their untreated counterparts for the ability to incorporate C\textsuperscript{14}-leucine into protein. The pancreatic RNase had no effect on the

\textsuperscript{7}S. Nishimura, Biochim. Biophys. Acta 45, 15 (1960).
\textsuperscript{8}S. Nishimura and H. Ozawa, Biochim. Biophys. Acta 55, 421 (1962).
supernatant, but it completely inactivated the ribosomes. *B. subtilis* RNase, on the other hand, had no effect on the ribosomes but led to a 50% loss of activity in the supernatant. This loss could be restored by the addition of an S-RNA preparation. However, these results are difficult to interpret in the light of current theory relating to the mechanism by which amino acids are incorporated into protein. Further studies now in progress are required before a meaningful interpretation of these data are possible.

**INACTIVATION OF S-RNA BY RIBONUCLEASE FROM BACILLUS SUBTILIS**

S. Nishimura  
G. D. Novelli

**Introduction.** — The finding that the incorporation of certain amino acids into protein in a cell-free system from *E. coli* was not completely inhibited when RNase from *B. subtilis* was added to the incubation system suggested that some species of RNA involved in the incorporation reaction might be resistant to attack by this enzyme. Since S-RNA, as a carrier of the activated amino acid, has been implicated in the incorporation reaction, the sensitivity of this species of RNA to *B. subtilis* was examined.

**Results and Discussion.** — The loading of S-RNA by amino acids, a process called charging, can be measured independently of amino acid incorporation by using a partially purified fraction of amino acid activating enzymes and by omitting ribosomes that are essential for incorporation. The amino acid to S-RNA bond is sensitive to dilute alkali and can therefore be easily differentiated from amino acids incorporated into peptide linkage. With this technique, the chargeability of S-RNA for leucine was measured after various treatments with either *B. subtilis* RNase or pancreatic RNase. With as little as 0.01 μg pancreatic RNase, S-RNA lost 99% of its original ability to accept C\(^{14}\)leucine, although over 92% of the S-RNA retained its property of acid precipitability. With 1 μg of *B. subtilis* RNase (100 times as much as pancreatic RNase), there was no loss of ability of S-RNA to accept leucine even though 22% of the S-RNA had been converted to acid-soluble material. Increasing the concentration of the *B. subtilis* enzyme to 5 μg results in a loss of 50% of the ability to accept leucine, with 46% of the S-RNA being converted to acid-soluble products. This resistance of S-RNA acceptor activity for leucine requires the presence of Mg\(^{2+}\) ions. For example, as mentioned above, leucine acceptor function of S-RNA was completely resistant to 1.0 μg of *B. subtilis* RNase in the presence of Mg\(^{2+}\), but at the same concentration of enzyme in the absence of Mg\(^{2+}\), acceptor activity for leucine was completely destroyed. The inactivation of acceptor activity of S-RNA has been measured for 12 other amino acids. At a concentration of 1.0 μg/ml of *B. subtilis* RNase acceptor, activity for methionine, valine, histidine, and lysine was inactivated at a maximum of 55%, while similar activity for threonine, serine, tyrosine, glutamic, and arginine was almost completely lost. These findings suggest that further studies with this enzyme may reveal structural differences between species of S-RNA responsible for their amino acid specificity.

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9 S. Nishimura and G. D. Novelli, this report.
EFFECTS OF RIBONUCLEASES ON AMINO ACID CHARGING AND INCORPORATION ACTIVITY OF ACCEPTOR RNA

N. P. Wood  S. Nishimura  G. D. Novelli

Introduction. — Information is needed on the effects of various ribonucleases on the terminal nucleotides of amino acid acceptor RNA. In this study terminal nucleotides are digested from S-RNA, charged with ATP-C\(^{14}\) by polyphosphorylase, subsequently digested with ribonuclease, and tested for charging and incorporation activity.

Results and Discussion. — A large batch preparation of amino acid acceptor RNA was prepared from 741 g of *E. coli* B by a method similar to that described by Ofengand, Diekman, and Berg.\(^6\) Dried cells were used in this procedure, but wet cells were found to be as good. Further purification of RNA was obtained by chromatography on DEAE-cellulose. The acceptor RNA was tested for charging activity (C\(^{14}\)-leucine) and then digested with purified snake venom diesterase to remove the terminal adenosine phosphate and the two adjacent cytosine nucleotides.

To incorporate ATP-C\(^{14}\) in the RNA digest, S-RNA polyphosphorylase was obtained from *E. coli* and purified by treatment with streptomycin sulfate, ammonium sulfate, and DEAE-cellulose chromatography. After the ATP-C\(^{14}\) was incorporated, most of the charging activity for leucine was regained. The study of the active ribonucleases is now in progress.

CELL-FREE SYNTHESIS OF TRYPTOPHAN SYNTHETASE

O. H. Smith  G. D. Novelli

Introduction. — In the program designed to demonstrate the *in vitro* synthesis of a specific protein, in this case the "A" protein of *E. coli* tryptophan synthetase, the experimental emphasis has been to determine optimal conditions for *in vitro* protein synthesis and to devise more sensitive methods of measuring the "A" protein.

Results and Discussion. — As previously reported,\(^10\) a fraction of soluble RNA was found to markedly stimulate the incorporation of C\(^{14}\)-amino acids into protein in a cell-free system composed of particulate and soluble components of *E. coli*. This stimulation was abolished by pretreatment of the RNA with metaperiodate or RNase. The increase of amino acid incorporation into an acid-precipitable form appears to reflect incorporation into protein since, at the end of the 60-min incubation period, treatment of a sample of the reaction mixture with RNase had no effect, while treatment with trypsin plus chymotrypsin released the C\(^{14}\)-amino acids into an acid-soluble form.

Addition of the DNA-dependent RNA polymerase prepared by the method of Chamberlin and Berg\(^11\) was found to result in further stimulation of amino acid incorporation into protein. In some experiments the stimulations due to soluble RNA and the RNA polymerase have been observed to be greater than additive when both components were included in a reaction mixture. Thus in one case addition of 2.5 mg S-RNA gave a


doubling in the amount of $^{14}\text{C}$-amino acid incorporated, while in a parallel reaction addition of 0.67 mg DNA and 0.4 mg RNA polymerase resulted in a threefold stimulation. However, when all three components were included in the reaction mixture, a tenfold stimulation of amino acid incorporation was observed. Observations such as these have indicated the necessity of adding these components to the protein synthesizing systems being utilized in our studies.

A sample of tritiated indole was prepared and after purification has allowed the development of a sensitive assay for the enzymatic activity of the "A" protein. The conventional colorimetric assay allows detection of about 0.5 µg of "A" protein, while the assay employing tritiated indole can be used to measure as little as 0.02 µg of the enzyme. Thus, accurate measurements of small increases in enzyme activity are now possible. In several recent experiments, increase in the enzyme activity of "A" protein was found to parallel the extent of amino acid incorporation and to show comparable stimulation upon addition of soluble RNA and RNA polymerase. Future experiments will attempt to confirm these observations in the effort to ultimately allow the isolation of the newly synthesized "A" protein.

**CELL-FREE SYNTHESIS OF ALKALINE PHOSPHATASE IN E. COLI K-12**

W. R. Finnerty  
G. D. Novelli

**Introduction.** — Mechanisms involved in the control of cellular function generally fall into two categories, repression and induction. A specific example of repression and the system to be discussed is alkaline phosphatase. Biosynthesis of this enzyme is repressed in *E. coli* K-12 by high levels of inorganic phosphate and synthesized to the extent of 6–8% of the total cellular protein when cells are grown under conditions of limiting phosphate.  

Repression of this enzyme is under the control of two regulator genes, one closely linked to the structural gene and the other located in a distant region. Genetic analysis suggests that each regulator gene plays an essential role in the formation of repressor for alkaline phosphatase, since a constitutive mutation in either regulator gene prevents repression of the enzyme in cells grown under conditions of high phosphate concentration.  

**Preliminary Studies.** — A cell-free system reflecting protein synthesis has been developed using *E. coli* K-12 and growing the organism under conditions of limiting phosphate. Reconstituting a 105,000 kg supernatant plus ribosomes with amino acids, nucleoside triphosphates, and an energy generating source, three- to fivefold increases in enzyme activity have been observed. $^{14}\text{C}$-leucine is incorporated into TCA precipitable protein, and the radioactivity precipitates with anti-alkaline phosphatase prepared against purified alkaline phosphatase. An increase in enzyme units is observed by addition of DNA extracted from derepressed cells, soluble RNA, and RNA polymerase. Using supernatant and ribosomes prepared from an alkaline phosphatase negative mutant, enzyme increases have been observed following preincubations with derepressed DNA, RNA polymerase, and the nucleoside triphosphates. These reactions are DNase and RNase sensitive, and $^{14}\text{C}$-leucine is incorporated into both TCA and antibody precipitable protein. The

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results suggest the conclusion that a specific protein has been synthesized from its constituent amino acids and that the alkaline phosphatase gene has directed the synthesis of specific "informational" RNA which mediated the formation of alkaline phosphatase on ribonucleoprotein particles.

**PREPARATION OF A DNA-DEPENDENT RNA POLYMERASE FROM *E. COLI*  
E. Glassman  J. L. FitzGerald  O. H. Smith  G. D. Novelli**

**Introduction.** — We have demonstrated that the synthesis of β-galactosidase in a cell-free preparation is markedly dependent upon a specific, information-containing RNA.¹ This RNA can be synthesized independently of protein synthesis by the DNA-dependent RNA polymerase described in the literature by several groups.¹⁴,¹⁵ The product can be isolated and added to the cell-free system from *E. coli*, and it will catalyze the synthesis of β-galactosidase if the priming DNA has been prepared from preinduced genetically competent cell. This RNA also stimulates amino acid incorporation into general protein. Several attempts have been made in this laboratory to prepare this enzyme by methods described in the literature, but yields have been low and not reproducible. Furthermore, the enzyme has proved to be extremely labile, losing 50% of its activity per week of storage in the deep-freeze. Since a major fraction of the work of our group depends upon the availability of this enzyme, we decided to make an attempt to develop a rapid, convenient method for the purification of RNA polymerase that would result in a stable product and give good yields.

**Results and Discussion.** — We first attempted to duplicate the procedure of Chamberlin and Berg.¹⁶ since its description was the most detailed available. Our experience was similar to that of others in our group, in that the yield was very low. One difficulty with the procedure is that it involves a 4-hr centrifugation in the Spinco preparative ultracentrifuge in order to remove ribosomes. This step makes it virtually impossible to process reasonably large quantities of starting material in a normal working day. A second difficulty with the method arises from the fact that our method of cell rupture, breaking cells in the French pressure cell, although giving much larger amounts of enzyme in the starting material, also gives a much greater amount of nucleic acid. The nucleic acid interferes with the fractionation procedure and in particular prevents the proper operation of the DEAE column used in purifying the enzyme. A third difficulty with the method is that the most critical step in the purification procedure involves the precipitation of the enzyme and nucleic acid with protamine sulfate and the selective elution of the enzyme from the precipitate. The two different batches of protamine sulfate we have do not behave like the one described by Chamberlin and Berg.¹⁶ For these reasons we first attempted to avoid 4-hr Spinco centrifugation by trying various methods to selectively remove ribosomes by precipitation. The use of streptomycin, protamine, or borium chloride to precipitate the ribosomes also caused precipitation of the enzyme.

In order to elute the enzyme from such precipitates, solutions of high ionic strength are required; these solutions also elute the ribosomes. One improvement has been made and that is to reduce the centrifugation time from 4 to 2 hr. Also, ammonium sulfate fractionation first at pH 8.4 and a second fractionation at

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pH 6.8 seems promising. Finally, a preliminary attempt to separate the enzyme from nucleic acid on calcium hydroxyl apatite looks promising.

**INCORPORATION OF LABELED RIBOTIDES INTO RNA**

W. H. Spell  
J. M. Eisenstadt  
G. D. Novelli

Ribotides labeled with $\text{P}^{32}$ were prepared by growing *E. coli* in a medium containing $\text{P}^{32}\text{O}_4^{2-}$. UTP and GTP were prepared from the ribotides enzymatically using a kinase system prepared from rat liver. These labeled compounds were used in the cell-free $\beta$-galactosidase system to study the disposition of messenger RNA during the course of $\beta$-galactosidase synthesis. The ribosomes were isolated at various time points during the course of the reaction and analyzed by centrifugation in sucrose density gradients. Although incorporation was not great, our results were similar to those of Risebrough *et al.*, who found that messengers RNA was attached to 100 S and 70 S particles.

The above-mentioned kinase systems from rat liver and from yeast were studied in order to determine the optimum conditions for the conversion of ribotides to the corresponding triphosphates.

Experiments are in progress to synthesize UMP$^{32}$ chemically according to the method of Straus and Goldwasser.

**AMINO ACID INCORPORATION BY CELL-FREE EXTRACTS OF SACCHAROMYCES FRAGILIS**

A. K. Williams  
G. D. Novelli

**Introduction.** — A cell-free system consisting of particles sedimenting at 105,000 g and a supernatant fraction obtained from *S. fragilis* were found to be capable of incorporating radioactive leucine into acid-soluble protein. The system was found to be very unstable, even at the temperature of liquid nitrogen. This instability of the system effectively prevented any detailed study of the mechanisms involved in the incorporation of the isotope into protein. For this reason, we have attempted to devise techniques whereby the system can be kept in a stable condition for extended periods of time.

**Results and Discussion.** — Log phase cells of *S. fragilis* are broken by suspending in trio-Mg$^2+$-$\beta$-mercaptoethanol buffer and stirring in the Waring Blender with glass beads. The resulting material is centrifuged at 10,000 g for 30 min to remove whole cells and debris. The resulting supernatant is fractionated into particulate and soluble components by centrifugation at 105,000 g for 90 min. The particles are washed once in the buffer and finally suspended in buffer. The supernatant fraction is dialyzed against the buffer. As stated previously, these preparations were found to be very unstable. When the particles were washed and suspended in glass-distilled water, however, they were found to be stable for at least six weeks when stored in liquid nitrogen. The stabilization of the supernatant was achieved by passage over a column of

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G-25 sephadex. The activity of the column eluate was unaffected by storage at -20°C for several weeks. Also, the activity of the eluate was approximately twice that of the dialyzed preparations. Utilizing these stabilized preparations, we are now attempting to study the effects of nucleic acids on incorporation and protein synthesis.

**STUDIES ON ANTIBODY SYNTHESIS**

C. J. Wust  G. D. Novelli

*Introduction.* — A cell-free system designed to study antibody synthesis and the role of antigen in induction has been reported previously. This system has been studied to determine optimal conditions for the incorporation of amino acids into the protein with the expectation that conditions for maximum incorporation will coincide with optimal conditions for antibody synthesis.

Another series of experiments have been performed to investigate the apparent time of antibody synthesis in the mouse prior to the time of its detection in serum.

*Results and Discussion.* — The incorporating system previously described²⁰ has been modified to some extent. Rat spleen ribosomes are prepared after homogenation of the organ in a medium buffered at pH 8.0 containing sucrose, Mg²⁺, and K⁺. The cellular debris, nuclei, mitochondria, and large microsomes are removed by sequential centrifugation; the final supernatant is treated with 0.65% sodium desoxycholate. Ribosomes are then collected by centrifugation at 105,000 g for 90 min. The yield is 3 to 4 mg ribosomal protein per g of organ weight in contrast to our earlier methods in which microsomes after collection were treated with desoxycholate. The latter procedure yielded 1.0 to 1.5 mg ribosomal protein per g of organ weight. Once the ribosomes have been isolated, they can be stored at -192°C (liquid nitrogen) with a 50% decay of 5 to 7 days.

Rat liver supernatant can be fractionated with streptomycin to yield a precipitate and a soluble phase. The soluble phase when concentrated by 0.75 saturation with ammonium sulfate stimulated incorporation of about 50% as well as the same fraction prepared with a prior streptomycin treatment. The streptomycin precipitate does not stimulate incorporation of amino acid into ribosomes; but, when added to the ammonium sulfate fraction of the soluble phase, complete activity is restored. In addition, this precipitate added to a liver supernatant fraction increases the amount of amino acid bound to S-RNA.

The ratio of ribosomal protein to soluble protein for maximum efficiency of incorporation is 1:4. The system is extremely sensitive to pancreatic ribonuclease but insensitive to desoxyribonuclease and chloramphenicol. Kinetic analysis indicates that 6% of incorporation occurs linearly within 6 min and continues at a reduced rate for at least 40 min. Further characterization of this system is in progress.

Time Course of *in vitro* Labeling of Antibody. — The appearance of circulating, precipitating, and neutralizing antibody to the enzyme triose phosphate dehydrogenase (TPD) occurs on and after the ninth day following a single injection in C3BF1/Cum mice. Separate groups of animals received C14-glycine every day from the 1st to the 14th day after a single injection of TPD. On the 14th day, when maximum serum titers of antibody were detected, all the animals were sacrificed, and the serum was collected from each group. Quantitative precipitin reactions were performed with each serum and a 0.45 ammonium sulfate fraction of each serum. The results showed that antibody had been labeled with C14-glycine from the first day after antigen injection. The findings indicated that, although antibody was not detected in the serum until the ninth day, it is being synthesized from the first day. The results also indicated that this early antibody was not degraded, since subsequent synthesis should have diluted out the radioisotope.

**ROLE OF S-RNA IN THE AMINO ACID INCORPORATING SYSTEM FROM MAIZE SEEDLINGS**

C. M. Purcell R. J. Mans

**Introduction.** — We have observed that the addition of soluble or transfer-RNA to the maize seedling incorporating system results in an inhibition of incorporation of C14-leucine into maize particle protein. In view of the general acceptance of S-RNA as an intermediate in protein biosynthesis and amino acid incorporation into protein,21 we are investigating this apparently anomalous result.

**Results and Discussion.** — Previously, S-RNA had been prepared by phenol extraction of the 105,000 g supernatant of a seedling homogenate as described by Kirby.22 We have since prepared S-RNA from corn seedlings and frozen kernels by modification of the detergent procedure of Ofengand et al.23 All three of the preparations inhibit amino acid incorporation when added to the cell-free system. Furthermore, the kernel S-RNA has been purified by chromatography on DEAE-cellulose paper (kindly performed by K. B. Jacobson of this group) and found to inhibit incorporation up to 65%.

All the preparations that inhibit incorporation are capable of being "charged" with C14-leucine, especially the chromatographically purified preparation. These results suggested that the isolated material may be chargeable but incompetent to participate in the subsequent transfer of the C14-leucine to the maize particle protein.

A preliminary experiment conducted with rechromatographed "charged" S-RNA indicates that about 4% of the amino acid can be transferred to acid precipitable protein. Consistent with findings in other systems, the transfer requires GTP, Mg2+, and receptor particles and is stimulated by 105,000 g supernatant and KCl. Therefore the material that is chargeable is also capable of some transfer. Attempts to elucidate this apparent anomaly by studying the relative chargeability and transfer activity of various preparations of S-RNA as well as examination of products obtained with and without added S-RNA are currently in progress.

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LARGE-SCALE GERMINATION AND HARVESTING OF MAIZE SEEDLINGS

Rusty J. Mans

Introduction. — In the investigation of protein biosynthesis in higher plants utilizing the cell-free amino acid incorporating system isolated from maize seedlings, a severe limitation had been encountered, that of obtaining sufficient quantities of metabolically active plant tissue required for biochemical preparations. By the adaptation of large-scale germination and harvesting methodology developed for pea seedlings by Bonner et al., adequate amounts of corn seedling tissue are now available.

Results and Discussion. — Corn seeds in 25-lb batches are soaked and then germinated in a barrel under a fine water spray at 23°C for three days. The stems and roots are broken away and separated from the rest of the developing seedling by passage over a vibrating-screen separator, continuously flooded with cold tap water. Approximately 1.9 kg wet weight of stems and roots is harvested per 25 lb of dry seeds in less than 1 hr. The material is then sterilized in dilute chlorox solution. The "crop" can be separated further into stem rich and root enriched fractions by flotation in dilute sucrose solutions. Further processing of the material, i.e., homogenization, extraction, rapid filtration, and differential centrifugation, depends upon the particular component to be isolated.

To date, pilot plant materials have been used as a source of 105,000 g supernatant, washed ribosomal particles, and transfer RNA, all of which are active in the maize amino acid incorporating system. In Table 34 is presented a comparison of materials obtained from a 25-lb batch of seeds via the pilot plant as compared with a 100 g batch germinated on trays and harvested manually. The times of preparation are essentially equal in each case.

The lower specific activity in the ribosomal and supernatant preparations from the pilot plant material is probably due to the method of homogenization of the stems. However, with the acquisition of a commercial food cutter, "gentle" homogenization of large amounts of material will be possible; it is anticipated that these isolated components will retain relatively more biological activity.

In addition to the preparations mentioned above, pilot plant materials are being utilized as a source for the isolation of maize seedling DNA and RNA polymerase.


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<th>Table 34. Comparison of Pilot-Plant- and Batch-Germinated Seeds</th>
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<td>Preparation</td>
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MAIZE TISSUE CULTURE AND CELL-FREE AMINO ACID INCORPORATION

J. E. Graebe

G. D. Novelli

Introduction. — One chief difficulty in studying the enzymology of protein biosynthesis in higher plants lies in the unavailability of sufficient material to isolate the individual steps. One way to circumvent this would be to use large-scale tissue culture as a source of cells.

Results and Discussion. — The growth of maize endosperm in tissue culture has been increased to a practical level by the use of submerged techniques. A cell-free system, which incorporates C\textsuperscript{14}-leucine into acid precipitable protein, has been prepared from the cells.

The tissue culture used was isolated by Straus and LaRue\textsuperscript{26} in 1954. It was maintained as a callus culture on a synthetic solid medium containing sucrose, minerals, vitamins, glycine, asparagine, and sugar. Attempts to obtain growth in submerged culture were unsuccessful until the growth rate on agar had been increased by frequent transfers and selection of the fastest growing tissue. The final yield thus obtained with the solid medium was up to 3 g per month from a 0.5 g inoculum. This tissue continued to grow after being transferred into liquid medium and incubated with shaking.

The production of tissue in submerged culture was scaled up by the serial transfers into increasing volumes of medium. In the most successful experiment of this kind, the liquid culture was started by inoculation of approximately 1 g of tissue into 30 ml of liquid medium. After 16 days of incubation on a shaker, the culture was poured into 400 ml of medium in a cylinder and aerated for an additional 13 days. The culture was then inoculated into 10 liters of medium in a 12-liter aerated flask. After 21 days in the large flask, the culture was harvested, yielding 250 g of fresh weight tissue.

The relatively slow growth rate necessitates the use of large inocula and increases the risk for contamination. A device has therefore been constructed by which any amount of a culture can be transferred from one growth flask to another with minimum handling. Although penicillin and Fungizone have no apparent effect on the tissue, pure cultures without antibiotics are preferred since in serial transfers a latent contaminant may ruin a whole series at a later stage. Streptomycin is inhibitory to the growth of the tissue.

Cell-free preparations were obtained by breaking the harvested and washed cells in a French pressure cell and removing the debris by centrifugation at 10,000, 30,000 and 105,000 g. The ability of the fractions to incorporate C\textsuperscript{14}-leucine into acid precipitable protein was measured in the incubation medium devised for cell-free systems from maize seedlings.\textsuperscript{27} The main incorporating activity was found in the fraction sedimenting at 10,000 g supplemented with the 105,000 g supernatant fraction. The best yield obtained was 22 \mu moles of leucine incorporated per mg of particle protein during 30 min. The incorporation obtained with the fraction sedimenting at 105,000 g, usually regarded as the microsomal fraction, was considerably lower.

\textsuperscript{26} J. Strauss, Am. J. Botany 47, 641 (1960).
The results show that it is possible to obtain tissue cultures of maize endosperm in sufficient amount to serve as starting material for amino acid incorporating systems. Work is in progress to define the cell-free systems gained from such tissue cultures and to explore their potentialities in the study of single enzymatic steps involved in protein biosynthesis.

**CELL-FREE AMINO ACID INCORPORATION IN *NEUROSPORA CRASSA***

W. E. Barnett C. J. Wust G. D. Novelli

*Introduction.* - A study has been initiated to develop and characterize an *in vitro* amino acid incorporating system from *Neurospora*. This system will be used in attempts at cell-free enzyme synthesis, and it is anticipated that it will be useful in investigating such phenomena as complementation at a subcellular level.

*Results and Discussion.* - An *in vitro* amino acid incorporating system has been developed. Incorporation requires both a microsomal and soluble fraction. Homogenized log phase cells are centrifuged at 10,000 g, and the sediment is discarded. The microsomal fraction is obtained by collecting the pellet from a centrifugation at 105,000 g. The soluble fraction is concentrated by precipitation at 0.9 saturation with ammonium sulfate. Incorporation is energy dependent and is stimulated by GTP, Mg$^{2+}$ ions, and a mixture of L-amino acids. The presence of $5 \times 10^{-3}$ M mercaptoethanol at all stages of preparation minimizes losses of activity. Microsomal and soluble fractions are stable at liquid nitrogen temperatures for at least one week.

This cell-free system has been prepared from log phase cultures that have been exposed to the purine analogue 8-azaguanine, and the soluble and microsomal fractions tested with their normal counterparts. Preliminary results indicate that 8-azaguanine affects both fractions, but there are differences in effects with different amino acids.

**STUDIES ON PROTEIN SYNTHESIS IN CELL-FREE PREPARATIONS FROM *DROSOPHILA MELANOGASTER***

E. Glassman C. J. Wust G. D. Novelli

*Introduction.* - The ultimate aim of this program is to study the genetic control of the synthesis of xanthine dehydrogenase in cell-free preparations from *Drosophila melanogaster*. Mutant flies incapable of making the enzyme are available. We intend to eventually use ribosomes and supernatant from mutant flies and DNA or messenger RNA from competent flies to attempt *in vitro* synthesis of the enzyme. Before attempting specific protein synthesis, it seemed advisable to determine optimal conditions for the incorporation of amino acids by cell-free preparations with the hope that such conditions would approach the optimal conditions for specific protein synthesis.

*Results and Discussion.* - Adult flies were homogenized in a medium buffered at pH 8.0 containing sucrose, Mg$^{2+}$, and K$^+$. The homogenate was centrifuged at 32,000 g for 20 min, and the resultant supernatant was centrifuged at 105,000 g to obtain microsomes and supernatant. Incorporation of C$^{14}$-leucine into

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28 Fungal genetics group.
TCA precipitable material could be achieved in incubation mixtures containing microsomes, 0.6 saturated ammonium sulfate fraction of the 105,000 g supernatant, GTP, Mg\(^{2+}\), K\(^+\), \(\beta\)-mercaptoethanol, and an energy generating system of creatinephosphate, creatine phosphate kinase. To compare the effectiveness of *Drosophila* microsomes to incorporate the labeled amino acid, they were tested with a 105,000 g supernatant of rat liver. The rat liver supernatant stimulated the incorporation more effectively than did *Drosophila* supernatant. Experiments are in progress to find the optimal conditions for incorporation with an all *Drosophila* system.

**HORMONAL CONTROL OF ENZYME SYNTHESIS – 1**

F. T. Kenney  
F. J. Kull

**Introduction.** – Our previous studies of the induction of tyrosine-\(\alpha\)-ketoglutarate transaminase of rat liver have established (1) that induction is mediated by adreno cortical hormone,\(^{29}\) (2) that induction requires de novo enzyme synthesis,\(^{30}\) and (3) that induction results from a large increase in the rate of enzyme synthesis.\(^{31}\) Since it would appear that this induction represents an instance of hormonal control of the synthesis of a specific protein, our present efforts in analysis of the mode of hormonal action have been directed toward the nucleic acid components thought to control specificity in protein synthesis.

**Results and Discussion.** – Methods have been elaborated that permit the isolation of hepatic DNA and RNA in presumably intact form. Livers are removed and immediately dropped into liquid nitrogen, then homogenized in the presence of aqueous phenol while still frozen, thus minimizing nucleic acid degradation due to nuclease action. Subsequent isolation of each of the nucleic acids utilizes modification of the gentle procedures introduced by Kirby\(^{22}\) and extended by Sarkar\(^{32}\) and Colter *et al.*\(^{33}\) Application of the methods to the livers of fasted, adrenalectomized rats yielded high molecular weight preparations of DNA and RNA free of obvious contaminants and "intact" by chemical criteria. However, glycogen deposition resulting from hydrocortisone treatment was found to prevent successful isolation of the nucleic acids from induced liver. The antibiotic puromycin was found to inhibit glycogen formation in dose levels similar to those required to inhibit enzyme induction. Since this inhibitor of protein synthesis is considered to act at the ribosomal level, it is reasonable to assume that changes in nucleic acids associated with induction will still occur in its presence. Indeed, it is possible that these changes may be made easier to detect, since the lability of some RNA species is thought to be due to their utilization in protein formation. Preliminary experiments have supported these assumptions, insofar as \(^{32}\) labeling of total liver RNA is nearly doubled by administration of puromycin. These experiments will be continued with the central aim of identifying and isolating the nucleic acid components presumed to be formed in response to hormonal treatment.


HORMONAL CONTROL OF ENZYME SYNTHESIS – II
F. T. Kenney                  J. M. Eisenstadt

Introduction. – Stimulation of the formation of a specific information-carrying RNA ("gene activation") appears to be the mechanism involved in the substrate control of certain microbial enzyme syntheses, and it could be operative in hormonal induction as well. An alternative mechanism can be construed, in which control is applied to the utilization of the gene product, rather than to its formation. This should result in a relatively non-specific stimulation of enzyme syntheses, and differential responses in the rate of synthesis of different enzymes could be attributed to existing differences in rates of formation of their respective RNA's. The present experiments were carried out to investigate the possibility of hydrocortisone effecting enzyme inductions by this type of mechanism.

Results and Discussion. – RNA polymerase was prepared from extracts of E. coli, as described by Chamberlin and Berg,16 and incubated with rat liver DNA under conditions designed to permit the accumulation of the RNA synthesized; hydrocortisone (10^-5 M) was added to some reaction mixtures and omitted from others. After 20 min incubation, all reaction mixtures were supplemented with the components required to carry out protein synthesis as described by Kameyama and Novelli;2 both the ribosomal and soluble components were from extracts of E. coli. Incorporation of C14-leucine into protein was measured at intervals throughout the subsequent 60-min incubation. The described preincubation with rat liver DNA effected a large increase in the incorporating activity of the E. coli preparation, and added hydrocortisone resulted in a significant (~30%) further increase in incorporation. In separate experiments hydrocortisone was found to have no effect on the activity of the RNA polymerase, and the steroid was similarly ineffective when added to the E. coli incorporating system unsupplemented with DNA and polymerase. These results are consistent with the alternative mechanism described above. Experiments planned and in progress are directed toward repeating these preliminary efforts and particularly toward developing preparations from rat liver in which to study these phenomena.

SEPARATION OF NUCLEOTIDES OF RNA ON ONE-DIMENSIONAL CHROMATOGRAPHY
K. B. Jacobson

Conditions have been developed so that the four main ribonucleotides of RNA may be separated on DEAE-cellulose paper sheets. Irrigation with 0.05 M formic acid separates cytidylic and adenylic acids from each other and from guanylic and uridylic acids. The latter are not resolved at 0.05 M formic, so the paper sheet is cut so as to separate the guanylic-uridylic areas and is irrigated in the reverse direction, resulting in resolution of these two nucleotides from each other and from orthophosphate. The time required for the first separation is 2½ hr and for the second, 45 min.

RNA CHROMATOGRAPHY ON DEAE-CELLULOSE PAPER SHEETS
K. B. Jacobson                  A. L. Dopirak

Introduction. – Chromatography of ribonucleic acid (RNA) on paper sheets of diethylaminoethyl (DEAE) cellulose offers the opportunity of (1) performing small-scale separations, (2) using the sheets to explore
Results and Discussion. — Various procedures for the chromatographic separation of various RNA fractions have been explored. Salt and urea resolve a fraction of ribosomal RNA not obtained by salt alone. An apparent resolution of S-RNA into two distinct chromatographic species was shown to be due, for the most part, to an anomalous affinity of a portion of the S-RNA for the DEAE-cellulose paper. On the other hand, S-RNA fractions from different organisms have been shown to have somewhat different mobilities.

The behavior of large molecular weight RNA is usually intractable in the usual procedure of using salt solutions to develop the chromatogram. From experiments on S-RNA the suspicion arose that ionic bonds were not the only force joining the RNA to the DEAE-cellulose and that non-ionic forces were also operative. The incorporation of 7 M urea into the usual salt solutions not only proved successful in causing ribosomal RNA to migrate but resulted in the fractionation of this RNA into two distinct components.

This technique of chromatography of various types of RNA seems sufficiently well developed to be applied to problems of RNA metabolism and function.

**STUDIES ON LACTIC DEHYDROGENASE ISOZYMES**

P. J. Fritz  
K. B. Jacobson

Introduction. — Lactic dehydrogenase (LDH) is an enzyme involved in a key reaction connecting glycolysis and the Krebs cycle. LDH is known to exist in five electrophoretically distinguishable forms, termed isozymes, in mice. In view of the metabolic significance of the enzyme and the existence of the multi-molecular forms, it was decided to undertake a study of the effect of closely linked metabolities on the activity of the individual isozymes in order to determine if any hitherto unknown metabolic control mechanisms were in operation.

Results and Discussion. — Homogenates were made from various organs of RF/Up and DBA/2 mice. The LDH patterns of the various organs were determined by electrophoresis on polyacrylamide gels followed by staining with an enzymatic technique involving the reduction of nitro blue tetrazolium. The individual isozymes could be separated by elution from the gel.

Initial studies on the separated isozymes indicated that all were inhibited, although to different extents, by $10^{-5} M$ oxaloacetic acid. However, the effect of oxaloacetic acid on brand No. 3 (the electrophoretically intermediate brand) could not be readily ascertained because of the interference of malic dehydrogenase. In general, the various isozymes were activated by DL aspartate ($10^{-5} M$) and inhibited by DL malate ($10^{-5} M$). Little difference could be detected between the two strains of mice or between the isozymes of different organs in the same mouse.

The inhibition by oxaloacetate and malate have been tentatively interpreted to indicate a metabolic control governing the release of pyruvate. The action of aspartate has not been explained.
STUDIES ON ADENOSINE 5'-PHOSPHOSULFATE REDUCTASE

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

Introduction. — Adenosine 5'-phosphosulfate (APS) reductase from Desulfovibrio desulfuricans catalyzes the reversible AMP-dependent oxidation of \( \text{SO}_3^{2-} \) to APS as follows:

\[
\text{AMP} + \text{SO}_3^{2-} \rightleftharpoons \text{APS} + 2\text{e}^-.
\]

The enzyme has been enriched some 75-fold and seems to be a flavoprotein that contains FAD as its prosthetic group. Although it has not as yet been possible to resolve the enzyme for FAD, the observation that \( \text{SO}_3^{2-} \) in the absence of AMP partially bleaches the flavoprotein indicates that FAD is intimately associated with the activity of the enzyme. This observation also suggests that an "enzyme-sulfate" or "cofactor-sulfate" may be involved in the mechanism of action of APS-reductase.

Results and Discussion. — The APS-reductase has been further purified and appears to be quite homogeneous in the ultracentrifuge. From the rate of sedimentation and the ratio of flavin to protein, the enzyme appears to have a molecular weight of 200,000.

Treatment of the enzyme with \( 1 \times 10^{-4} \text{ M} \) \( p \)-hydroxy-mercuri-benzoic acid (\( p \)-CMB) results in an inhibition of activity and an apparent complete bleaching of the enzyme. This apparent bleaching is the result of the liberation of FAD from the protein by the inhibitor. Although \( \text{SO}_3^{2-} \) has no effect upon the sedimentation pattern obtained with the enzyme in the ultracentrifuge, \( p \)-CMB effects a change in the sedimentation pattern of the enzyme. This change appears to be the result of disassociation of the reductase into smaller components by the action of the inhibitor.

The observations made thus far suggest that an "enzyme-sulfate" or "cofactor-sulfate" is involved in the mechanism of action of APS-reductase; however, as yet it has not been possible to devise a definitive experiment to demonstrate the intermediate. It is anticipated that further studies with inhibitors and the physical properties of the enzyme will yield information regarding the mechanism of action of APS reductase.

SULFATE REDUCTION BY CELL-FREE EXTRACTS OF DESULFOVIBRIO ORIENTIS

Sister M. Regina Lanigan H. D. Peck, Jr.

Introduction. — Most microorganisms, capable of reducing sulfate, are "assimilatory sulfate reducers" and reduce sulfate in the form of \( 3' \)-phosphoadenosine 5'-phosphosulfate (PAPS). Two of the three known strains of "dissimilatory sulfate reducing" bacteria, Desulfovibrio desulfuricans and Clostridium nigrificans, have recently been shown to reduce sulfate in the form of adenosine 5'-phosphosulfate (APS). It was therefore of interest with regard to this biochemical difference to determine whether the third known dissimilatory sulfate reducer, \( D. \) orientis, reduced sulfate via the PAPS or APS pathway.

Results and Discussion. — *D. orientis* was grown as described by Adams and Postage, and cell-free extracts were prepared by means of the French pressure cell. When cell-free extracts are incubated with ATP and S\textsubscript{35}O\textsubscript{4}\textsuperscript{2−}, a single radioactive nucleotide is observed that has the same electrophoretic mobility as APS. The presence of ATP-sulfurylase was indicated by the molybdate dependent release of orthophosphate from ATP and APS-reductase, by the AMP-dependent reduction of Fe(CN)	extsubscript{6}\textsuperscript{3−} by SO\textsubscript{3}\textsuperscript{2−}. The product of the latter reaction was identified as APS.

Although *D. orientis* does not show hydrogenase activity, ATP-dependent H\textsubscript{2} utilization and SO\textsubscript{4}\textsuperscript{2−} reduction can be demonstrated when these extracts are supplemented with hydrogenase from *Escherichia coli* and the artificial electron donor, methyl viologen. Crude extracts of *Clostridium pasteurianum* that contain hydrogenase also stimulate the reduction of SO\textsubscript{4}\textsuperscript{2−} by H\textsubscript{2} in extracts of *D. orientis*, but it is in the absence of methyl viologen. As partially purified preparations of ferredoxin increase the rate of sulfate reduction in the presence of *C. pasteurianum*, this iron-containing protein may be involved in the electron transport system of *D. orientis*; the nature of the stimulation observed with ferredoxin is now being investigated.

It has been demonstrated that *D. orientis* reduces SO\textsubscript{4}\textsuperscript{2−} via the APS pathway as do the other known dissimilatory sulfate reducing bacteria. *D. orientis* differs from *D. desulfuricans* in that it does not produce cytochrome C\textsubscript{3}; however, sulfate reduction is stimulated by ferredoxin in extracts of *D. orientis* and may function in a manner similar to that of the cytochrome C\textsubscript{3}.

### STUDIES ON THE FORMIC HYDROGENLYASE OF *DESULFOVIBRIO DESULFURICANS*

Joy P. Williams H. D. Peck, Jr.

Introduction. — Cell-free preparations from *D. desulfuricans* and *E. coli* each contain enzymes that catalyze the formic hydrogenlyase reaction, that is, the decomposition of formate to CO\textsubscript{2} and H\textsubscript{2}. Although, in addition to formic hydrogenlyase, both preparations exhibit formic dehydrogenase and hydrogenase activity; only the formic hydrogenlyase of *D. desulfuricans* requires cytochrome C\textsubscript{3} for activity. This, and other results, indicated that the formic hydrogenlyase of *E. coli* and that of *D. desulfuricans* are not identical, and the possible points of difference are being investigated.

Results and Discussion. — As formic dehydrogenase preparations from coli-aerogenes bacteria as well as *D. desulfuricans* reduce cytochrome C\textsubscript{3} in the presence of formate, the major point of difference between these two systems is believed to lie in the oxidation of reduced cytochrome C\textsubscript{3} to H\textsubscript{2}. Extracts of *D. desulfuricans* produced H\textsubscript{2} from the reduced cytochrome, but hydrogenase preparations from coli-aerogenes bacteria or *Clostridium pasteurianum* do not catalyze this oxidation. The reduced form ferredoxin, a brown protein involved in anaerobic electron transport, is oxidized by the hydrogenases from *C. pasteurianum*.

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and *D. desulfuricans* but not by hydrogenase preparations from *E. coli*. In addition, ferredoxin is not involved in the oxidation of reduced cytochrome C₃ to H₂ in extracts of *D. desulfuricans*. Studies on the fractionation of the enzyme system evolving H₂ from reduced cytochrome C₃ suggest that addition electron transport factors are not involved in this reaction but rather that the differences observed with cytochrome C₃ and ferredoxin are related to the specificities of the respective hydrogenases.
PRIMARY REACTIONS IN BACTERIAL PHOTOSYNTHESIS

R. K. Clayton  Rose P. Feldman

The study of primary photochemical reactions in bacterial chromatophores has been continued. The primary electron acceptor in bacterial photosynthesis has been identified spectrophotometrically as coenzyme Q. Light-induced reactions of coenzyme Q (CoQ), cytochrome (cyt), and bacteriochlorophyll (BChl) are related kinetically in a way consistent with the following reaction sequence:

1. BChl + hv → BChl* 
2. BChl* + CoQ → BChl* + CoQ-
3. BChl* + cyt → BChl + cyt*

According to this model, light affords a primary separation of oxidizing and reducing power in the form of oxidized cytochrome and reduced coenzyme Q.

These reactions take place at a reaction center involving a small fraction of the total BChl in the chromatophore. The bulk of the BChl serves to harvest light energy and transfer it to the reaction center. This has been shown by the following experiments, performed with a carotenoidless mutant of Rhodopseudomonas spheroides. When these cells are incubated anaerobically in the light, about 90% of their BChl is converted to bacteriopheophytin (BPh). Nevertheless, the component of BChl that reacts photo-

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chemically is unaffected, as shown in Table 35. The photochemical reduction of CoQ is also unimpaired in the pheophytinized cells.

Action spectra for the photochemical oxidation of BChl show that light absorbed by BPh is utilized with 50% efficiency, compared with light absorbed by BChl. One-half of the BPh is excreted from the cells as free microcrystals; the other half appears to be retained in the same form within the cells.

It is hoped that an improvement of the conditions for pheophytinization will lead to cells in which the light-gathering (bulk) BChl is lost and only the photochemically reacting ("reaction-center") BChl remains. Primary photochemical reactions can then be observed with greater ease and sensitivity.

### Table 35. Conversion of Bacteriochlorophyll to Pheophytin in \textit{Rhodopseudomonas sphaeroides}, with Preservation of the Light-Reacting Component of Bacteriochlorophyll

<table>
<thead>
<tr>
<th></th>
<th>&quot;Fresh&quot; Cells</th>
<th>Pheophytinized Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BChl, (\mu)moles</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Bacteriopheophytin, (\mu)moles</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>BChl, (\mu)moles, oxidized reversibly upon illumination</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

### THE EFFECT OF NEAR ULTRAVIOLET IRRADIATION ON THE PEROXIDE CONTENT OF SOLUTIONS OF OXIDIZED OR REDUCED DIPHOSPHOPYRIDINE NUCLEOTIDE

M. I. Dolin

Oxidized and reduced pyridine nucleotides are widely used in biochemical assay techniques. The purity and stability of these nucleotides is an important concern since interpretation of experimental results may be markedly influenced by the presence of unknown compounds in the presumably pure nucleotide solutions.

It has been recognized recently that frozen solutions of DPNH may contain inhibitors of pyridine nucleotide-linked dehydrogenases. One such inhibitor is a reduced pyridine nucleotide similar in its spectral properties to DPNH. This report prompted a reinvestigation of previous findings that solutions of DPNH may contain appreciable concentrations of \(H_2O_2\).

With DPNH preparations of the highest purity obtainable, it has been confirmed that significant concentrations of \(H_2O_2\) may be found in both fresh and frozen solutions of the reduced nucleotide. The highest concentration of \(H_2O_2\) (~ \(10^{-4}\) M) was found in frozen solutions that had been stored at \(-20^\circ C\) for 4 days under conditions recommended for preservation of DPNH solutions, that is, at pH 10.5. It

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appears that the peroxide found in fresh solutions does not arise through autoxidation reactions. Photo-oxidation may occur however, since on irradiation of DPNH solutions at 360 mp there is a marked and rapid rise in the H\textsubscript{2}O\textsubscript{2} level.\textsuperscript{5}

Fresh solutions of oxidized DPN, unlike those of the reduced nucleotide, do not contain appreciable concentrations of peroxide. Storage at −20°C of alkaline (pH 10.5–11.5), but not neutral, DPN solutions does, however, result in the accumulation of H\textsubscript{2}O\textsubscript{2}. This result is accompanied by the formation of a highly fluorescent modification product of DPN, which is different from any previously reported. The modification product does not form at 4°C. Whereas the irradiation of DPN solutions at 360 mp does not result in peroxide formation, the irradiation of solutions containing the modification product results in high peroxide concentrations.\textsuperscript{5}

An investigation of the mechanism by which the modification product is synthesized in frozen solutions should contribute to an understanding of the chemical potentialities of the DPN molecule. As a practical matter, since the modification product is approximately 5 times as fluorescent as DPN, the properties of this modification product should be understood so that valid chemical estimates of DPNH by fluorescence can be assured.

There are several modification products formed under the conditions described. In cooperation with K. B. Jacobson, the major fluorescent compound has been isolated in a pure state and its chemical, spectral, and fluorescent properties are currently being investigated.

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AN INTRAMOLECULAR REARRANGEMENT IN THE METHYLMALONYL ISOMERASE REACTION DEMONSTRATED BY MASS ANALYSIS OF SUCCINIC ACID\textsuperscript{6}

E. F. Phares \hspace{1cm} Mary V. Long \hspace{1cm} S. F. Carson

Introduction. — Marston and co-workers\textsuperscript{7} have proposed that the cobamide coenzyme-dependent isomerization of methylmalonyl CoA to succinyl CoA occurs by an intermolecular reaction involving a cyclic dimer, 2,5-dioxo-cyclohexane-1, 4-dicarboxylic acid (succino-succinic acid) (Fig. 29). Bilateral cleavage of such a ring on alternate sides of the carbonyl group would lead to the formation of two molecules of either methylmalonic or succinic acid. These authors suggested that such a mechanism would also explain the glutamic-methyl aspartic isomerization, which likewise requires a cobamide coenzyme;\textsuperscript{8} however, they were unable to label a pool of the proposed intermediate. The same scheme for methylmalonyl isomerase was suggested independently by Hegre et al.\textsuperscript{9} The dimer theory offers an elegant explanation for the two similar isomerization reactions.

We have tested the possibility of this mechanism by the use of an equal mixture of C\textsuperscript{13}-carboxyl- and C\textsuperscript{13}-thioester-labeled methylmalonyl CoA as a substrate for the isomerase. An intermolecular transcarboxylation, as with the dimer mechanism, would label a predictable fraction of the product molecules with two C\textsuperscript{13} atoms, which can be detected by mass spectrometry (of the intact succinate molecule). The mass spectrum of the enzymic succinate has been compared with calculated intermolecular and intramolecular spectra, as well as with that from a model chemical synthetic intermolecular reaction. The results show that the transcarboxylation occurs by intramolecular rather than intermolecular rearrangement.

**Experimental.** – Methylmalonic acid-1-C\textsuperscript{13} was prepared by carbonation of t-buty1 propionate anion with C\textsuperscript{13}O\textsubscript{2} containing 52 at. % excess C\textsuperscript{13}; conversion to the CoA ester resulted in a mixture containing 26% each methylmalonyl-1-C\textsuperscript{13} CoA and methylmalonyl-3-C\textsuperscript{13} CoA, and 48% with no excess C\textsuperscript{13}. This mixture, 56 \textmu moles total, was incubated for 10 min at 30°C with 2 mg of a 10-fold purified methylmalonyl isomerase preparation from Propionibacterium shermanii in the presence of 10\textsuperscript{-4} M adenylcobamide co-enzyme (from Micrococcus lactilyticus) and 0.1 M phosphate, pH 6.8. The final volume was 5 ml. Residual CoA esters were hydrolyzed with 0.01 M alkali, and the succinate product, 18 \textmu moles, was isolated from a Celite partition column by elution with chloroform-butanol.

To test the mass analysis methods, succinic acid 1,4-C\textsuperscript{13} (C\textsubscript{2} \textsuperscript{13}, i.e., a definite fraction of the molecules contained two C\textsuperscript{13} atoms) was synthesized by a chemical method which produced a labeling pattern equivalent to that of the proposed enzymic intermolecular reaction.

Mass analyses were made on a 6-in.-radius spectrometer equipped to use solid samples (of crystalline succinic acid) for either positive or negative ion detection. Positive ion spectra at masses 100 to 103 and negative ion spectra at masses 117 to 119 were obtained from the chemically synthesized C\textsubscript{2} \textsuperscript{13}-succinate. These experimental values agree with the calculated spectra. The negative ion data prove the reliability of the more complicated spectrum of the positive ion.
Results and Discussion. – The experimental results with the enzymically derived succinic acid compare closely with the values calculated for an intramolecular reaction. They are distinctly different from the theoretical and the experimentally found values for the model chemical intermolecular reaction. Hence the cyclic dimer is not an intermediate in the isomerization reaction; some type of intramolecular reaction is involved. Eggerer et al.\textsuperscript{10} proposed an intramolecular rearrangement involving a free-radical intermediate. An additional intramolecular rearrangement, in which a carbanion intermediate is stabilized by cobalt, has been suggested;\textsuperscript{11} here the cobamide coenzyme is called a "biological Grignard reagent." No experimental evidence for these proposed mechanisms is available.

\textsuperscript{10}H. Eggerer et al., J. Am. Chem. Soc. 82, 2643 (1960).
Plant Physiology and Photosynthesis

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| T. J. Long | Helen J. Luippold | bORINS research participant. |
| D. E. Foard | H. A. Roesel | cConsultant. |
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NUCLEAR FUNCTIONS IN GAMMA-PLANTLET ROOTS GROWING WITHOUT DNA SYNTHESIS AND MITOSIS

A. H. Haber  
D. E. Foard

Introduction. — After high doses of gamma-radiation (800 kr) are given to wheat grain, the embryos can germinate and grow without tissue-cell division into small seedlings called "gamma-plantlets." The apparently normal cytoplasmic differentiation\(^1\) might suggest that gamma-plantlet nuclei probably retain some important functions despite inhibition of DNA synthesis and mitosis. This study, with gamma-plantlet roots lacking both DNA synthesis and mitosis, gives more direct evidence for normalcy of certain nuclear functions.

Results. — Absence of DNA synthesis was shown by exposing lower lateral seminal roots of 0-, 1-, 2-, and 3-day-old, intact gamma plantlets to solutions of H\(^3\)-thymidine for 16 hr. In autoradiographs none of the approximately 15,000 cells of cortical parenchyma showed evidence of accumulation of insoluble label. In the same experiment some of the 3-day-old gamma plantlets were put in solutions of H\(^3\)-uridine and fixed after 30 min, 1 hr, and 2 hr. Autoradiographs of cortical parenchyma cells of lower lateral seminal roots in these plants, on the other hand, showed accumulation of H\(^3\) in insoluble material (RNA) after only 30 min in the H\(^3\)-uridine solution. Nuclear RNA synthesis was indicated by a high ratio of nuclear to cytoplasmic label after only 30 min in the H\(^3\)-uridine. Movement of nuclear RNA into the cytoplasm was indicated by a decreasing ratio of nuclear to cytoplasmic label with increasing time in the H\(^3\)-uridine solution.

On the anatomic level there are two striking examples of nuclear differentiation normally occurring during maturation of wheat roots: (1) the nucleus of the trichoblast migrates into the root hair and changes shape, and (2) the nucleus in the mature sieve-tube element disappears. In both these instances of nuclear differentiation, the gamma-plantlets behave in a manner indistinguishable from unirradiated controls.

Discussion. — Nuclear RNA synthesis and the apparently normal nuclear differentiation at the anatomic level in the gamma-plantlet roots indicate that the nucleus has not been completely inactivated by the irradiation, but has lost only some of its functions (e.g., DNA synthesis and mitosis). Although the nuclei still make RNA, we do not imply that the RNA so made is necessarily identical with RNA made in nuclei of unirradiated controls. Apparently normal nuclear and cytoplasmic differentiation in gamma-plantlets strongly suggests, but does not conclusively prove, that the RNA synthesized is functional both in the nucleus and after it has moved into the cytoplasm.

DOES GENETIC INFORMATION SPECIFY ORGAN FORM BY MEANS OF CELL FORMS?

A. H. Haber

Introduction. — The extent to which organ form is determined by genetic specifications of cellular parameters is one of the basic questions of morphogenesis. In this study a comparison has been made of sizes, shapes, and numbers of corresponding types of cells in normal wheat leaves and in gamma-plantlet leaves growing without increase in cell number. Since the first foliage leaf of gamma-plantlets and unirradiated control leaves grow with the same degree of polarization, such a study can be made with leaves of the same size and shape.

Results. — Ten-day-old gamma-plantlet (800 kr) and three-day-old unirradiated control leaves with the same length also have the same shape. The sizes, shapes, and numbers of corresponding epidermal and chlorenchymatous mesophyll cells in these leaves are drastically different, as would be expected since the cell numbers did not increase in gamma-plantlets but increased manyfold in unirradiated controls. It can be shown that if the gamma-plantlet and unirradiated control leaves had grown in such a manner as to assume similar sizes and shapes of corresponding cells, then the leaves themselves would have had significantly different shapes when they attained the same length.

Discussion. — Although organ form can always be described in terms of sizes, shapes, numbers, and arrangements of its constituent cells, this study shows that organ form is not determined by sizes, shapes, numbers, and arrangements of its constituent cells. In other words, organ form is not a dependent variable that is uniquely fixed by the independent variables: cell sizes, cell shapes, extents of cell divisions, and orientations of cell divisions. Thus, although genetic information specifies both organ form and cell forms, it does not specify organ form by means of cell forms. Since the development of the wheat leaf primordium into a seedling leaf proceeds with little relation to the manner in which it is partitioned into cells, we may seriously question whether the individual cells are in fact always units of function in growth and morphogenesis.


GROWTH OF GAMMA-PLANTLET AND UNIRRADIATED EMBRYOS ON SYNTHETIC MEDIA

T. J. Long

Introduction. – Previous studies of seedlings grown from heavily gamma-irradiated grain have shown that they can grow without increase in tissue-cell number and are capable of photosynthesis, protein synthesis, and RNA synthesis. This study with embryos excised from their endosperm reserves compares the growth of gamma-plantlets with the growth of unirradiated controls on chemically defined media when photosynthesis is prevented by darkness.

Results. – In the absence of nitrogen and carbon sources, growth of gamma-plantlets and unirradiated controls is slight. With 15 g/liter glucose in absence of a nitrogen source, both gamma-plantlet and unirradiated embryos grow and double their protein content. The order of decreasing growth on three common nitrogen sources with glucose as carbon source is the same for gamma-plantlets and unirradiated controls: NO₃ > NH₄ > casein hydrolysate ~ no nitrogen. The protein content of such seedlings is only slightly higher when a nitrogen source is added. By contrast, the growth of embryos is very dependent on glucose concentration, with an optimum between 15 and 20 g/liter, irrespective of irradiation. When unirradiated embryos with 15 g/liter glucose are given 5 g/liter galactose, growth is almost completely inhibited in the first foliage leaf, coleoptile, and roots. In gamma-plantlets, addition of 5 g/liter galactose to the 15 g/liter glucose similarly affects the roots but only slightly inhibits coleoptile growth and has little or no effect on the first foliage leaf.

Discussion. – The finding that the early growth of gamma-plantlet and unirradiated embryos is more dependent on carbohydrate than on an external nitrogen supply indicates that the embryo itself contains relatively little carbohydrate. The considerable growth and protein synthesis in absence of an external nitrogen source indicate that the embryo contains considerable quantities of nitrogenous compounds. Since the growth of gamma-plantlets is ultimately, but not immediately, limited by radiation-induced mitotic inhibition, these methods of embryo culture are not adequate for nutritional studies with nitrogen sources. On the other hand, such studies with carbon sources are feasible, as suggested by differential effects of galactose on irradiated and unirradiated plants in presence of optimal concentrations of glucose.

SEPARATION OF "INITIATION" AND "MATURATION" ASPECTS OF CELLULAR DIFFERENTIATION IN ROOT VASCULAR BUNDLES

D. E. Foard  A. H. Haber

Introduction. – The many instances of normal cellular differentiation in gamma-plantlets raise the question: What generalizations can be made concerning cellular differentiation during such growth without cell division? In normally growing roots, immature cells of specialized tissue-cell types are initiated near the apical meristem; progeny of these immature cells mature in orderly linear sequence as they are

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displaced from the root apex during growth. In this study of root vascular bundles, which contain distinctive and easily recognizable cell types, a comparison has been made between such normal cellular differentiation and the differentiation that occurs during growth without cell division in gamma-plantlets.

**Results.** – The nonessentiality of an increase in cell number for cell and tissue maturation in the vascular bundle can be seen by comparing cross sections of gamma-plantlet (800 kr) roots with corresponding sections of the embryo in the unsown grain. Whereas basal sections of gamma-plantlet roots have the full complement of eight mature sieve-tube elements and eight mature protoxylem points, the corresponding section of the root primordium in the embryo of the unsown grain has these cells only in immature form. More-apical sections of gamma-plantlet roots, however, have only one or a few mature sieve-tube elements and only one or a few mature protoxylem points. At these more-apical levels the other sieve-tube elements and protoxylem points are not present, not even as immature cells. In other words, in cross sections of mature gamma-plantlet roots, these vascular elements are either absent or mature. By contrast, in any cross section of unirradiated roots where the full complement of cells of these specialized types is present, the cells are at very similar stages of differentiation.

**Discussion.** – The normal maturation of the immature sieve-tube and protoxylem elements in gamma-plantlets indicates that the cell divisions that normally occur during such maturation are not essential for this aspect of differentiation. The absence of initiation of new cells of these specialized cell types is most simply interpreted as a consequence of the absence of cell division. This interpretation, that absence of cell division prevents initiation of new elements but does not prevent maturation of immature elements, thus explains why in cross sections of mature gamma-plantlet roots these vascular elements are either absent or mature.

**ACTIONS OF LIGHT AND GIBBERELLIN ON COLEOPTILE ELONGATION**

**H. A. Roesel**

**A. H. Haber**

**Introduction.** – Although there have been many studies of effects of light on overall elongation of various organs, little is known concerning effects of light on the pattern of elongation within a given organ, i.e., the contributions of various regions of the organ to the total elongation. This study was performed with wheat seedlings after cell division had ceased in the coleoptiles. Because gibberellins have been implicated in the mechanisms by which light affects elongation, this system has further been used to investigate interactions of light and gibberellin.

**Results.** – Seedlings with straight shoots and well-developed roots were selected, and their coleoptiles were marked into three segments at a time after cell division had ceased; overall elongation and elongation of different segments were recorded after 24 hr. Growth of etiolated coleoptiles ceases first in the basal region and last in the apical region. The growth pattern of light-grown coleoptiles is the opposite: growth first ceases in the apical region and last in the basal region. On the other hand, short exposures of three-day-old etiolated coleoptiles to red, blue, or white light did not essentially

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alter the growth pattern during the next 24 hr, but they did decrease elongation of the basal and increase elongation of the apical regions. Red light, even after long exposures, did not change total coleoptile length, since the decrease in elongation in the basal region was balanced by the increase in elongation in the apical region. Increasing doses of blue or white light decreased total coleoptile elongation despite stimulation of the apical region. De-etiolation effects, whether stimulatory or inhibitory, produced by short exposures to red or blue (uncontaminated with red) light were mutually reversible with far-red radiation.

There are instances in which gibberellin sensitivity can be altered by changing only one of the following three characteristics of the de-etiolating light treatments: intensity, duration, and quality. Gibberellin sensitivity tends to be greater when light treatments of etiolated coleoptiles result in lesser total elongation but in most cases is nearly the same in all segments of an individual coleoptile. Whereas light sometimes promoted and sometimes decreased elongation, gibberellin in these experiments always increased elongation.

Discussion. — This study demonstrates an inadequacy of measuring only total length of an organ in photobiological studies. Thus from measuring only total elongation of coleoptiles de-etiolated with red light, one might have come to the erroneous conclusion that the light treatment had no effect, whereas in fact the red light produced both a stimulatory and an inhibitory effect. Results with elongation in the various regions of etiolated and de-etiolated coleoptiles are consistent with the theory that light mimics aging.6 The opposite growth patterns in light-grown compared against etiolated and de-etiolated coleoptiles, however, suggest that such theories be restricted to de-etiolating light treatments. These results with light effects on an organ growing without cell division are consistent with the suggestion that cell expansion, and not cell division, is the initial site of action in photomorphogenesis.7 The finding that gibberellin promotes elongation irrespective of whether light increases or decreases elongation demonstrates that in this system the regulation of endogenous gibberellin levels is not the mechanism by which light affects elongation.

PHOTOSYNTHESIS

W. A. Arnold W. F. Bertsch

For several years, experiments in this laboratory have been aimed at understanding the initial energy conversion steps of photosynthesis. Evidence has accumulated that a solid-state physical phenomenon is involved in the process by which energy from light quanta is made available for utilization in the biochemical reduction of carbon dioxide to sugars.8 A number of authors have recently presented evidence that two photoreactions are involved in photosynthesis, but the exact nature of these photoreactions remains unclear.9 The present experiments on delayed-light production in living green plants indicate

that the delayed light is emitted by functionally active chlorophyll and that the presence of two pigment systems is reflected in details of the delayed-light emission. Since this delayed light has the characteristics of emission due to untrapping of electrons in a semiconductor, we feel that a more complete understanding of the delayed light might elucidate the way in which the photoreactions are linked together and thereby allow a specific interpretation of the physical events of photosynthesis.

Several authors have recently reported that transients in oxygen production are produced when a plant in the dark is first illuminated. Wavelengths which selectively excite either of the two different photoreactions show differently shaped oxygen transients. Similarly, a shift from one wavelength to the other also produces an oxygen transient. Since we have observed both types of transient in the delayed-light production of plants, it seems certain the delayed light comes from chlorophyll that is functionally involved in photosynthesis.

We are studying in detail the various shaped dark-decay curves of delayed light. The shapes of these decay curves are altered by factors such as wavelength of exciting light, temperature of the cells, time and intensity of irradiation, and availability of oxygen. Under certain sets of conditions the decay becomes extremely complex, even showing an increase in intensity in certain portions. It is clear that several processes are involved in the decay which we observe.

The fact that a saturating dose of 6500 Å light produced a different decay curve from a saturating dose of 7200 Å implies that the two colors of exciting light are not absorbed by the same pigment system. It is known that these two wavelengths selectively excite the two pigment systems, and it might be expected that the two different decay curves would add, if both wavelengths were given simultaneously. On the contrary, we found that simultaneous excitation of the two photoreactions at saturation resulted in a decay which was more or less intermediate between the decay curves from the individual wavelengths. Thus the two photoreactions are linked together, presumably through a chain of enzymatic reactions.

By lowering the temperature of the cells to 5°C, we found the decay of delayed light produced by simultaneous excitation to be nearly additive, as compared to the individual decays produced by each wavelength given separately. Thus the enzymatic link between the two photoreactions appears to function very inefficiently at low temperatures.

We have also found the delayed light excited by the two wavelengths to be additive at very low exciting intensities, these intensities being below the point where measurable photosynthesis takes place. This result probably reflects the presence of photosynthetic units (or groups of cooperating chlorophyll molecules), the existence of which has long been suggested by the observation that the absorption cross section for photosynthesis is the size of several hundred chlorophyll molecules. We interpret the additivity of delayed light produced by the two photoreactions at very low intensities of exciting light as indicating that an individual enzymatic link between the two photoreactions is present for every photosynthetic unit. The additivity at low exciting intensities would then be explained, since there would be a very small probability of both pigment systems in an individual unit undergoing an absorption act within the turnover time of the enzymatic link.
Biophysics

J. S. Kirby-Smith  
J. Jagger  
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Amleto Castellani  
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aData foreign investigator from abroad (Italian Atomic Energy Commission).  
Data research participant assigned to Cell Physiology.  
Data summer student trainee.  
Data loanee.

BIOLOGY DIVISION SPACE RELATED PROGRAMS

J. S. Kirby-Smith

Three more or less independent activities, involving a number of different groups in this laboratory, dealing with space related radiobiological and biological research have been undertaken. The first of these programs, involving the exposure of a number of radiation-sensitive biological test systems in space probes and balloon flights, has not yielded data to date because of failure of the flight vehicles. The other two programs, both laboratory based and dealing with (1) the basic radiobiology of high-energy protons and (2) the biological effects of high-intensity magnetic fields, have been successful in their preliminary phases, and work is continuing.

Effects of High-Energy Protons.—Investigations of the action of high-energy protons on several organisms as well as on chain breakage in DNA have been carried out using particles of approximately 130 Mev from the Harvard Cyclotron. (Introduction of scattering blocks in the primary 160-Mev primary beam from this facility in order to ensure uniformity in beam section was responsible for the 130-Mev rather than the 160-Mev figures.) The irradiations were carried out in collaboration with A. M. Koehler and William Preston of the Harvard University Physics Department. Biological facilities required for the experiments were made available through Dr. Sbarra and St. Margaret's Hospital, Boston, and particularly through the efforts of Dr. Kjellberg and members of Dr. Sweet's staff at the Massachusetts General Hospital. Specific experiments and results to date may be summarized as follows:

1. Chain Breakage in DNA. P<sup>32</sup>-labeled DNA from <i>E. coli</i> was exposed to the protons at a dose level of 50 kr, and chain breakage was determined by measuring the ratio of bound phosphorus to phosphorus liberated from free ends after enzymatic digestion by alkaline phosphatase. The results of this experiment involving two independent samples indicate that the RBE of 130-Mev protons as compared to cobalt gamma rays is 0.7.
2. Radiation Killing of E. coli. Survival curves of E. coli, with and without the addition of the protective compound cysteamine, were obtained over dose ranges up to 100 kr. In both systems the survival curves indicate an RBE for bacterial killing of approximately 0.65 as compared to a value of 1 for 250-kvp x-rays.

3. Radiation Effects on the Germinal Epithilium in the Male and in Oocytes in the Female Mouse. Irradiation of mouse testes and ovaries with the 130-Mev proton beam was carried out in the dose range 5 to 200 rad. X-ray control experiments were also carried out at the Massachusetts General Hospital. Counts of the normal spermatogia remaining in the male testes 72 hr after irradiation indicate an RBE of 0.8 for protons compared with a value of 1 for 250-kvp x-rays.

4. Chromosomal Aberration in Human Peripheral Blood. Several samples of human peripheral blood were exposed to high-energy protons. Although preliminary examination of this material shows the presence of chromosomal aberration, this experiment should be repeated under more ideal conditions.

**Proton Studies for the Immediate Future.**—From predictions based on the LET of 130-Mev protons, one would expect an RBE more closely approximating 1 than the figures obtained in the obtained experiments, which range in RBE from 0.65 to 0.8. Two physical factors exist, however, which may have some bearing on this question: (1) the high dose rates existing in the individual proton pulses and (2) a possible systematic difference in the value of the rad as measured by the Harvard ion chambers. With the development of ion chambers at ORNL by the Neutron Shielding Group and the Instrument Division, it is expected that this latter question will be resolved in the near future, that is, when these chambers are intercompared with the existing Harvard equipment. A repeat of the DNA chain-breakage experiment is also planned for late August. Further duplications of the more biological experiments, in particular the chromosomal breakage studies in human peripheral blood, are projected for late September or early October using the Harvard Machine; they are to be followed, if possible, with similar experiments at higher energy when suitable facilities are available.

Experimental work with protons is expected to begin in Oak Ridge in the near future, as soon as the external beam from the 22-Mev, 86-in. cyclotron is in operation. Work with the 80-Mev ORIC protons, using the biological systems above, as well as studies of the pathological and physiological effects of these particles in mice will begin as soon as the facility is available. Current estimates for this date are in December 1962.

**Preliminary Exposures of Biological Material to an Intense Magnetic Field.**—Preliminary exposures of two kinds of biological material, virus and mice, have been made in an intense constant magnetic field in cooperation with personnel of the Electronuclear Division, utilizing their high-flux solenoid.

In the virus experiments, vaccinia and cocksackie B-3 organisms were exposed for about 6 hr to a field strength of approximately 55 to 60 kg, the range representing the field gradient. No effect was noted on the ability to grow and infect the host cell cultures.

In the mouse experiments, 6 male and 6 pregnant female mice were exposed for about 5 hr each, 2 mice per exposure. The field gradient from head to tail was about 6 kg. Control mice were maintained in similar positions outside the magnet in each experiment. The male mice were subsequently mated to normal females. First and subsequent generations of all these mice are being observed for alterations in litter size and physical abnormalities. Preliminary counts show some apparent decrease in litter size from normal unexposed females mated to irradiated males; however, it must be emphasized
that these results are incomplete and fragmentary at present. Further experiments using bacteria as the test system with particular emphasis on studies of the production and release of bacterial phage from lysogenic bacteria are scheduled for the latter part of August 1962, with studies involving irradiated Tradescantia pollen and inflorescences to be scheduled later.

GENERAL ASPECTS OF THE MECHANISM OF PHOTOPROTECTION IN BACTERIA

John Jagger W. C. Wise R. S. Stafford

Introduction. — Photoprotection (PP) is the prevention of far ultraviolet radiation (uv) effects in a biological system by prior irradiation at longer wavelengths. Our previous work has shown that the early events in PP are purely photochemical\(^1\) and that the action spectrum for PP from killing in bacteria lies entirely in the near ultraviolet.\(^2\) Now that the phenomenon is quite well characterized, we are trying to learn what sort of mechanism is involved.

Results and Discussion. — We have made complexes of phage T2 and Escherichia coli B and have irradiated these with PP radiation followed by uv. Under our conditions host cells alone showed excellent PP, but phage survival showed none. The experiments involved differing doses of PP radiation and uv, and phage development was stopped either by cyanide treatment or by cooling to 3°C. This result, combined with previous indications in the literature, suggests that viruses cannot be photoprotected. In cooperation with Jane Setlow, we attempted to photoprotect purified transforming deoxyribonucleic acid (DNA) that can endow cells of Haemophilus influenzae with heritable streptomycin resistance. During all irradiations, the DNA was in phosphate buffer. No PP of transforming activity was found. However, Setlow has preliminary evidence of a small PP of this DNA in the presence of crude yeast extract containing the photoreactivation enzyme.

Thus the evidence to date indicates that a direct reversal of uv damage to DNA is probably a quite minor part of the phenomenon observed in cells. It seems more likely that some sort of indirect effect is the major mechanism.

Cells of E. coli B held in a liquid medium for several hours between UV irradiation and plating exhibit a “holding restoration.”\(^3\) The similarity of the curves for this effect to those for PP, as well as the fact that neither works on strain B/r, led us to examine overlap in the two phenomena. It was found that, for stationary and log-phase cells, there exists a definite amount of modification of uv damage that can be achieved either by holding restoration or by PP, or by any combination of the two. We conclude that these two treatments act upon the same uv damage and that their mechanisms may therefore well have something in common. It is known that holding restoration induces a division delay of the cells after plating.\(^4\) One might suppose that PP radiation also slows division after plating and thus permits a similar restoration to take place. In agreement with this supposition are the following observations we have

\(^1\)J. Jagger, *Radiation Research* 13, 521 (1960).
made on *E. coli* B: (a) PP radiation induces a division delay on nutrient agar plates, (b) PP radiation induces a similar division delay and growth delay in nutrient broth, and (c) a preliminary action spectrum for growth delay in broth shows a close similarity to the action spectrum for PP. It thus seems probable that PP operates by inducing a division delay in the cells after the uv treatment.

We have found that irradiation of cells of *E. coli* B with PP radiation during growth in a manometric respirometer causes a large and almost immediate depression of the rate of oxygen uptake. Also, the action spectrum for PP, after correction for killing by the PP radiation alone, fits fairly well the absorption spectrum of reduced nicotinamide-adenine dinucleotide\(^2\) (NAD\(\text{H}_2\), formerly called DPHN). These results suggest that PP in *E. coli* B may produce its division delay by inhibiting the respiratory system. It is of interest that the only other bacterium we have found to show striking PP, *Pseudomonas aeruginosa*, is an obligate aerobe.

**FIRST COLLISION DOSE RATES PRODUCED BY FAST-NEUTRON FLUXES OF WIDE ENERGY DISTRIBUTION**

M. L. Randolph

**Introduction.** — Although provisions are commonly made for measurement of the neutron flux produced by fast-neutron facilities, they are seldom made for measurement of the absorbed dose in tissue. The ratio\(^5\) of dose rate to fast-neutron flux is available for monoenergetic neutrons but not for continuous distributions of neutron energies. Here we sketch the calculation of this ratio and give some typical results.

**Method.** The first collision dose rate per unit flux \(f(E)\) for monoenergetic neutrons of energy \(E\) is

\[
f(E) = \sum_i \sum_j n_i \sigma_{ij} \epsilon_{ij},
\]

where \(n_i\) is the concentration of nuclei of isotope \(i\), \(\sigma_{ij}\) is the probability of nuclear interaction \(j\) of a neutron with a nucleus of isotope \(i\), and \(\epsilon_{ij}\) is the kinetic energy of resulting ionizing particles. Although \(f(E)\) strictly bears no simple analytical dependence on \(E\), our method rests on the observation that, for neutron energies \(0.03 \leq E \leq 10\) Mev, \(f(E)\) can be approximated adequately by

\[
f(E) = A + B\sqrt{E} + CE,
\]

where \(A\), \(B\), and \(C\) are empirically determined constants. For irradiation by a neutron distribution, \(N(E)\) neutrons having energy \(E\), the ratio of dose rate to flux is

\[
F = \int_0^\infty N(E) f(E) dE/\int_0^\infty N(E) dE.
\]

Often the limits of integration may be narrowed to the range wherein eq. (2) may be applied. Then, using standard mathematical devices, we have made our calculations for different classes and for examples of fast-neutron spectra.

Results. — Examples of our specific values of the dose rate to flux value in tissue and bone are given in Table 36 for various kinds of neutron spectra. The dose rates in other typical hydrogenous materials are found to be directly related to those in tissue. For the cases investigated and bone composition assumed, the ratio of dose in bone to that in tissue was found to be 0.45. A fast-neutron dose rate of 10 to 20 mrem/day in the upper atmosphere was estimated.

Conclusion. — The details of a method for calculating first collision dose in hydrogenous materials from total fast-neutron flux of five kinds of wide distributions have been worked out, and about 200 specific cases were calculated. The method makes immediately available reasonably reliable biological dosimetry at various fast-neutron facilities where the neutron flux and spectral distribution are known.

Table 36. Values of the Ratio, $F_1$, of the Dose Rate in Tissue to Neutron Flux for Several Neutron Spectra

<table>
<thead>
<tr>
<th>Neutron Spectrum</th>
<th>Source of Nuclear Data</th>
<th>$F_1$ (rads sec $10^9$ neutrons per cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>In Tissue$^a$</td>
</tr>
<tr>
<td>Thermal neutron fission of $^{235}\text{U}$</td>
<td>Cranberg et al.$^b$</td>
<td>2.71</td>
</tr>
<tr>
<td>$^{109}\text{Ag}(p,n)\text{Cd}^{109}$</td>
<td>Cranberg et al.$^b$</td>
<td>2.75</td>
</tr>
<tr>
<td>$^{9}\text{Be}(d,n)\text{B}^{10}$, at $E_d = 1.6$ Mev, $^0\text{O}$</td>
<td>Bonner$^c$</td>
<td>2.19</td>
</tr>
<tr>
<td>Class of Spectrum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxwellian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaporation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multigroup (Each assumed Gaussian)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper atmosphere (100 km)</td>
<td>Hess et al.$^e$</td>
<td>3.24</td>
</tr>
</tbody>
</table>

$^a$Composition given in Reference 1 assumed.

ELECTRON SPIN RESONANCES PRODUCED BY ULTRAVIOLET IRRADIATION OF AMINO ACIDS AND PROTEINS

M. L. Randolph

Introduction. — Our attempt to understand the nature and kinetics of radiation-induced electron spin resonances (ESR) in dry biochemicals has been extended to a comparison of studies on the effects of ultraviolet irradiation at low temperatures versus ionizing radiation at room temperature. Our new data are a direct continuation of previously reported work.$^6$

Methods. — Polycrystalline amino acid samples in air were irradiated within the ESR cavity by ultraviolet (uv) light, while the samples were cooled by a flow of nitrogen gas near its boiling point. The uv intensity produced by a Phillips 500-watt high-pressure mercury arc lamp with filtration against visible light was measured by a thermocouple within a sample tube as about 1100 erg mm$^{-2}$ sec$^{-1}$. Of this about 10% was above 3700 A. Generally a 5-gauss modulation was employed, although this could seriously overmodulate lines less than 2 gauss wide.

Results and Discussion. — As expected, the yield of resonances produced per unit incident energy by uv seems less than that for ionizing radiations. In general, the lifetimes of these free radicals, even at room temperature, were of the order of hours. For bovine serum albumin, which seemed the most sensitive of the materials investigated, the energy expended per resonance seen was estimated to be 300 to 7000 ev. Approximate relative yields for other materials are indicated in Table 37. The yield values must still be regarded as preliminary because of the oxygen present during these measurements and possibilities of power saturation and overmodulation. Furthermore, for our values to hold, the irradiation of samples which are thick compared to the penetration of uv requires a linear dose curve to very high doses in the surface layer. For glycyl-glycine irradiated with uv, more rapid decay and singlet rather than doublet absorption suggest that uv may produce predominately a resonance not noticed with ionizing radiation. The observations that more resonances are produced in proteins or conjugated amino acid than in simple amino acids suggest such hypotheses as: (1) bonds between amino acids may be very likely sites for unpaired electrons; (2) amino acid compounds may better store and transfer energy to yield unpaired electrons than simple amino acids; and (3) radical lifetimes may be much longer in the compounds.

Table 37. Approximate Relative Yields of Free Radicals Produced by a Wide Spectrum of Ultraviolet Light

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative Number of Radicals Produced at Saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine serum albumin</td>
<td>30</td>
</tr>
<tr>
<td>Glycyl glycine</td>
<td>10</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>7</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>$\leq 2$</td>
</tr>
<tr>
<td>Cystine</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>$&lt; 2$</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Salmine should be at some high position in this list.
RADIATION EFFECTS ON "PRIMER" DNA – NEAREST-NEIGHBOR FREQUENCIES

R. B. Setlow W. L. Carrier F. J. Bollum

Normal and ultraviolet irradiated DNA's of several species have been used as primers for DNA synthesis using calf-thymus polymerase (fraction E)\(^7\), and the frequencies of the "nearest neighbors," \(pXpY\), have been determined in the product DNA. A careful statistical analysis has not been completed, but it is apparent that the nearest-neighbor frequencies in the products of normal primer DNA are similar to those obtained by Josse et al.\(^8\) using an E. coli polymerase system. Particularly noticeable are infrequent sequences such as \(pCpG\) in calf thymus DNA, originally observed by Sinsheimer\(^9\) using direct chemical analysis.

The uv irradiation of DNA results in a decrease in primer activity\(^10\) – a decrease that seems to be associated, in large part, with the photochemical dimerization of adjacent thymine residues in polynucleotide primers. If such dimers, \(-pTpT-\), act as blocks to polymerization, there should be fewer complementary sequences, \(pApA\), in the product made using irradiated primer than in the product of normal DNA. The anticipated drop in \(pApA\) sequences is observed for \(pTpT\) sequences. The decrease in \(pApA\) is counterbalanced not by an increase in all other sequences but by increases in sequences of the type \(pGpX\).

M. lysodeikticus DNA has very few \(pTpT\) sequences, is relatively resistant to uv inactivation, and shows no loss in \(pApA\) sequences in product DNA. It is hoped that a complete analysis of the dinucleotide frequencies will provide a clue to the base composition of the uv lesions in this type of DNA.

EVIDENCE THAT UV-INDUCED THYMINE DIMERS IN DNA CAUSE BIOLOGICAL DAMAGE

R. B. Setlow J. K. Setlow

Ultraviolet light is known to dimerize adjacent thymine residues in polynucleotide chains. We have obtained evidence that the formation of such dimers results in the biological inactivation of transforming DNA of H. influenzae.\(^11\) The principle of the experimental method used is that dimerization is a photochemically reversible reaction, \(-pTpT- \underset{\text{hv}}{\xrightarrow{\text{rev}}} -pTpT-\), of known wavelength dependence.\(^12,13\) The steady state is far to the right for 2800 A and to the left for 2390 A. Thus a DNA irradiated with a large dose of 2800 A followed by 2390 A will have fewer dimers than one irradiated with 2800 A alone.

If thymine dimers lead to biological inactivation, transforming DNA inactivated by 2800 A should be reactivated by a subsequent 2390-A irradiation; it is. The kinetics and wavelength dependence of this

\(^7\)F. J. Bollum, G. E. Houts, and P. A. Williams, this report.
ultraviolet reactivation are the same as those for the splitting of thymine dimers. The magnitude of the reactivation indicates that, at large doses of 2800 Å, between 50 and 70% of the biological inactivation arises from the production of thymine dimers, and one inactivating "hit" at high doses is the equivalent of the production of a dimer every 160 nucleotides.

**DELAYED EFFECT OF OXYGEN ON THE PHOTODYNAMIC INHIBITION OF LYSOZYME**

Amleto Castellani  J. S. Kirby-Smith

**Introduction.**—It is well known that visible light in the presence of a photosensitizer may produce profound changes in biological material. Both light alone and the photosensitized substance in the dark are inactive as well as oxygen alone; only their combined action will give rise to the so-called photodynamic effect. In order to explain the mechanism of action of this effect, we have to consider at least three steps: (1) formation of a complex between photosensitizer and the substrate, (2) absorption of energy by the sensitizer and internal energy transfer in the photosensitizer-substrate complex, and (3) interaction with oxygen. Our current studies have been concerned mainly with this last step in the complete photodynamic system as well as with investigations by ESR techniques of the influence of oxygen on light-induced and naturally occurring free radicals in the dry photosensitizer. Lysozyme was used as substrate, and Hæmatoporphyrin (Hp) hydrochloride in phosphate buffer pH 7.5 as photosensitizing substance. The radiation used was monochromatic light of wavelength 4047 Å. Irradiations were performed in the presence and in the absence of air as well as at different oxygen pressures.

**Results and Discussion.**—The lysozyme, irradiated in the presence of Hp and oxygen, shows a decrease in its enzymatic activity. This inhibition is an exponential function of the irradiation time. If the oxygen is removed before the irradiation, the inhibition is very strongly decreased. Theoretically, no inhibition would take place if all the oxygen were removed. The small inhibition, which takes place also after the removal of oxygen, would be due to some traces of oxygen which are still present. If the oxygen is added to a sample irradiated in vacuo (up to 30 min of delay), the inhibition of the enzyme begins to take place, although the effect is not as great as that obtained when the oxygen is initially present during the irradiation. These results indicate the formation in the presence of oxygen or in vacuo of long-lived excited states which can either interact immediately with the oxygen present during irradiation or with oxygen admitted considerably later.

Electron spin resonance studies of the dry Hp were carried out in the solid state. These spectra of Hp (free base) show two different signals, one narrow and the other very broad. The narrow signal was found to increase upon exposure to radiation from a high-pressure mercury arc in quartz as shown in Fig. 30. The decay of this signal is very slow (20% in 5 days). The response of Hp to various experimental conditions is shown in Fig. 31.

Currently our experimental results may be interpreted and formalized by the following relations:

1. Inhibition studies,

   \[ \text{Lys + Hp + O}_2 + \nu \longrightarrow \text{Lys-}(\text{HpO}_2)_m \text{ (Inhibited form of lysozyme)}, \]  
   \[ \text{Lys + Hp + } \nu \longrightarrow \text{Lys + Hp}^* \text{ (no inhibition)}, \]  
   \[ \text{Lys + Hp + } \nu \longrightarrow \text{Lys + Hp}^* \text{ (no inhibition)}; \]  
   \[ \text{Lys + Hp}^* + \text{O}_2 \longrightarrow \text{Ly(HpO}_2)_m. \]
Fig. 30. Increase upon Exposure to Radiation from a High-Pressure Mercury Arc in Quartz.

2. ESR studies on HP in solid state,

\[ \text{HP} + h\nu \longrightarrow \text{Hp}^* \text{ (excited state no signal),} \]  
\[ \text{Hp}^* + \text{O}_2 \longrightarrow \text{HpO}_2^* \text{ (paramagnetic material, free radical),} \]  
\[ \text{Hp} + h\nu + \text{O}_2 \longrightarrow \text{HpO}_2^* \text{ (large increase of signal).} \]

On this basis we relate the ESR experiment to the inhibition studies,

\[ m \text{ HpO}_2^* + \text{Ly} \longrightarrow \text{Ly(HpO}_2^*)_m \nu \text{ (large increase of signal).} \]

Studies are in progress to determine the exact nature of the Ly(HpO_2)_m complex. Projected ESR studies of HP m solution will be required to further clarify these hypothetical mechanisms.
NMR SPECTRA OF PROTEINS

R. H. Dinius  R. R. Becker  H. G. Jones

Introduction. – The proton resonance spectra observed in proteins are characterized by broad overlapping bands which have limited, to some extent, the application of NMR techniques to these compounds. In urea solution, however, the protein spectra are sharpened and are changed in shape. These results have been explained in terms of a loosening of the tertiary structure of the molecule, with resultant gain in configurational freedom, but raise the additional special questions concerning the NMR spectra of protein: (1) are the envelopes of the spectra a multitude of narrow lines or are they indeed broad, single lines? and (2) if the interpretation of the urea experiments is correct, what is the extent of configurational freedom that might be induced on heating? The first question has been approached by carrying out order of magnitude saturation experiments, the second by studying temperature effects in a heated NMR probe.

Results and Discussion. – The proteins studied were ribonuclease and chymotrypsin, and the spectra were obtained with the spectrometer system described previously. Protein samples were dried over

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P₂O₅ for five days prior to dissolving in 99.7% D₂O. The side band technique was used to determine frequencies, and the imposed oscillation was measured with an electronic frequency counter. The broad spectra envelope of ribonuclease and chymotrypsin was examined by the saturation technique. By this method one may determine whether a broad line is due to a multitude of narrow lines or is truly a very broad line. It was not possible to "eat a hole" of narrow width in any of the broad bands. We conclude that these envelopes are, indeed, very broad lines and not a multitude of closely spaced narrow lines. This implies that the relaxation processes responsible for the line widths are active uniformly throughout the protein molecule. This is basically consistent with currently proposed models of the protein molecule although not to be expected entirely.

The NMR spectra of ribonuclease and chymotrypsin have been recorded throughout a wide temperature range (4 to 77°C). Both proteins demonstrate rather similar behavior, although the temperature at which changes take place differs markedly. Both showed a steady increase in band width with increasing temperature until, in a narrow temperature interval, the broad spectra envelope broke apart and was resolved into several bands of varying broadness. The transitions occur in a rather narrow temperature interval (2 to 4°C) and are reversible with temperature if the coagulation point of the protein is not exceeded.

These spectral changes seem to indicate that the broad envelope observed at room temperature is possibly due equally to the configurational interactions and to spin coupling with the nitrogen nuclei. This would seem to imply that the hydrogen bonds of the protein molecule distribute these relaxation couplings throughout the molecule and thus have a greater coupling action than heretofore expected. The work is continuing with the study of the temperature dependence of the NMR spectra of polypeptidyl derivatives of ribonuclease and chymotrypsin. It is hoped that Arrhenius plots of the spectral changes will yield energies for the structural changes involved. It would be worthwhile to carry out spin decoupling energies of the various types of protons to the nitrogen nuclei.

**ESR STUDIES OF ORGANIC PHOTOSENSITIZERS**

L. P. Simpson, J. S. Kirby-Smith, M. L. Randolph

**Introduction.** – The ESR studies on fluorescent dyes reported previously have been extended; experimental emphasis has been on methylene blue. The purpose of these studies is to gain insight into the mechanisms of excitation by visible light and energy transfer in organic photosensitizers, with the hope of ultimately elucidating the physicochemical basis of photodynamic action.

Previous work has shown that the dark signals in polycrystalline and aqueous MB were not saturated at the usual power levels. It was also shown that illumination of colorless MB leucobase caused a huge increase of the signal together with a photooxidation of the leucobase into the blue form. Several experiments have shown that visible light could induce signals in Mb and riboflavin adsorbed on

silica in vacuo. Oxygen reversibly quenched the light-induced signals, with the line width remaining constant. This indicates that the oxygen probably forms a quasi-chemical bond with the unpaired spin.\textsuperscript{19,20}

Results and Discussion. — Curie law dependence held for the dark signal in the purified polycrystalline dye between 153 and \( -189^\circ C \), the dependence being completely reversible. Heating to 271 and then to 315\(^\circ C \) caused the signal to change from 10 to 138 to 45. This corresponded to the melting and decomposition of MB.

Electron spin resonance spectra from a single crystal of MB showed no hyperfine structure, although changing the crystal orientation in the magnet field caused the signal to change in size and shape. ESR signals were not seen in frozen MB aqueous solutions in the presence of air.

The known structures of the dyes examined do not include unpaired electrons; therefore it is of primary importance to show that the resonances are not due to impurities. The occurrence of the dark signal in recrystallized MB and in a single crystal, the change of the signal with the orientation of the crystal, and the correlation of the reappearance of the signal in aqueous solution with the reappearance of the blue dye all provided evidence in this regard.

The reversible quenching effect of oxygen and the lack of hyperfine structure in the crystal indicate that the unpaired electrons are highly delocalized. However, the fact that the signal appears at all in solution means that it is not due to such delocalization alone. The Curie law dependence shows that the dark signal is not due to conduction band electrons. Finally, the signal widths and \( g \)-values indicate that the resonances are due to free radicals.

The appearance of ESR absorptions in organic dyes seems to be a quite general phenomenon\textsuperscript{21,22} which has implications for the existence of one common mechanism for dye-sensitized photooxidations. Our results give a preliminary indication that the dye molecule is excited by light to form a free radical which then can form a low-energy bond with oxygen. Presumably, the oxygen would then be transferred to oxidize the substrate.

In conclusion, it must be emphasized that the molecular nature of the observed signals, dark- and light-induced, is not understood, although evidence indicates that the MB molecules have the unpaired electrons in their outer orbitals.

**UV-INDUCED CROSS-LINKING OF DNA MEASURED BY REVERSIBLE DENATURATION**

D. L. Drake

Introduction. — Cross-links between complementary strands of DNA have been produced by uv irradiation\textsuperscript{23} and by chemical means.\textsuperscript{24} The cross-linked molecules have been detected by density-gradient centrifugation. This procedure is cumbersome; a quicker method would be useful.

\textsuperscript{20} This work was done at Princeton University under H. F. Blum and J. Turkevich.
\textsuperscript{21} Chernyakovskii, Kalmanson and Blyumenfeld, *Optics and Spectroscopy*, LX, 414 (1960).
Geiduschek\textsuperscript{24} has used absorbance measurements to show that thermal denaturation of DNA in which cross-linkage has been produced chemically is reversible by quick cooling, whereas denaturation of uncross-linked DNA is not. In the work described here this method was applied to determine uv-produced cross-links.

**Results and Discussion.** — The irreversible denaturation of a sample of DNA was taken to be proportional to the percentage increase in absorbance at 2600 A after heating to 95°C and quick cooling; this increase was greater in unirradiated than in irradiated samples. The ratio of the absorbance increase in an irradiated sample to that in an unirradiated sample was used to measure the amount of uncross-linked DNA. The ratio was dose-dependent and decreased approximately exponentially. The sensitivity of calf thymus DNA to cross-linking at 2600 A was approximately $2 \times 10^{-5}\text{mm}^2/\text{erg}$; the sensitivity of a sonically degraded sample of one-sixteenth the molecular weight was about $1 \times 10^{-5}\text{mm}^2/\text{erg}$.

To test whether thymine dimerization is responsible for cross-linking, samples were irradiated at 2800 A and then at 2390 A; irradiation at the latter wavelength tends to break thymine dimers. If thymine dimerization is significant in cross-linking, such a sample should show a larger absorbance increase than would a sample irradiated only at 2800 A. In fact, the observed increase was slightly smaller.

The cross-section was largest at 2600 A, decreasing by a factor between 3 and 4 at 2300 and 2900 A. Cross sections were reproducible only to ±30%, but agreed, within this error, with those found by density-gradient centrifugation.\textsuperscript{25}

### NMR SPECTRA OF POLYPEPTIDYL PROTEINS

**F. C. Scaduto\textsuperscript{26} H. G. Jones R. R. Becker**

**Introduction.** — Previous experiments\textsuperscript{27,28} on NMR of polypeptidyl proteins have been concerned with correlations of the broadness of the spectra with the configurational freedom of the various residues present in the proteins. The work described here is a study of the spectra of poly-L-valyl ribonuclease to determine whether the positions of the various peaks are significantly shifted in the protein derivative as compared to the native.

**Materials and Methods.** The compounds used and the techniques applied have been described,\textsuperscript{28} with the exception that 1% tetramethyl silane in carbon tetrachloride was used as a reference in the determination of the $\tau$ values.\textsuperscript{29}

**Results and Discussion.** — Table 38 summarizes the results in the form of $\tau$ values for ribonuclease and the poly-L-valyl derivative in the presence and absence of urea. The peak assignments\textsuperscript{29} are: (1) methyl hydrogens of valine, isoleucine, and leucine; (2) methyl, methylene, and methine hydrogens of isoleucine, methyl hydrogens of alanine and threonine, methylene hydrogens of leucine, lysine, and methine hydrogen of leucine; (3) methyl hydrogens of methionine and methylene hydrogens of proline

\textsuperscript{25} V. Glisin, unpublished results.
\textsuperscript{26} From Cell Physiology group.
Table 38. NMR Peak Positions (τ Values)\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>RNase</th>
<th>Poly-L-valyl RNase</th>
<th>RNase + Urea</th>
<th>Poly-L-valyl RNase + Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.0</td>
<td>9.0</td>
<td>9.1</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>8.4</td>
<td>8.5</td>
<td>8.4</td>
<td>8.4</td>
</tr>
<tr>
<td>3</td>
<td>7.0</td>
<td>7.9</td>
<td>7.9</td>
<td>7.9</td>
</tr>
<tr>
<td>4</td>
<td>5.6</td>
<td>5.8</td>
<td>6.1</td>
<td>5.8</td>
</tr>
<tr>
<td>5</td>
<td>3.1</td>
<td>3.2</td>
<td>3 (split)</td>
<td>3.1 (split)</td>
</tr>
<tr>
<td>H₂O:</td>
<td>5.3</td>
<td>5.4</td>
<td>5.3</td>
<td>5.4</td>
</tr>
<tr>
<td>Urea:</td>
<td></td>
<td></td>
<td>4.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Using 1\% (CH₃)₄Si in CCl₄ as reference.

and arginine; (4) methylene hydrogens in which the carbon atom is adjacent to a nitrogen, an oxygen, a sulfur atom, a carboxyl group, or an aromatic ring; (5) hydrogens alpha to a carboxyl group in valine, histidine, leucine, alanine, methionine, lysine, glutamic acid; phenylalanine, tyrosine, aspartic acid, arginine, proline, serine, and theonine, and (6) ring hydrogens of tyrosine, phenylalanine, and one histidine ring hydrogen.

The only unusual observation is the appearance of a peak in poly-L-valyl ribonuclease at 7.9 τ. This peak was observed by Kowalsky in ribonuclease in the presence of 8 M urea and was assigned to the methyl group of methionine. It is possible that the introduction of the valyl residues has produced an increase in the configuration freedom of methionine residues; however, it is difficult to understand the reasons for it.
Tissue Culture

M. A. Bender  P. E. Eide
G. Marin  F. M. Faulcon
P. C. Gooch  

<sup>a</sup>Visiting investigator from abroad (Italian Atomic Energy Agency).

**ANALYSIS OF LEUKOCYTE CHROMOSOME ABERRATIONS IN HUMANS SHORTLY AFTER IRRADIATION**

M. A Bender  P. C. Gooch

**Introduction.** — Analyses have been made of chromosome aberration levels induced in the peripheral leukocytes of four men irradiated in a nuclear excursion. The studies were undertaken because our knowledge of the radiosensitivity of human somatic chromosomes has been derived previously entirely from measurements made *in vitro* with various cell culture systems. The present cases provided our first opportunity to check our *in vitro* observations<sup>1,2</sup> against material irradiated *in vivo*.

**Material.** — Peripheral blood samples were obtained from four irradiated men 4 hr after the criticality had occurred, and periodically thereafter from three of them. The doses for these three men were estimated to be 19, 43, and 110 rem of whole-body exposure, using an arbitrary RBE of 2 for the neutron component. In the high-dose case the irradiation was not homogeneous over the whole body. The dose estimated for the fourth man, who was only sampled once, was 1.4 rem. Leukocyte cultures and chromosome preparations were made by our standard methods.<sup>2</sup>

**Results and Discussion.** — Samples obtained at 4 hr, 2 weeks, and 4 weeks have been scored to date. Table 39 shows the results. Scoring for the individual receiving 1.4 rem is not yet complete; so far we have seen no effect of this low dose on his chromosomes. The other men show significant levels of chromosomal aberration. We have examined numerous unirradiated control individuals, and whereas chromatid type aberrations arise spontaneously in culture,<sup>2,3</sup> chromosome type aberrations rarely, if ever, occur.


Since the data for the first four weeks show no significant, or even consistent, differences between samples from the same man, we have pooled the data for each case in order to make estimates of the effective dose received and of the aberration rates induced. Table 40 gives the results. Since dose may be estimated either from deletion frequency or from dicentric frequency, both methods were used. The expected aberration frequencies are based on coefficients of aberration production derived from experiments in which whole, fresh, human blood was irradiated with hard x rays. No allowance for the RBE of the neutron component of the dose was made.

Table 39. Chromosomal Aberrations in the Blood of Men Irradiated in a Criticality Accident

<table>
<thead>
<tr>
<th>Case</th>
<th>Estimated Time of Sampling</th>
<th>Cells Scored</th>
<th>Chromatid Aberrations</th>
<th>Chromosome Type Aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2n ≠ 46</td>
</tr>
<tr>
<td>W</td>
<td>4 hr</td>
<td>100</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>19 ± 6.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>100</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>100</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4 hr</td>
<td>100</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>L</td>
<td>2 weeks</td>
<td>100</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>43 ± 8.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>100</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>100</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4 hr</td>
<td>100</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>A</td>
<td>110 ± 14.0</td>
<td>2 weeks</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43 ± 8.5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1.4 ± 0.14</td>
<td>4 hr</td>
<td>37</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 40. Aberration Rates and Dose Estimates for Men Involved in a Criticality Accident

<table>
<thead>
<tr>
<th>Individual</th>
<th>Physical Estimate (rem)</th>
<th>Deletion Frequency</th>
<th>Dose Estimate from Deletions (rem)</th>
<th>Dicentric Frequency</th>
<th>Dose Estimate from Dicentrics (rem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>19 ± 6.7</td>
<td>0.006</td>
<td>52.3</td>
<td>0.003</td>
<td>25.2</td>
</tr>
<tr>
<td>L</td>
<td>43 ± 8.5</td>
<td>0.093</td>
<td>82.3</td>
<td>0.010</td>
<td>43.8</td>
</tr>
<tr>
<td>A</td>
<td>110 ± 14.0</td>
<td>0.080</td>
<td>70.4</td>
<td>0.033</td>
<td>80.9</td>
</tr>
</tbody>
</table>

*Biological estimates based on pooled data for the first four weeks.

Using an arbitrary RBE of 2.

From \(Y = 0.0025 + 0.0011D\); \(D = (Y - 0.0025)/0.0011\).

From \(Y/0.52 \times 10^{-5} = D\).
The results of these calculations are in remarkably good agreement with the results of *in vitro* experiments. The dose estimates derived from the aberration frequencies are quite close to the physical dose estimates. The greatest deviation occurs in case "A." This may be possibly due to the inhomogeneous dose received.

**REPRODUCTIVE DEATH IN HE LA S3 CELLS AFTER INCORPORATION OF H3-LABELED THYMIDINE**

G. Marin M. A. Bender

**Introduction and Methods.** – To determine whether the survival kinetics of mammalian cells which have incorporated H3-thymidine are in agreement with the expectation that cell killing is due only to intracellular irradiation from the beta-decay of tritium, we have exposed single HeLa S3 cells for 15 hr to various levels of labeled precursor. The survival of these cells was measured by the fraction that had developed into macroscopic colonies after 12 days. The degree of labeling was measured by grain counting on liquid-emulsion autoradiographs. Since preliminary experiments had shown that the uptake of thymidine was not a linear function of its concentration in the medium, the cells were exposed to a constant concentration of precursor, and the dose was varied by changing the specific activity – under these conditions the uptake of label becomes a linear function of the activity in the medium.

**Results and Discussion.** – The survivals from three separate experiments are shown in Fig. 32. After exposure to the radioactive precursor, a small fraction (5 to 10%) of the cells consistently appeared to be

![Fig. 32. Survival Curve for Exposure of HeLa S3 Cells to Different Levels of H3-Thymidine. Data from three separate experiments are reported.](attachment:image)
unlabeled. This could not account, however, for any difference in survival, since no correlation was found between dose and fraction of unlabeled cells. Moreover, cells which did not take up the label were likely to be nonviable, since almost one-half were consistently giant cells. The curve given in Fig. 32 represents, therefore, the survival kinetics of cell populations that may be regarded as being uniformly exposed to internal irradiation. The points seem to fit the same "multiprocess" killing model that gives the best fit to most of the survival data for mammalian cells in vitro. In this particular case, however, since irradiation extends throughout the entire period of colony development, the cells have a finite, although decreasing, probability of dying after any given number of generations. Therefore, several independent kinetics with increasing extrapolation number may contribute to the parameters of the final survival curve.

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