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SEMIANNUAL PROGRESS REPORT

FOR PERIOD ENDING FEBRUARY 15, 1954



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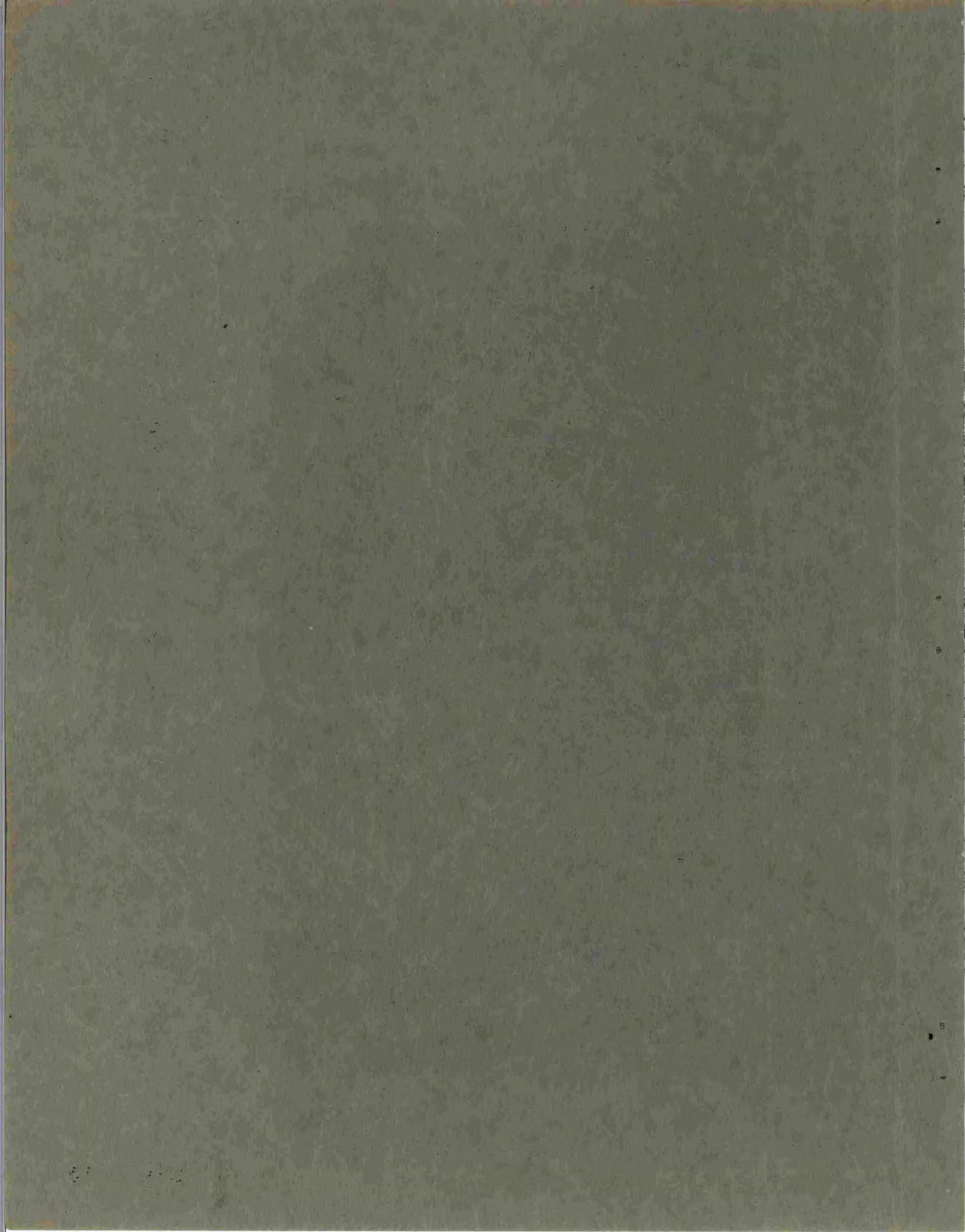


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BIOLOGY DIVISION
SEMIANNUAL PROGRESS REPORT
for Period Ending February 15, 1954

Alexander Hollaender, Director

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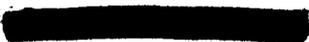
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75. T. T. Odell
76. Sheldon Wolff
77. M. L. Randolph
78. A. D. Conger
79. J. N. Dent
80. R. C. von Borstel
81. C. O. Doudney
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86. J. V. Passonneau
87. G. C. Williams

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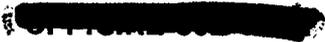
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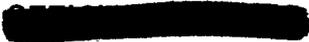


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BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications. The list of 36 publications from the Biology Division for the past six months consists of 33 open literature articles and three project reports. In addition to these, two papers published by the Mathematics Panel were coauthored by Biology Division members.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	Studies on isolated cell components. VI. The effects of nucleases and proteases on rat liver nuclei	<i>Exptl. Cell Research</i> 5, 361-374 (1953)
_____	Degree of polymerization of deoxyribonucleic acid	<i>Nature</i> 107, 807-808 (1953)
Atwood, K. C. and F. Mukai	Indispensable gene functions in <i>Neurospora</i>	<i>Proc. Natl. Acad. Sci. U.S.</i> 39, 1027-1035 (1953)
Baker, W. K. and E. S. Von Halle	The production of dominant lethals in <i>Drosophila</i> by fast neutrons from cyclotron irradiation and nuclear detonations	<i>Science</i> 119, 46-49 (1954)
Ball, E.	Histological effects of absorbed radioisotopes upon the callus of <i>Sequoia sempervirens</i>	<i>Botan. Gaz.</i> 114, 353-363 (1953)
_____	Hydrolysis of sucrose by autoclaving media, a neglected aspect in the technique of culture of plant tissue	<i>Bull. Torrey Botan. Club</i> 80, 409-411 (1953)
Bowen, G. H.	Studies of ultraviolet irradiation phenomena – An approach to the problems of bacteriophage reproduction	<i>Cold Spring Harbor Symposia Quant. Biol.</i> 18, 245-253 (1953)
Brumfield, R. T.	Curvatures in timothy roots induced by ultraviolet radiation	<i>Am. J. Botany</i> 40, 615-617 (1953)
Burnett, W. T., Jr., A. W. Burke, Jr., and A. C. Upton	Protective effect of acetyl- β -methylcholine, carbamylcholine, and atropine on X-irradiated mice	<i>Am. J. Physiol.</i> 174, 254-258 (1953)
Cohn, W. E.	The separation of biochemically important substances by ion-exchange chromatography	<i>Ann. N. Y. Acad. Sci.</i> 57, 204-224 (1953)
Conger, A. D.	The relative biological effectiveness of radiation from a nuclear detonation on <i>Tradescantia</i> chromosomes	<i>Science</i> 119, 36-42 (1954)
_____	Culture of pollen tubes for chromosomal analysis at the pollen tube division	<i>Stain Technol.</i> 28, 289-293 (1953)

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AUTHOR(s)	TITLE OF ARTICLE	PUBLICATION
Conger, A. D. and L. M. Fairchild	A quick-freeze method for making smear slides permanent	<i>Stain Technol.</i> 28, 281-283 (1953)
Cormier, M. J. and B. L. Strehler	The identification of KCF: Requirement of long-chain aldehydes for bacterial extract luminescence	<i>J. Am. Chem. Soc.</i> 75, 4864-4865 (1953)
Doermann, A. H.	The vegetative state in the life cycle of bacteriophage: Evidence for its occurrence, and its genetic characterization	<i>Cold Spring Symposia Quant. Biol.</i> 18, 3-11 (1953)
Furth, J., E. L. Gadsden, and A. C. Upton	ACTH secreting transplantable pituitary tumors	<i>Proc. Soc. Exptl. Biol. Med.</i> 84, 253-255 (1953)
Furth, J. and A. C. Upton	Histopathology and carcinogenesis	<i>Ann. Rev. Nuclear Sci.</i> 3, 303-338 (1953)
Gadsden, E. L. and J. Furth	The effect of thyroid hormone on the growth on thyrotropin-secreting pituitary tumors	<i>Proc. Soc. Exptl. Biol. Med.</i> 83, 511-514 (1953)
Gaulden, M. E.	Proceedings of the Fourteenth Annual Meeting of the Association of Southeastern Biologists	<i>J. Tenn. Acad. Sci.</i> 28, 172-174 (1953)
_____	Telophase behavior of extranuclear chromatin and its bearing on telophase changes in chromosomes	<i>Experientia</i> 10, 18-20 (1954)
Hampton, J. K. and J. B. Kahn, Jr.	Uptake and storage of radioactive iron by mouse liver	<i>Am. J. Physiol.</i> 174, 226-230 (1953)
Hollaender, A.	Biology Division Semiannual Progress Report for period ending August 15, 1953	Project Report ORNL-1614
Kirby-Smith, J. S. and C. P. Swanson (with appendix by C. W. Sheppard and E. B. Darden, Jr.)	The effects of fast neutrons from a nuclear detonation on chromosome breakage in <i>Tradescantia</i>	<i>Science</i> 119, 42-45 (1954)
Noggle, G. R.	The use of cation-exchange resins for the hydrolysis of sugars in plant extracts	<i>Plant Physiol.</i> 28, 736-740 (1953)
Noggle, G. R. and L. P. Zill	The biosynthesis of carbon-14-labeled compounds. III. The separation and isolation of sugars by ion-exchange chromatography	<i>Plant Physiol.</i> 28, 731-735 (1953)
_____	Ion exchange as a tool for studying plant carbohydrates	The Role of Atomic Energy in Agr. Research, Proc. 4th Ann. Oak Ridge Summer Symposium (1952), TID-5115, 1953, pp. 378-403.

PERIOD ENDING FEBRUARY 15, 1954

AUTHOR(s)	TITLE OF ARTICLE	PUBLICATION
Schwartz, D.	An interesting phenomenon associated with irradiation of dry maize seeds	<i>Science</i> 119, 45-46 (1954)
St. Amand, G. S. and S. R. Tipton	The separation of neuroblasts and other cells from grasshopper embryos	<i>Science</i> 119, 93-94 (1954)
Sheppard, C. W., W. E. Cohn, P. J. Mathias	The estimation of choline esters by ion exchange (Comm. to Editor)	<i>Arch. Biochem. and Biophys.</i> 47, 475-477 (1953)
Sheppard, C. W. and E. B. Darden, Jr.	Radiation measurement problems (confidential)	Project Memo 53-7-168
Strehler, B. L. and M. J. Cormier	Factors affecting the luminescence of cell-free extracts of the luminous bacterium, <i>Achromobacter fischeri</i>	<i>Arch. Biochem. and Biophys.</i> 47, 16-33 (1953)
Strehler, B. L. and C. S. Shoup	The chemiluminescence of riboflavin	<i>Arch. Biochem. and Biophys.</i> 47, 8-15 (1953)
Swanson, C. P. and D. Schwartz	Effect of X rays on chromatid aberrations in air and nitrogen	<i>Proc. Natl. Acad. Sci. U.S.</i> 39, 1241-1250 (1953)
Totter, J. R. and W. T. Burnett, Jr. (with R. A. Monroe, I. B. Whitney, and C. L. Comar, UT-AEC)	Evidence that molybdenum is a nondialyzable component of xanthine oxidase	<i>Science</i> 118, 555 (1953)
Upton, A. C. and J. Furth	Induction of pituitary tumors by ionizing irradiation	<i>Proc. Soc. Exptl. Biol. Med.</i> 84, 255-257 (1953)
Volkin, E. and W. E. Cohn	On the structure of ribonucleic acids. II. The products of ribonuclease action	<i>J. Biol. Chem.</i> 205, 767-782 (1953)

Published by Mathematics Panel; coauthored by Biology Division members:

Moshman, J. and A. C. Upton	Depigmentation of hair as a biological radiation dosimeter	<i>Science</i> 119, 186-187 (1954)
Sangren, W. C. and C. W. Sheppard	A mathematical derivation of a labeled substance between a liquid flowing in a vessel and an external compartment	<i>Bull. Math. Biophys.</i> 15, 387-394 (1953)

Publications in Press. Among the publications in press are 58 papers, 31 abstracts, and the proceedings of the 1953 Spring Conference in Oak Ridge. These proceedings will be published, as in the past, as a supplement to the *Journal of Cellular and Comparative Physiology*; the table of contents follows:

SYMPOSIUM ON EFFECTS OF RADIATION AND OTHER DELETERIOUS AGENTS ON EMBRYONIC DEVELOPMENT

Introduction – A. Hollaender

J. G. Wilson. Differentiation and the reaction of rat embryos to radiation

R. Rugh. The effect of ionizing radiations on amphibian development

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- J. N. Dent and E. L. Hunt. Radiotracer techniques in embryological research
 L. B. Russell and W. L. Russell. An analysis of the changing radiation response of the developing mouse embryo
 S. P. Hicks. The effects of ionizing radiation, certain hormones, and radiomimetic drugs on the developing nervous system
 D. Bodenstein. Effects of radiomimetic substances on embryonic development, with special reference to nitrogen mustards
 J. Warkany. Disturbance of embryonic development by maternal vitamin deficiencies
 F. C. Fraser, H. Kalter, B. E. Walker, and T. D. Fainstat. The experimental production of cleft palate with cortisone and other hormones
 W. Landauer. On the chemical production of developmental abnormalities and of phenocopies in chicken embryos
 B. H. Willier. Phases in embryonic development
 J. N. Yamazaki, S. W. Wright, and P. M. Wright. A study of the outcome of pregnancy in women exposed to the atomic bomb blast in Nagasaki
 P. Weiss. Summarizing remarks

Scientific Society Lectures and Traveling Seminars. Research in the Biology Division has been discussed in 38 professional society lectures and 23 traveling seminars during this half year. Speakers and their subjects are listed.

Anderson, N. G.	Duke University, Durham, N. C.	Physical studies on isolated nuclei and their constituents
Anderson, E. H.* and D. Billen	Genet. Soc. Am. (AAAS), Boston	The influence of postirradiation temperature treatment of X-ray-induced mutations in <i>E. coli</i>
Arnold, W. A.	Sigma Xi, University of Tennessee, Knoxville	Population and energy
_____	Spring Hill College, Spring Hill, Ala.	Problems connected with photosynthesis
_____	Biology Division, University of Georgia, Athens	As above
_____	Physics Seminar, ORNL	Delayed light emission and photosynthesis
Atwood, K. C. and F. Mukai	Genet. Soc. Am. (AAAS), Boston	High spontaneous incidence of a mutant of <i>Neurospora crassa</i>
Baker, W. K.	Genet. Soc. Am. (AAAS), Boston	Evidence on the chromosome distance over which V-type position effect may act
Billen, D. and E. Volkin	Kentucky-Tennessee Branch, Soc. Am. Bacteriologists, Knoxville, Tenn.	The effect of X irradiation on the macromolecular organization of <i>Escherichia coli</i>
Brumfield, R. T.	Botanical Soc. Amer. (AIBS), Madison, Wis.	The inhibition of curvatures in timothy roots by 2,4,6-trichlorophenoxyacetic acid

*Deceased

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Carson, S. F.	6th International Congress of Microbiology, Rome, Italy	Significant advances in the study of carbon metabolism of microorganisms
_____	Biochemistry Department, Columbia University, New York	Succinic decarboxylase system
Cohn, W. E.	Philadelphia Biochemists Club, Philadelphia	Application of ion-exchange chromatography to nucleic acid chemistry
_____	Biochemical Research Foundation, Newark, Del.	Biochemical applications of ion exchange
_____	Institute of Enzyme Research, University of Wisconsin, Madison	Nucleic acid and ion-exchange chromatography
Conger, A. D.	Genet. Soc. Am. and Soc. Am. Naturalists, Symp. "Some Biological Effects of Radiation from Nuclear Detonations" (AAAS), Boston	Chromosomal breakage in <i>Tradescantia</i>
Furth, J.	Sigma Xi, University of Tennessee, Knoxville	Induction of tumors by sustained imbalance of physiological growth regulators
_____	Armed Forces Institute of Pathology, Washington, D. C.	Clinical pathology
_____	Hammersmith Hospital Radiotherapeutic Research Unit, London, England	Experimental pituitary tumors
_____	Institut du Cancer Néerlandais, Amsterdam, Netherlands	As above
_____	Gordon Research Conference on Cancer, New London, N. H.	Character of 131 I-induced pituitary growth, hormonal and histological changes accompanying acquisition of autonomy
Furth, J. and A. C. Upton	Ciba Conference, London, England	Leukemogenesis by ionizing irradiation
Furth, J., A. C. Upton, K. W. Christenberry, and W. H. Benedict	Radiol. Soc. N. Am., Chicago	Experimental studies on the late effects of nuclear detonation
Gaulden, M. E.	Knoxville Science Club, Knoxville, Tenn.	"Let's Talk Turkey, etc."
_____	Zoology Department, University of Tennessee, Knoxville	Recent scientific findings in European laboratories
_____	University of Cambridge, Cambridge, England	Effects of radiation on living cells
Gaulden, M. E. and J. R. Totter	International Genetics Congress, Bellagio, Italy	Effects of X rays on nucleic acid synthesis and on mitosis

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Geckler, R. P. and R. F. Kimball	Genet. Soc. Am. (AAAS), Boston	The effects of hypoxia on X-ray destruction of kappa in <i>Paramecium aurelia</i>
Hollaender, A.	AAAS and Soc. Am. Naturalists Symp. "Physics in Biology," Boston	The reversal of biological effects of radiation
_____	Sigma Xi, University of Florida, Gainesville	Basic studies on biological effects of radiation
Horn, E. C. and N. G. Anderson	Am. Soc. Zool. (AAAS), Boston	Changes in basic proteins, deoxyribonucleic acid, and total nitrogen during the development of the chick to hatching
Kimball, R. F.	International Genetics Congress, Bellagio, Italy	The role of oxygen in the production of various effects of X rays on <i>Paramecium aurelia</i>
Kimball, R. F. and N. Gaither	Genet. Soc. Am. (AAAS), Boston	The dominant lethal problem in <i>Paramecium aurelia</i>
Lovelace, R.	Genet. Soc. Am. (AAAS), Boston	The shattering of <i>Tradescantia</i> chromosomes by ultraviolet radiation (2650 Å)
Mickey, G. H. and A. F. Yanders	Genet. Soc. Am. and Soc. Am. Naturalists, Symp. "Some Biological Effects of Radiation from Nuclear Detonations" (AAAS), Boston	Comparison of rates of visible mutations produced by fast neutrons and by X rays at specific loci in the third chromosome of <i>Drosophila melanogaster</i>
Oakberg, E. F. and J. E. Crowell	Genet. Soc. Am. (AAAS), Boston	Degeneration of spermatogenic cells following exposure of mice to X rays
Phares, E. F., S. F. Carson, and E. A. Delwiche	Am. Chem. Soc., Chicago	Origin and fate of the C ₁ fragment produced from succinate by propionic acid bacteria
Pittenger, T. H.	Genet. Soc. Am. (AAAS), Boston	Pseudo-wild type strains in <i>Neurospora</i> (Work done at Cal. Inst. Technol.)
Russell, L. B. and M. H. Major	Genet. Soc. Am. (AAAS), Boston	Dominant lethals in mouse oocytes induced by X rays in air and 5% oxygen
Russell, W. L.	Genet. Soc. Am. and Soc. Am. Naturalists, Symp. "Some Biological Effects of Radiation from Nuclear Detonations" (AAAS), Boston	Genetic and developmental effects in mice
Russell, W. L., L. B. Russell, J. S. Gower, and W. C. Sheppard	Genet. Soc. Am. (AAAS), Boston	Neutron-induced dominant lethals in the mouse
Schwartz, D.	Genet. Soc. Am. (AAAS), Boston	Studies on the mechanism of crossing over

PERIOD ENDING FEBRUARY 15, 1954

Sheppard, C. W.	Minn. Heart Assoc., Symp. "Recent Advances in Cardiovascular Physiology and Surgery," Minneapolis	Mathematical considerations of indicator dilution techniques
_____	Department of Biological Sciences, Stanford University, California	Recent advances in circulatory physiology
_____	AAAS and Soc. Am. Naturalists Symp. "Physics in Biology," Boston	The physics of circulatory mixing
Stapleton, G. E., D. Billen, and A. Hollaender	Kentucky-Tennessee Branch, Soc. Am. Bacteriologists, Knoxville, Tenn.	Some aspects of recovery of bacterial cells from ionizing radiation effects by postirradiation treatment
Strehler, B. L.	Zoology, Bacteriology, Biology Departments, University of Tennessee, Knoxville	Bioluminescence
Tolbert, N. E.	Biochemistry Seminar Group, Vanderbilt University, Nashville, Tenn.	Cell-free photosynthesis
_____	Department of Biological Sciences, University of Tennessee Medical School, Memphis	As above
_____	Departments of Botany and Zoology, University of Texas, Austin	As above
Tolbert, N. E. and L. P. Zill	Am. Soc. Plant Physiol. (AIBS), Madison, Wis.	Photosynthesis by cut cells and cell-free sap from <i>Chara</i> and <i>Nitella</i>
	Am. Soc. Plant Physiol. (AIBS), Madison, Wis.	Metabolism of sedoheptulose-C ¹⁴ by plants and yeast
Totter, J. R.	AAAS, Medical Sciences Section, Symp. "Antimetabolites and Cancer," Boston	Antimetabolite studies on bone marrow <i>in vitro</i>
_____	XIX International Physiological Congress, Montreal, Canada	Studies on the effects of irradiation on bone marrow
_____	Am. Chem. Soc., Chicago	The metabolism of formate in bone marrow suspensions
Upton, A. C.	Natural Science Division, Tuskegee Institute, Alabama	The production of cancer by ionizing irradiation
Wheeler, H. E.	Am. Phytopath. Soc. (AIBS), Madison, Wis.	Linkage groups in <i>Glomerella cingulata</i>
Whiting, A. R.	Genet. Soc. Am. (AAAS), Boston	Frequencies of dominant and recessive lethals induced in <i>Habrobracon</i> eggs by X rays in air and in nitrogen
Wilde, W. S. and J. M. O'Brien	XIX International Physiological Congress, Montreal, Canada	Time relation between potassium K ⁴² outflux, action potential and contraction phase of heart muscle as revealed by the effluogram

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Wolff, S.	Genet. Soc. Am. (AAAS), Boston	Effects of chemical protective agents on the restitution rate of radiation-induced chromosomal aberrations in <i>Vicia faba</i>
Zill, L. P. and N. E. Tolbert	Am. Soc. Plant Physiol. (AIBS), Madison, Wis.	Biosynthesis of sedoheptulose-C ¹⁴

Visiting Lecturers. The following prominent scientists have given lectures in the Biology Division seminar program during the period covered by this report.

Dr. A. D. Barton	University of Wisconsin, Madison	Enzyme distribution within cells
Dr. J. W. Boag	National Bureau of Standards, Washington, D. C.	Linear energy transfer with particular reference to fast neutron beams irradiating tissue
Dr. D. L. Lindsley	Department of Zoology, University of Missouri, Columbia	Some consequences of mitotic exchange in <i>Drosophila</i>
Dr. C. S. Bachofer	Department of Biology, University of Notre Dame, South Bend, Ind.	Discussion of work on protection of bacteriophage against X rays and related problems
Dr. R. E. Zirkle	Institute of Radiobiology and Biophysics, University of Chicago, Chicago	Mitotic aberrations produced by partial-cell proton irradiation
Dr. C. E. Carter	Department of Pharmacology, Yale University, New Haven, Conn.	The pyrimidine problem
Dr. B. L. Horecker	National Institutes of Health, Bethesda, Md.	An oxidative pathway for carboxylations
Dr. C. B. Anfinsen, Jr.	National Institutes of Health, Bethesda, Md.	Mechanism of protein biosynthesis
Dr. K. Dittmer	Department of Chemistry, Florida State University, Tallahassee	The mechanism of unsaturated amino acid antagonism in biological systems
Dr. A. Kornberg	Department of Microbiology, Washington University Medical School, St. Louis, Mo.	Orotic acid and the biosynthesis of pyrimidines

University of Virginia Course in Radiation Biology. The University of Virginia offered a course in Radiation Biology at their Mountain Lake Biological Station during the past summer. This Laboratory assisted in teaching this course by supplying a number of lecturers, as follows: "Radiation Physics," C. W. Sheppard; "Radiation Biochemistry," J. R. Totter; "Radiation Genetics," R. F. Kimball; "Radiation Cytology," M. E. Gaulden; and "Radiation Biology of Microorganisms," G. E. Stapleton.

Seventh Annual Biology Research Conference in Oak Ridge. Announcements of the seventh in the series of annual spring conferences sponsored by the Biology Division of Oak Ridge National Laboratory and supported by the Atomic Energy Commission have been sent out. This conference, which convenes in Oak Ridge on April 19 to 21, 1954, will feature a symposium on "Genetic Recombination." W. K. Baker and D. Schwartz, of the Cytology and Genetics Section, have given active assistance in arranging the program for this meeting, and both will take part in the discussions. The program follows.

- First Session – Chairman: M. Demerec, Carnegie Institution of Washington
“Bacterial Transformation” – R. D. Hotchkiss, The Rockefeller Institute for Medical Research
“Bacterial Transduction” – N. Zinder, The Rockefeller Institute for Medical Research
- Second Session – Chairman: A. D. Hershey, Carnegie Institution of Washington
“The Mechanism of Genetic Recombination in Bacteriophage” – A. H. Doermann, University of Rochester
“Recombination in Bacteria” – J. Lederberg, University of Wisconsin
- Third Session – Chairman: A. Weinstein, Harvard University
“Chromosome Replication (and ‘Recombination’) in Relation to the Structure of DNA Molecule” – J. D. Watson, California Institute of Technology
“Materials for Extending the Study of Crossing Over” – D. D. Perkins, Stanford University
- Fourth Session – Chairman: M. M. Rhoades, University of Illinois
“Some Observations on Chromosome Behavior During Meiosis in *Drosophila*” – E. Novitski, University of Missouri
“Studies on Crossing Over in Maize and *Drosophila*” – D. Schwartz, Oak Ridge National Laboratory
- Fifth Session – Chairman: K. Cooper, University of Rochester
“Effect of Ionizing Irradiation on Crossing Over” – M. B. Whittinghill, University of North Carolina
“Interchromosomal Effects on Recombination” – H. L. Carson, Washington University
- Sixth Session – Chairman: G. W. Beadle, California Institute of Technology
“Evaluation of Recombination Theory” – A. Weinstein, Harvard University; A. H. Sturtevant, California Institute of Technology; and K. Sax, Harvard University
- Evening Session (April 19) “The Oxygen Effect of X-Ray-Induced Chromosome Aberrations (Recombination Versus Initial Breakage Hypothesis)” – N. H. Giles, Jr., Yale University; W. K. Baker, Oak Ridge National Laboratory; C. P. Swanson, The Johns Hopkins University

Biology Division Speakers at Future Meetings of Scientific Societies. The speakers from the Division who will present papers at the *Federation of American Societies for Experimental Biology* in Atlantic City on April 11 to 16 are as follows: N. G. Anderson, W. T. Burnett, Jr. (coauthors, B. Anderson, E. L. Gadsden, and J. Furth), E. A. Delwiche (coauthors, E. F. Phares and S. F. Carson), D. G. Doherty (coauthor, L. A. Thomas), M. I. Dolin, J. X. Khym (coauthor, W. E. Cohn), T. T. Odell (coauthors, F. G. Tausche and J. Furth), C. W. Sheppard (coauthor, P. J. Mathias), A. C. Upton (coauthor, J. Furth), E. Volkin, L. P. Zill (coauthor, N. E. Tolbert).

At the *American Association for Cancer Research*, which meets simultaneously with the Federation, A. C. Upton will present a paper of which J. Furth and W. H. Benedict are coauthors. At the Conference on “Ionizing Radiation and the Cell” to be held under the auspices of the New York Academy of Sciences on April 23 and 24, three papers will be given, by A. Hollaender, R. F. Kimball, and G. E. Stapleton. G. S. St. Amand will give a talk at the *Association of Southeastern Biologists* in Baton Rouge, La., on April 16. Coauthors on this work are M. E. Gauden and J. R. Totter. The program of the *Radiation Research Society*, in Cleveland on May 17 to 19, will include talks by several Biology Division members. J. N. Dent will present a paper to the *American Association of Anatomists*, which meets in Galveston on April 7 to 9. D. Billen will speak before the *Society of American Bacteriologists*, which meets in Pittsburgh on May 2 to 7.



CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball	D. Schwartz
K. C. Atwood	S. Wolff
G. H. Bowen	N. Gaither
A. J. Bridgman ¹	A. H. Johnston
A. D. Conger	M. K. King
T. H. Pittenger	A. D. Phelps

Effect of X Rays on the Behavior of Nuclei at Conjugation in *Paramecium aurelia*

R. F. Kimball	N. Gaither
M. K. King	

In a previous report² a study was reported of the effects of high (33 kr) doses of X rays on the behavior of nuclei at conjugation in *Paramecium*. Normally, each of the two members of the conjugant pair forms two haploid "pronuclei" by the mitotic division of one haploid nucleus. These two nuclei are therefore identical in genotype and are spoken of as the "migratory" and "stationary" pronuclei. The migratory pronucleus from each member passes into the other member and fuses with the stationary pronucleus there, a sort of mutual cross-fertilization. The two members of the conjugant pair thus come to have identical nuclei which are derived equally from the two parents, but there is little if any exchange of cytoplasm. The conjugants separate and each can be developed into a clone by vegetative reproduction. By use of appropriate gene markers, the behavior of nuclei in crosses can be followed, and the cytoplasmic origin of the two members can be recognized after conjugation.

The main experiment involved a cross of heavily irradiated (33 kr) animals of one genotype with unirradiated animals of another. As shown previously², one of the major abnormalities resulting from such a cross is that the irradiated member often fails to contribute a functional pronucleus to its mate. However, in quite a few pairs the stationary pronucleus of the irradiated member functions and fuses with a migratory pronucleus from the unirradiated mate. Such "one-sided" hybrids are more likely to die than are hybrids produced in pairs in which the migratory as well as the station-

ary pronucleus is functional. Further investigations have established this phenomenon on a still stronger basis and have, in particular, demonstrated that the unirradiated member of one-sided pairs usually has haploid micronuclei, presumably derived from the single stationary pronucleus remaining in the unirradiated member. Cytological examination showed that the micronuclei were appreciably smaller than normal in these cases, and most of these animals died at the next autogamy, as would be expected when a haploid attempted to undergo self-fertilization.

In ORNL-1614, it was suggested that the correlation between dominant lethality in the hybrid and failure of the migratory pronucleus to function could be explained, by the formation of chromosome bridges, as a result of chromosome aberrations. Such chromosome bridges would be associated with a loss of parts of chromosomes which could function as dominant lethals in the stationary pronucleus but the bridges themselves might interfere with the movement of the nuclei sufficiently to prevent the migratory pronucleus from functioning. However, recent work has suggested another and perhaps more likely interpretation. A cross was made between a haploid and a diploid which differed in several marker genes. From the distribution of the genes, it was clear that the haploid formed pronuclei which were grossly deficient in the chromosomes which they contained, presumably by random assortment of the single set of chromosomes. In a number of cases, the deficient pronucleus from the irradiated member failed to function as a migratory pronucleus although it did function as a stationary pronucleus. This suggests that simple loss of parts of chromosomes may interfere with the functioning of nuclei and that it is unnecessary to invoke the mechanical interference of bridges although this may also be involved.

If the movement of a pronucleus with a deficient chromosome set is distributed by the deficiencies, the action of the genes on this movement is autonomous, since there is a macronucleus with a complete set of genes in the same cytoplasm as the deficient pronuclei. The case is similar in many respects to the class of autonomous nuclear lethals in *Neurospora* demonstrated by Atwood and Mukai.³

¹Research Participant.

²R. F. Kimball, N. Gaither, and M. K. King, *Biol. Semiann. Prog. Rep. Aug. 15, 1953, ORNL-1614, p 11.*

³K. C. Atwood and F. H. Mukai, *Biol. Semiann. Prog. Rep. Aug. 15, 1953, ORNL-1614, p 25.*

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Role of Oxygen and H_2O_2 in the Induction of Mutations in *Paramecium aurelia*

R. F. Kimball N. Gaither
M. K. King

Evidence was presented in the previous semi-annual report⁴ that H_2O_2 could not be responsible for the effect of oxygen on mutation production by X rays. The argument was based on a calculation of the amount of H_2O_2 which penetrated into the cell. Unfortunately, the value used for the catalase activity was too low, partially because of an error in calculations of the dimensions of the cells. Determinations of the catalase activity of *Paramecium aurelia* have now been made by use of approximately 100 dried paramecia for each determination and by measuring colorimetrically the H_2O_2 utilized at ice-bath temperatures. The activity was found to be high. It has also proved feasible to measure the amount of very dilute (approximately 4×10^{-5} M) H_2O_2 used by living paramecia. From this measurement, the permeability constant for H_2O_2 can be calculated since the diffusion coefficient and the dimensions of the cell can be considered to be known, and the use is nearly independent of catalase concentration when the concentration is high. The permeability constant is reasonably

close to that previously calculated from the constant for water for another protozoan. With all the necessary constants determined, it seems probable that the concentration inside the cell is a small percentage of that outside.

For this reason, certain critical experiments were repeated with 0.003 M KCN present to poison the catalase. Under these circumstances the inside concentration should be a large fraction of the outside concentration. The results of experiments with 0.003 M KCN plus low concentrations of H_2O_2 are shown in Table 1. In one of the experiments, the paramecia were exposed to X rays during the exposure to H_2O_2 (2 min of X rays during the 10-min exposure to H_2O_2) in order to check for possible sensitization to peroxide by X rays as reported by Alper⁵ for bacteriophage. The results were negative. In the X-ray experiment there was slightly more effect when H_2O_2 was present, but the increase was not statistically significant and was much too low to account for the oxygen effect at this dose. Actually, the amount of H_2O_2 inside the cell was probably many times greater than would have been produced by the X-ray doses usually used for mutation production. Thus it can be estimated from Savage's data⁶ that 3 kr of X rays

⁴R. F. Kimball, N. Gaither, and M. K. King, *Biol. Semian. Prog. Rep. Aug. 15, 1953*, ORNL-1614, p 12.

⁵T. Alper, *Nature* 169, 964-965 (1953).

⁶D. J. Savage, *Analyst* 76, 224-226 (1951).

TABLE 1. LACK OF EFFECT OF LOW DOSES OF H_2O_2 ON KCN-POISONED *PARAMECIUM*

DOSE OF X RAYS (r)	FLUID	NUMBER OF TREATED PARAMECIA	PERCENTAGE OF NORMAL EXAUTOGAMOUS CLONES
None	10% Ringer's solution	60	93
None	2.4×10^{-4} M H_2O_2 (commercial)	60	96
None	3.5×10^{-4} M H_2O_2 (commercial)	51	96
None	0.003 M KCN	56	99
None	2.4×10^{-4} M H_2O_2 and 0.003 M KCN	58	96
None	3.5×10^{-4} M H_2O_2 and 0.003 M KCN	47	98
None	10% Ringer's solution	30	96
3380	0.003 M KCN	42	72
3380	1.8×10^{-4} M H_2O_2 (radiation produced) and 0.003 M KCN	52	67

produces about 5×10^{-6} M of H_2O_2 in pure aerated water.

The possibility remained that high concentrations of H_2O_2 , present momentarily in ionization tracks, were effective although small concentrations were not. No accurate estimation of the concentrations in the tracks can be made. However, Gray⁷ gave diagrams for the distribution of radicals in the paths of particles of different ion densities. While these calculations must be considered very rough approximations, nonetheless it can be guessed that it is unlikely that concentrations of H_2O_2 as great as 0.1 M would be encountered except with extremely densely ionizing particles. Since there is no oxygen effect for such particles, these densities are immaterial. Data from experiments in which the paramecia were exposed to 0.003 M KCN for several minutes before a half-minute exposure to 0.003 M KCN plus 0.2 or 0.15 M H_2O_2 are shown in Table 2. The equilibrium concentration of H_2O_2 inside the cell should have been reached in less than a half minute; so the negative results of this experiment can be taken to mean that very high concentrations of H_2O_2 for brief periods of time are not effective.

Thus the conclusion is reached that whatever the mechanism of the action of oxygen on mutagenesis, it is not a matter of producing H_2O_2 which then produces the biological effect. In the reactions occurring in irradiated nuclei, H_2O_2 must therefore be considered as a harmless substance. This leads to a consideration of other hypotheses such as oxygen acting to increase the effective concentration of OH by reacting with H atoms to form

H_2O_2 , and thus decreasing the back reaction of H and OH to water.

One curious exception to the conclusion that H_2O_2 is ineffective in the mutagenic process must be noted. In three experiments in which an X-ray treatment was followed by an exposure to H_2O_2 which was sufficient to slow division in the first day after treatment, the amount of mutation was less in the H_2O_2 controls than in the irradiated controls (Table 3). In all three experiments there was some peroxide-induced death, but it seems most unlikely that selection of nonmutants could be brought about in this way since this would require that mutations in the micronucleus have an immediate effect on the animal, a most unlikely event. The results might be taken to mean that there is a period immediately after irradiation during which it is possible to modify the mutagenic effect by chemical means. However, it remains possible that the action is less direct and involves, in some fashion, the division delay which H_2O_2 produces. Further studies will be required to elucidate this matter.

We are greatly indebted to J. Z. Hearon of the Mathematics Panel who formulated for us the problem of penetration of H_2O_2 into *Paramecium*. His formulation and calculations are the basis for the experiments and conclusions of this section.

**Radiation Studies on *Tillina Magna*
and Other Ciliates**

A. J. Bridgman R. F. Kimball

In a previous report⁸ on the effect of X rays on

⁷L. H. Gray, *J. Cellular Comp. Physiol.* 39, Suppl. 1, 57-74 (1952).

⁸A. J. Bridgman and R. F. Kimball, *Biol. Quar. Prog. Rep.* Nov. 10, 1952, ORNL-1456, p 15.

TABLE 2. LACK OF A MUTAGENIC EFFECT ON *PARAMECIUM* OF A 30-sec EXPOSURE TO 0.2 M H_2O_2 IN THE PRESENCE OF 0.003 M KCN

FLUID	NUMBER OF TREATED PARAMECIA	PERCENTAGE OF NORMAL EXAUTOGAMOUS CLONES
10% Ringer's solution	38	99
0.2 M H_2O_2 and 0.003 M KCN	20	99
0.003 M KCN	72	89
0.15 M H_2O_2 and 0.003 M KCN	27	88

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TABLE 3. EFFECT OF H₂O₂ TREATMENT AFTER IRRADIATION ON MUTAGENESIS

DOSE OF X RAYS (r)	POSTTREATMENT	NUMBER OF TREATED PARAMECIA	PERCENTAGE OF NORMAL EXAUTOGAMOUS CLONES
None	None	8	98
2800	0.003 M KCN	40	62
2800	0.003 M KCN and 0.00015 M H ₂ O ₂	40	73
None	None	52	94
2680	None	50	67
2680	0.15 M H ₂ O ₂	44	78
None	None	63	99
3330	None	62	53
3330	0.003 M KCN and 0.2 M H ₂ O ₂	37	69

the ciliate, *Tillina magna*, evidence was presented that death occurred a number of divisions after irradiation. The existence of this delayed death has been confirmed and investigated in more detail. Most of the death occurs between the fourth and fourteenth divisions, and it is not associated with a reduction in division rate in surviving lines as was the case for delayed death after ultraviolet irradiation in *Paramecium aurelia*.⁹ Those lines which die usually show a greater depression in the division rate in the first two days after irradiation than do the survivors. However, recovery to almost the normal rate occurs before the final decline leading to death. Thus the changes which lead to death are already expressed immediately after irradiation. This makes unlikely the assumption that macronuclear mutations are involved (this strain of *Tillina* has no micronucleus), since these would not be expected to express themselves until later. As a matter of fact, calculations by A. W. Kimball and A. S. Householder of the ORNL Mathematics Panel have shown that, on the simplest theory of segregation of macronuclear mutations, death should have been much more delayed than it was. A more complex theory might allow earlier death but would still not explain the high correlation with low division rate in the first two days.

⁹R. F. Kimball, R. P. Geckler, and N. Gaither, *J. Cellular Comp. Physiol.* 40, 427-459 (1952).

This means that the delayed death in *Tillina*, despite the all-or-none character of the response and the long period intervening between irradiation and death, is not due to mutational changes, in the ordinary sense.

In the previous report, it was shown that *Tillina* suffered less delay in division when irradiated in nitrogen than when irradiated in 20% oxygen. Also it was shown that considerable division delay could be brought about by H₂O₂ added to the liquid surrounding the animals. However, unlike *Paramecium*,¹⁰ the presence of bacteria in the medium during irradiation does not greatly decrease the oxygen effect on division delay. Since bacteria in the medium should prevent H₂O₂ from accumulating outside the cell, the oxygen could be involved in radiation-induced reactions taking place locally within the cell; in this respect, *Tillina* differs from *Paramecium*. Experiments with two species of *Colpoda* have yielded very similar results to those with *Tillina* whereas *Tetrahymena* reacts very much as does *Paramecium*. *Tillina* and *Colpoda* are closely related genera which divide into four or eight daughter cells in a division cyst. *Paramecium* and *Tetrahymena* divide, in the free-swimming form, into two daughter cells. It seems possible that the difference in the reaction to oxygen is related to

¹⁰R. F. Kimball and N. Gaither, *Proc. Soc. Exptl. Biol. Med.* 82, 471-477 (1953).

the different mechanisms of division.

Delayed death is also influenced by the oxygen tension present during irradiation, being greater when oxygen is present. However, H_2O_2 in the medium appears to be ineffective in inducing this change. The situation is thus comparable to that for micronuclear mutations in *Paramecium* for which there is an oxygen effect but no effect of H_2O_2 in the medium.

Lack of an Effect of Oxygen on the Fusibility of Radiation-Induced Chromosomal Breaks

A. D. Conger

A. H. Johnston

A small amount of disagreement has developed over which of two explanations will be accepted for the observation that more chromosomal aberrations result from irradiation when oxygen is present than when it is absent. A direct answer for this cannot be found, because the aberrations which are observed result from two unseen events – the initial breakage of chromosomes by the radiation, followed by nonfusion, or fusion of these broken ends into new arrangements which are later detected as chromosomal aberrations. The following question has arisen. Does the oxygen cause more aberrations by increasing the number of breaks produced by a given dose of radiation, or does it affect the frequency with which the broken ends rejoin? The latter explanation is quite acceptable, for it is known that, ordinarily, most of the broken ends never appear as aberrations, but re-fuse in their original arrangement (“restitution”) and so are undetectable.

It is possible in *Tradescantia* to obtain an estimate of the fusibility of broken ends, that is, the fraction of broken ends produced by radiation which are capable of fusing with other broken ends. The application of this knowledge to the question of how oxygen causes higher aberration yield is obvious.

A few examples will illustrate how the estimate can be derived. Since that large proportion of broken ends which re-fuse in the original condition (“restitute”) cannot be detected, it is necessary to estimate somehow, from the events which result in observable aberrations, the fraction of broken ends which are fusible. Consider an ionization event which produces an “isochromatid break” that may result in an isochromatid aberration. A (large) fraction of the breaks are thought to resti-

tute, and such breaks are never seen as aberrations. But the remaining breaks result in the isochromatid aberration which can be analyzed under the microscope. Ordinarily, all that is measured is the total number of these isochromatid aberrations produced, which has been found to be roughly 3 to $3\frac{1}{2}$ times as great in air (21% oxygen) as in nitrogen, for the same dose of radiation. However, with careful observation it is possible to distinguish the different kinds of isochromatid aberrations with a fair degree of accuracy, and the proportion of these kinds depends on how much fusion of the ends has occurred. The different kinds of aberrations are illustrated in Fig. 1. Of the observed isochromatid aberrations, one class represents two fusions (SU), two classes represent only one of the two that could occur (NUp and NUd), and one represents no fusions at all (NUpd). If, for example, the higher yield of aberrations produced by irradiation in the presence of oxygen is caused by the oxygen decreasing the fusibility of ends and not by an increase in the initial number of breaks, it is apparent that a larger proportion of the isochromatid aberrations observed would show the incomplete fusion as indicated in the NUp, NUd, or NUpd types. Furthermore, the higher total (observable) yield would result because, with lower fusion, a smaller proportion of the breaks result in the (invisible) restitution class, which is actually just another fusion class like the others. Certain other numerical relations should hold between the proportions of these different classes, but these arguments are secondary and will not be considered here.

The same sort of estimate can be derived from another class of aberrations – the interchanges between two different chromosomes, both of which suffered a chromatid break. When such an exchange occurs, the fusions may be complete or incomplete; if complete, both proximal and distal ends fuse, if incomplete, only one or the other of the pair fuses, as shown in Fig. 2. These complete-incomplete fusion data from interchange aberrations also yield an estimate of fusibility.

The results of several experiments in which *Tradescantia* was irradiated while in oxygen or nitrogen and microscope slides made at different intervals after irradiation are given in Table 4. The numbers of isochromatid and interchange aberrations in each of the fusion classes just discussed are shown. This microscopic classification is difficult and subject to some error, since

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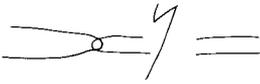
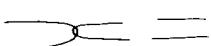
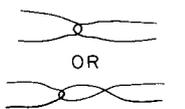
INITIAL ISOCHROMATID BREAK 		
RESULTS AFTER FUSION	CLASS OF FUSION	NUMBER OF FUSIONS
OBSERVED ISOCHROMATID ABERRATIONS		
	SISTER UNION (SU)	2
	NONUNION proximal (NUp)	1
	NONUNION distal (NUd)	1
	NONUNION proximal and distal (NUpd)	0
ISOCHROMATID ABERRATIONS NOT OBSERVED		
 OR	RESTITUTION	2

Fig. 1. Fusion Fate of an Isochromatid Break.

the observer must decide not only whether he is looking at an isochromatid or an exchange aberration, which is simple, but also which of the fusion types it is. The accuracy of the latter observation is poorer than the former. The slides were encoded before scoring was begun and were not decoded until it was completed, thus eliminating any bias in the scoring of the aberrations from oxygen or nitrogen treatments, even though not improving the accuracy. For each of the aberration types the fraction of aberrations which show fusion is calculated. The meaning of the "fusion fraction" from isochromatid aberrations is apparent from its derivation. Each isochromatid break will, if fusion

is complete, result in two fusions, one on the chromosome end and one on the fragment end; these make up the SU class. Thus the isochromatid fusion fraction is the proportion of fusions that did occur to the number that could occur. Had fusion been complete, this fraction would be unity, and all isochromatid aberrations would be the SU type; had fusion been zero, all would be the non-fusion NUpd type.

There are no significant differences between the fusion fractions in oxygen and in nitrogen, either in the paired groups observed at the same time or in the (more reliable) totals of all the oxygen and nitrogen data. Similarly, there is no difference in

TABLE 4. FUSION IN INTERCHANGE AND ISOCHROMATID ABERRATIONS AFTER IRRADIATION IN O₂ OR N₂

GAS	X-RAY DOSE	TIME AFTER IRRADIATION (hr)	CHROMATID/CHROMATID INTERCHANGES			ISOCHROMATID ABERRATIONS					FUSION FRACTION 2(SU) + NU _p + NU _d	
			Total	Complete Fusion	Fraction Complete	SU 	NU _p 	NU _d 	NU _{pd} 	Total	2 (all isochromatids)	
											Number	Fraction
O ₂	75	8	39	24	0.62	63	9	15	9	96	150/192	0.78
N ₂	75	8	23	13	0.56	53	14	21	20	108	141/216	0.65
O ₂	76.5	22	40	21	0.52	52	15	6	7	80	125/160	0.78
N ₂	229	22	45	20	0.44	52	22	10	19	103	136/206	0.66
O ₂	153	48	23	14	0.61	96	20	18	17	151	230/302	0.76
N ₂	459	48	26	17	0.65	127	30	18	24	199	302/398	0.76
O ₂	76.5	48	5	5	1.00	32	4	16	10	62	84/124	0.68
N ₂	229	48	16	11	0.69	43	15	23	21	102	124/204	0.61
O ₂	Total		107	64	0.60	243	48	55 (0.141)	43	389	589/778	0.757
N ₂	Total		110	61	0.56	275	81	72 (0.141)	84	512	703/1024	0.686

PERIOD ENDING FEBRUARY 15, 1954

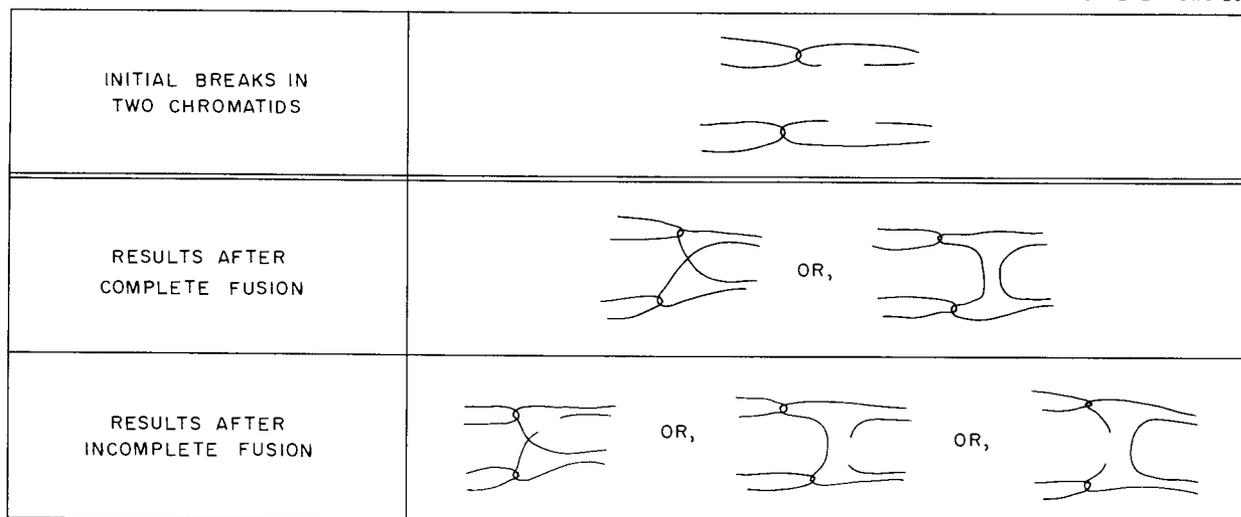


Fig. 2. Complete and Incomplete Fusion in Chromatid/Chromatid Interchange Aberrations.

the fraction of exchange aberrations which have complete fusion. One particular isochromatid aberration, the NUd type, has been selected because it can be distinguished more accurately under the microscope than any other. The proportion of these (0.141) among all isochromatid aberrations is the same in oxygen and in nitrogen. The slight differences between fusion fractions in oxygen and nitrogen shown in the total columns (Table 4) are not thought to be real; but if they are, they are in the wrong direction to account for increased aberration yield on the hypothesis that oxygen depresses fusibility. Furthermore, of more importance on the basis of the fusion hypothesis, the fusibility would have to be 3 to 3½ times as great in nitrogen as it is in oxygen, and this very clearly cannot be true, even allowing for the grossest possible errors in scoring.

The fusion fraction can be altered by some kinds of treatment. For example, Kotval and Gray¹¹ demonstrated that fusibility was about four times as high with X or gamma rays as it was with alpha particles. The lack of a difference in the present results cannot, therefore, be attributed to some inherent invariability of fusion.

It is apparent from these data that in *Tradescantia* the fusibility of broken chromosome ends is the same whether irradiation was in nitrogen or oxygen.

¹¹J. P. Kotval and L. H. Gray, *J. Genet.* 48, 135-154 (1947).

Therefore the increased aberration yield cannot be due to an effect of oxygen on the fusibility of the broken ends induced by the radiation.

Oxygen and Ultraviolet Experiments with Mouse Ascites Tumor

A. D. Conger

A. H. Johnston

The Ehrlich mouse ascites tumor grows as a free-cell suspension in the peritoneal cavity, increasing about a hundredfold in cell number and fluid volume in about six days. For example, a single mouse inoculated with 0.1 ml of fluid containing 10⁷ cells will yield approximately 5 to 10 ml of fluid containing about 10⁹ cells in six days. The fluid can be held out of the body for 8 hr or more for experimental treatments, injected into fresh mice, and samples drawn at any time thereafter for cytological analysis. The total number of cells in an animal also can be counted, thus giving information on the effect of treatment on cell growth. This tumor was used to determine if the effect of oxygen on radiosensitivity was the same for mammalian cells as that found for plants and lower animals. The effects were found to be the same, qualitatively and quantitatively; the cells were about three times as radiosensitive in air as in anoxic conditions, and radiosensitivity was increased little if any by an increase in oxygen concentration from air to pure oxygen. The results are based on two criteria of cell damage to the cells: chromosomal damage (as measured by

the fraction of anaphases which show chromosomal bridges and fragments) and reduction in the amount of cell growth which occurs in five to six days after treatment. The cells are quite radiosensitive – 50% of the cells are affected, cytologically and growthwise, by a dose of about 100 r *in vitro* in air.

Exploratory work has shown that it will be possible to perform ultraviolet experiments with this material. Washed cells suspended in a salt solution have been irradiated with ultraviolet, and cytological abnormalities have been observed subsequently. The cells are appreciably affected by moderate ultraviolet doses.

On the Mechanism of Chromosome Recombination

D. Schwartz

A. D. Phelps

In an earlier report¹² experiments were described which indicated that crossing over between homologous chromosomes involved only the newly formed chromatids. Somatic crossing over between attached-X chromosomes of *Drosophila* were studied, since in somatic tissue little or no sister-strand crossing over occurs. Brown and Hannah¹³ have shown that aging of females as virgins before mating is responsible for a high degree of instability of ring chromosomes in the F₁. One of the hypotheses advanced is that the instability is caused by sister-strand exchanges in the ring chromosomes. On the basis of this report, the effect of aging on the frequency of twin spotting in attached-X flies heterozygous for *y* and *sn*³ was studied. The data are given in Table 5. The female parents were aged for 13 days before mating.

¹²D. Schwartz, *Biol. Semiann. Prog. Rep. Aug. 15, 1953*, ORNL-1614, p 23.

¹³S. W. Brown and A. Hannah, *Proc. Natl. Acad. Sci. U.S.* 38, 687-693 (1952).

If crossing over occurs between any of the four chromatids, as has generally been accepted, sister-strand crossing over and hence aging should have no effect on the frequency of twin spotting. However, on the hypothesis that crossing over between homologous chromosomes is limited to the new chromatids occurring as a result of the process of duplication, the frequency of twin spotting would be greatly increased by sister-strand crossing over. A somatic crossover in the region of the centromere between the new chromatids would give rise only to wild-type tissue. Such a crossover associated with a proximal sister-strand exchange would form a twin spot of yellow and singed tissue (Fig. 3). The results strongly support the proposed hypothesis.

The alternative possibility that aging merely increases the frequency of somatic crossing over between homologous chromosomes was ruled out by experiments with unattached-X chromosomes. If the effect of aging is on somatic crossing over, a ninefold increase in the frequency of twin spotting would be expected in the progeny of aged as compared to unaged unattached-X females. However, if the effect of aging is limited to sister-strand exchanges, no difference would be expected. The data (Table 5) show no significant difference between the frequency of twin spotting in aged and unaged classes.

The results of these experiments suggest that crossing over involves two events: (1) exchanges between the new chromatids of homologous chromosomes, and (2) exchanges between sister chromatids. Both types of exchanges are involved in meiosis, whereas in mitosis only crossing over between the new chromatids normally occurs.

TABLE 5. FREQUENCY OF TWIN SPOTTING IN FEMALES HETEROZYGOUS FOR *y* AND *sn*³

Mutant genes located in opposite chromosome arms

CHROMOSOME CONSTITUTION	TOTAL FLIES SCORED	NUMBER OF TWIN SPOTS	PERCENTAGE OF TWIN SPOTS
Attached-X (not aged)	1041	8	0.77
Attached-X (aged)	709	48	6.77
Unattached-X (not aged)	180	27	15.00
Unattached-X (aged)	196	26	13.27

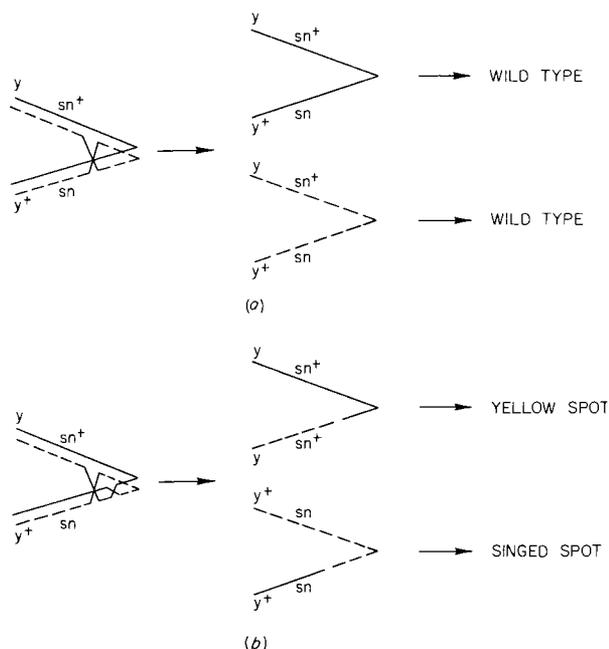


Fig. 3. Diagrammatic Representation of Somatic Crossing Over Between the Newly Formed Chromatids (a) Not Associated with and (b) Associated with Sister-Strand Exchanges. Broken lines represent newly formed chromatids.

Independent X-ray Effects on Chromosome Breakage and Reunion in *Vicia faba*

S. Wolff

K. C. Atwood

It has previously been determined (by dosage-fractionation experiments) that chromosome breaks produced by X raying the seed of *Vicia faba* remain open for a period of 2 hr before rejoining. If the seed are soaked in BAL previous to irradiation, this delay in rejoining is shortened. These phenomena manifest themselves in aberration yield since breaks that stay open for 2 hr will interact with other breaks produced later within this period of time. The number of two-hit aberrations will then increase as the square of the total dose. If the first breaks rejoin before the second ones are produced, the two-hit aberrations will be the sum of those produced by the various fractions.

In order to study further the relation between breakage and reunion and their effect on aberration

yield, a series of dosage fractionation experiments were performed. These were carried out under varying protocols of irradiation and of BAL treatment.

The experiments are summarized in Tables 6 and 7, and the following conclusions are indicated:

1. It may be noted from experiments 5, 6, and 12 that breaks produced in the presence of BAL interact normally with those produced in its absence.

2. Rejoining of breaks produced in the presence of BAL is delayed by previous irradiation in its absence (experiments 5 and 6 contrasted to experiments 7 and 8).

3. BAL decreases the aberration yield and the reunion delay by different amounts (experiments 12 and 13).

4. The time interval between breakage and reunion is dose dependent (Table 7) and is decreased if BAL is present during irradiation (Table 6).

The quantitative aspects of the experiments also lead to the conclusion that the reunion delay time per se has little or no influence on the aberration frequency.

Enzymatic Aspects of Chromosome Rejoining

S. Wolff

The assumption was made that radiation-induced chromosome breaks would require an enzyme system to perform the necessary biosynthesis which results in rejoining of broken ends. To test this assumption, fractionated-dosage experiments are being performed both in the presence and absence of enzyme inhibitors.

Table 8 shows that in *Vicia faba* after an irradiation of 600 r *in vacuo* the broken ends rejoined within 75 min at room temperature (experiment 3). However, if the temperature was lowered to 0°C immediately after the first irradiation, the ends did not rejoin in the 75-min rest period (experiment 4).

Similar results were obtained if 2×10^{-3} M KCN was substituted for cold treatment. Therefore at this time it may be said that the rejoining of chromosome breaks is cyanide sensitive in *Vicia faba*.

Both these findings are consistent with the assumption that the rejoining is mediated by an enzyme system.

X-ray Inactivation of a Triple Heterokaryon

K. C. Atwood

T. H. Pittenger

The X-ray inactivation of the conidia of two-

TABLE 6. RESULTS OF DOSAGE-FRACTIONATION STUDIES

EXPERIMENT NO.	FRACTION			OBSERVED ABERRATION FREQUENCY PER CELL	EXPECTED FREQUENCY	
	First at Time t	Second at $t + 30$ min	Third at $t + 60$ min		When all Breaks Interact	When all Fractions are Additive
1	400			0.087	0.078	
2	BAL 400			0.043	0.041	
3	800			0.333	0.335	
4	BAL 1000			0.263	0.258	
5	400	BAL 400		0.263	0.233	0.119
6	400	BAL 200	BAL 200	0.270	0.233	0.098
7	BAL 400	BAL 400		0.153	0.165	0.082
8	BAL 400	BAL 200	BAL 200	0.090	0.165	0.061
9	BAL 500	BAL 400		0.213	0.209	0.105
10	BAL 500	BAL 200	BAL 200	0.147	0.209	0.084
11	400		400	0.353	0.335	0.156
12	400		BAL 400	0.233	0.233	0.119
13	BAL 600		BAL 400	0.133	0.258	0.134
14	BAL 400	BAL 400	BAL 200	0.293	0.258	0.091
15	BAL 600			0.93	0.092	

component heterokaryons has been discussed previously.¹⁴ Obligate three-component heterokaryons are also possible, and their properties should be predictable from the behavior of the two-component systems. A triple heterokaryon has been prepared from three double-mutant stocks designated *a*, *b*, and *c*. Stock *a* requires pantothenate and *p*-aminobenzoic acid (PABA); stock *b*, pantothenate and nicotinic acid; and stock *c*, nicotinic acid and PABA. None of these stocks will grow by itself unless both of the indicated factors have been added to the medium. The heterokaryon formed by any combination of two of these stocks will require only a single growth factor, and complete independence of the growth factors is achieved by a combination of all three stocks. Since this triple combination is the prerequisite to growth on minimal medium, it may be properly regarded as an obligate triple heterokaryon.

When multinucleate conidia are formed by the

¹⁴K. C. Atwood and F. H. Mukai, *Biol. Quar. Prog. Rep.* Nov. 10, 1951, ORNL-1167, p 29.

TABLE 7. NUMBER OF ABERRATIONS PER CELL PRODUCED AS INTENSITY OF RADIATION DECREASES

DOSE GIVEN IN t MINUTES (r)	TIME t (min)	NUMBER OF ABERRATIONS PER CELL
600	3	0.177
	60	0.193
	120	0.157
	180	0.093
900	5	0.420
	60	0.436
	180	0.433

triple heterokaryon, seven different nuclear combinations are possible, whereas only three combinations are possible with a double heterokaryon. The seven classes of conidia comprise three homokaryotic types (the original three stocks), three

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kinds of double heterokaryon, and the triple heterokaryon. The relative frequencies of the seven types in the cell population can be determined by plating on eight different media: one minimal, three singly supplemented, three doubly supplemented, and one triply supplemented, respectively. If the three nuclear components are designated by *a*, *b*, and *c*, then the classes of multinucleate cells capable of growth on the various media are as shown in Table 9. As an illustration of the way in which the relative frequencies of the cell types may be determined by differential

plating, consider the experiment shown in Table 10, where the same volume of an appropriate dilution of a conidial suspension from the triple heterokaryon was plated on each of the eight media. The smallest number of cells can grow on the minimal medium and represents the cells containing all three components. The frequencies of each of the three doubly heterokaryotic types are then computed by taking the difference between the count on each singly supplemented medium and the count on the minimal medium. The frequencies of the three homokaryotic types are then computed by

TABLE 8. RESULTS OF DOSAGE-FRACTIONATION STUDIES

EXPERIMENT NO.	FRACTION		OBSERVED ABERRATION FREQUENCY PER CELL	EXPECTED FREQUENCY	
	First at Time <i>t</i>	Second at <i>t</i> + 75 min		When all Breaks Interact	When all Fractions are Additive
1	600 <i>in vacuo</i>		0.115		
2	400 air		0.087		
3	600 <i>in vacuo</i>	400 air	0.220	0.380	0.195
4	600 <i>in vacuo</i> , 0°C	400 air	0.367	0.380	0.195
5	600 <i>in vacuo</i> + KCN		0.123		
6	400 air + KCN		0.080		
7	600 <i>in vacuo</i> + KCN	400 air	0.367	0.410	0.203

TABLE 9. GROWTH REQUIREMENTS OF CONIDIAL TYPES DERIVED FROM A TRIPLE HETEROKARYON

CONSTITUENTS OF THE MEDIUM	NUCLEAR CONSTITUENTS OF CELLS CAPABLE OF GROWTH*
Minimal	(<i>abc</i>)
+ Pantothenate	(<i>abc</i>) (<i>ab</i>)
+ PABA	(<i>abc</i>) (<i>ac</i>)
+ Nicotinic acid	(<i>abc</i>) (<i>bc</i>)
+ Pantothenate + PABA	(<i>abc</i>) (<i>ab</i>) (<i>ac</i>) (<i>a</i>)
+ Pantothenate + nicotinic acid	(<i>abc</i>) (<i>ab</i>) (<i>bc</i>) (<i>b</i>)
+ PABA + nicotinic acid	(<i>abc</i>) (<i>ac</i>) (<i>bc</i>) (<i>c</i>)
+ Pantothenate + PABA + nicotinic acid	(<i>abc</i>) (<i>ab</i>) (<i>ac</i>) (<i>bc</i>) (<i>a</i>) (<i>b</i>) (<i>c</i>)

**a* = Pantothenate-PABA,

b = Pantothenate-nicotinic acid,

c = PABA-nicotinic acid.

TABLE 10. RESULTS OF DIFFERENTIAL PLATING OF THE CONIDIA FROM A TRIPLE HETEROKARYON

Summation of the separate frequencies: 506

CELL TYPE*	TYPE OF MEDIUM (minimal plus additives)							
	Minimal	Pantothenate	PABA	Nicotinic Acid	Pantothenate + PABA	Pantothenate + Nicotinic Acid	PABA + Nicotinic Acid	Pantothenate + PABA + Nicotinic Acid
(a)					59			59
(b)						77		77
(c)							52	52
(ab)		48			48	48		48
(ac)			48		48		48	48
(bc)				146		146	146	146
(abc)	76	76	76	76	76	76	76	76
Plate counts**	76	124	124	222	231	347	322	495

*a = Pantothenate-PABA,
 b = Pantothenate-nicotinic acid,
 c = PABA-nicotinic acid.

**Counts are the average of approximately 10 plates of each medium.

extension of the same method. When frequencies determined in this way for the seven different cell types are summed, the result is in remarkably close agreement with the observed total count on triply supplemented medium. This agreement serves as a check on the validity of the assumptions underlying the procedure.

By reference to frequency distributions of conidia with different numbers of nuclei, it is seen that the average nuclear number for cultures grown on minimal or vitamin-supplemented media lies between two and three nuclei per cell.¹⁵ Moreover, the typical form of the distributions is such that cells with three different components are most often exactly trinucleate and cells with two different components most often binucleate. Thus the form of the survival curves is most strongly influenced by the inactivation of trinucleate or binucleate cells, the presence of conidia with higher numbers of nuclei being a factor of secondary importance. This simplifies the interpretation of the survival curves. With two-component heterokaryons the survival curve on doubly supplemented medium is sigmoidal and rapidly approaches (on a semilogarithmic plot) a slope representing the nuclear inactivation constant.

On minimal medium the survival is exponential, with approximately double the nuclear-inactivation constant. This is predominantly a reflection of the properties of the binucleate heterokaryotic cell, in which the inactivation of either nucleus renders the cell inviable on minimal but viable on supplemented medium. Nuclear inactivation among conidia of the triple heterokaryon should lead, as a first approximation, to the following results:

1. An exponential survival curve on minimal medium with three times the nuclear inactivation constant ($\frac{3}{2}$ times more sensitive than the double heterokaryon).
2. On the singly supplemented media, exponential survival curves with twice the nuclear inactivation constant (resembling the double heterokaryon on minimal medium).
3. On triply supplemented medium, a sigmoidal survival curve like that of the double heterokaryon on doubly supplemented medium.
4. On doubly supplemented media, the survival curves should be intermediate between (2) and (3).

These predictions are confirmed by experiment. The survival curves obtained by X irradiation of an

¹⁵K. C. Atwood and F. H. Mukai, *Biol. Quar. Prog. Rep.* May 10, 1952, ORNL-1297, p 25.

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aqueous suspension of the triple heterokaryon followed by plating on the differential media are shown in Fig. 4. It is noteworthy that none of the growth factors involved has any effect on the radiation sensitivity of homokaryotic or wild-type *Neurospora*. These results may be taken as a striking confirmation of the hypothesis that the nuclei are the units of inactivation.

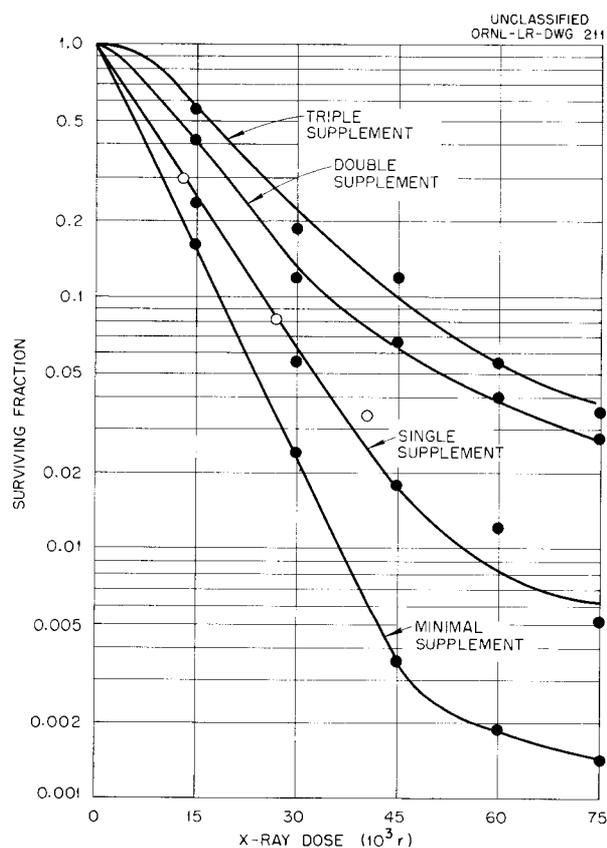


Fig. 4. Survival Curves of a Triple Heterokaryon on Variously Supplemented Media. Conidia in aqueous suspension. X rays at 250 kvp with 1 mm of Al filtration. The open circles are surviving fractions of the two-component heterokaryon, arginine-methionine, on minimal medium, given for comparison. All surviving fractions are referable to the control (unirradiated) count on the corresponding medium. Surviving fractions have been averaged for the three types of single supplement and the three types of double supplement.

INSECT CYTOLOGY AND GENETICS

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Effect of X Irradiation on Nucleic Acid Synthesis in Grasshopper Embryos

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This investigation was initiated to determine whether synthesis of nucleic acids in embryos of the grasshopper *Chortophaga viridifasciata* is altered by X radiation and, if so, whether a correlation could be established between inhibition of nucleic acid synthesis in the embryo and inhibition of mitosis in the neuroblasts.

It was determined that the grasshopper embryo readily incorporates formate, labeled with C¹⁴, into the nucleic acids. Furthermore, it was shown that the small amount of beta radiation emitted by the C¹⁴ which is fixed by the cell does not affect the mitotic rate of the neuroblast cells. These results indicated that the fixation of labeled formate could be used to study the effects of X rays on nucleic acid synthesis in the grasshopper embryo.

Groups of 30 fourteen-day-old embryos (separated from membranes and yolk) were placed in a modified Tyrode's solution and subjected to the following doses of X rays: 64, 128, 256, 400, 700, 1,000, 2,000, 3,000, 4,000, 6,000, 8,000, 12,500, and 20,000 r. Several groups of embryos were irradiated at most of the doses. When mitotic activity (presence of metaphases and anaphases) reached zero, 1 hr after irradiation at all doses, the X-irradiated embryos (and controls) were incubated at 38°C for 30 min in a formate solution (total volume of culture medium was 2.1 ml containing 0.94 μmole of potassium formate; 7.6 μc of C¹⁴). A temperature of 38°C was used because all studies of mitotic activity of grasshopper neuroblasts have been made at this temperature. Embryos were fractionated by a modified Schmidt-Thannhauser method, the deoxyribonucleic and ribonucleic acids (DNA and RNA) being separated by perchloric acid extraction.

The results are plotted in Figs. 5 and 6 as mean specific activities (counts/sec/unit) of DNA and RNA after varying doses of X rays. The mean specific activity of DNA in embryos exposed to 64 r did not differ from that of control embryos

¹⁶Consultant.

(1.214 counts/sec/unit). At doses between 128 and 6000 r the specific activity of DNA was less than that of the controls, the mean specific activity decreasing linearly with logarithmic increase in dose. After doses of 8,000, 12,500, and 20,000 r the specific activity of DNA was almost zero.

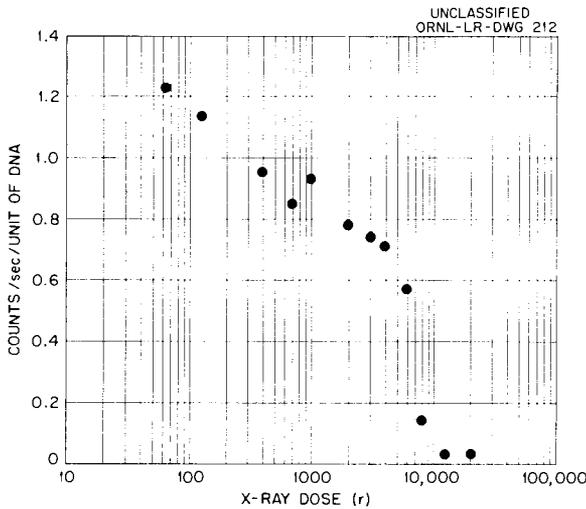


Fig. 5. Effects of X Rays on Utilization of Formate in the Synthesis of Deoxyribonucleic Acid.

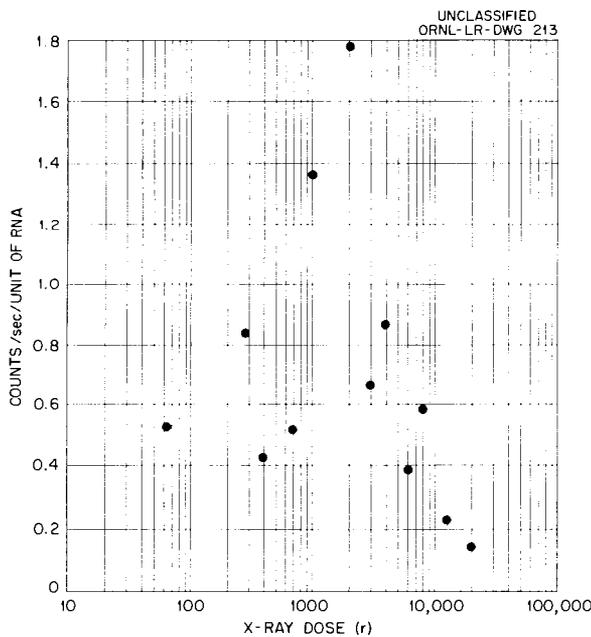


Fig. 6. Effects of X Rays on Utilization of Formate in the Synthesis of Ribonucleic Acid.

It can be seen from Fig. 6 that the synthesis of RNA was less sensitive to X rays than synthesis of DNA. Furthermore, there was no definite trend in the fixation of formate into RNA as the radiation dose increased. The lack of definite effect of X rays on RNA may be due to poor separation of this fraction or it may be real; the data do not permit clarification of this point.

Previous work has shown that an X-ray dose of as little as 3.5 r depresses mitotic activity of the neuroblasts to about 50% of the control activity. Doses of 32 r and above depress mitotic activity to zero; the duration of the inhibition period and time of recovery is directly related to dose. The results herein reported indicate that inhibition of mitosis in the grasshopper embryo at low doses of X rays is not related to an alteration in nucleic acid synthesis. It must be remembered, however, that there are several types of cells in the embryo - they do not divide synchronously and probably have different mitotic rates. In other words, at any given time the embryo contains not only a population of different kinds of cells but a population of different mitotic stages. This fact may account for the seeming lack of sensitivity of nucleic acid synthesis at the low doses. A more sensitive technique than that used in these experiments is required to determine unequivocally the relation, or lack of it, between inhibition of nucleic acid synthesis and inhibition of mitosis.

Nuclear Division in Broken Eggs and Egg Exudates

R. C. von Borstel

A procedure is being devised whereby mitosis may be studied in a system deprived of all limiting cell membranes.

Briefly, the normal condition is as follows: In *Habrobracon*, the unfertilized egg develops and becomes a haploid male. The egg when laid is in the metaphase of the first meiotic division. Meiosis proceeds at the anterior end of the egg on the convex side. Meiosis is completed and the pronucleus starts migrating into the center of the egg at 30 min (20°C) after oviposition. At 38 min after oviposition, the pronuclear membrane disappears and the first nuclear division ensues. Nuclear cleavage continues at the approximate rate of one mitotic cycle every 20 min. The mitotic divisions are synchronous and no cell membranes are formed until 2000 to 8000 nuclei are present.

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When the newly oviposited, unfertilized egg is punctured at the posterior end (end away from the nucleus), much of the egg content flows out and the egg partially collapses. Mineral oil was used to cover the egg to prevent evaporation of water from the exudate. All such eggs passed through meiosis at the normal rate. It is clear that complete structural integrity of the egg is not required for normal chromosome separation to take place.

Pronuclear migration and mitosis also proceeded in eggs which were punctured at the first meiotic metaphase. Of the eggs examined, 89% developed normally to the four-nucleus stage, 78% developed normally to the 64-nucleus stage, and 47% continued development until nuclear migration to the egg periphery at the third hour after oviposition.

The procedure has been further modified to obtain nuclear division in the exudate from broken eggs. In the cases cited, nuclei occasionally migrated into the exudate and continued dividing, but this occurred too rarely to be utilized experimentally. In order to get the single egg nucleus into the exudate, the egg was incubated for 35 min after oviposition (the pronucleus was then undergoing migration), and then the egg was torn laterally, spilling the nucleus along with the egg cytoplasm and yolk. Nuclear division continued in the egg exudate in approximately 50% of the cases. Experiments are now under way to increase the yield.

Most of the technical difficulties have been solved for obtaining a membrane-free mitotic system. It is now possible to study the mitotic effects of chemicals which under normal conditions could not penetrate the egg.

DROSOPHILA GENETICS

W. K. Baker E. S. Von Halle
D. R. Parker¹⁷ C. W. Edington¹⁸

Chromosome Segregation in a Translocation Heterozygote

W. K. Baker E. S. Von Halle

A translocation which was induced in *Drosophila virilis* by X radiation has been used to study chromosome segregation in *Drosophila* males carrying abnormal numbers of Y chromosomes. This rearrangement is a translocation between chromosomes 5 and Y, where the break in 5 is just distal

to the locus of the gene *peach* (*pe*) and thus at the distal end of the centromeric heterochromatin of 5. When the rearranged chromosome carrying the wild-type allele of *pe* is heterozygous with a normal chromosome 5 bearing the mutant allele of *pe*, a variegated eye is produced. This phenotypic change permits the chromosome segregation pattern to be followed in flies of different genotypes.

The genetic scheme used in determining segregation is too involved to present in this report. In essence, the method involves a system of progeny testing by which it is possible to determine from the classes of offspring recovered from a test cross the type of segregation which took place in a P₁ male of a particular genotype.

The data showing the types of segregation observed and their relative frequencies are presented in Table 11. In this table the notation 5^Y indicates the translocated chromosome producing the variegated position effect. The subscript, f or m, denotes translocated chromosomes which are genetically related but which theoretically might be different.

It is conventional to interpret segregation data derived from cases in which three chromosomes share homologous regions on the assumption that two of the chromosomes pair and form a bivalent and the third remains unpaired and passes at random to either pole. If the data are interpreted on this type of pairing, the results shown in Fig. 7 are obtained. It is obvious from a study of this figure that such an interpretation of the pairing relations is invalid. For example, in the X5_f5_f males the pairing would be expected to be as strong between the two translocated chromosomes as it was between the two Y's in the XYY males. However, the data indicate that, on this interpretation, little or no pairing takes place between the two 5^Y chromosomes.

An alternative consideration remains: instead of the formation of a bivalent and a univalent, a trivalent is formed and the pairing relations within this structure direct the segregation. There is precedent for this interpretation in the cytological analysis by Cooper¹⁹ of the meiotic stages of *D. melanogaster* males with abnormal numbers of Y chromosomes. A study of the metaphase I configurations in the translocation heterozygotes has been initiated. Preliminary qualitative studies on

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¹⁸ORINS Predoctoral Fellow.

¹⁹K. W. Cooper, *J. Morphol.* 84, 81-129 (1949).

males of the type $XY5^Y$ indicate that most frequently a trivalent is formed, thus confirming the conclusions reached from the genetic data.

Radiation-Induced Separation of Attached-X Chromosomes in Females of *Drosophila melanogaster*

D. R. Parker

It has been known for some time that X rays can

bring about the separation of attached-X ($X.X$) chromosomes, even in the absence of any Y chromosome, whereas spontaneous detachments of attached-X's will occur only in the presence of a Y, the separation being a result of crossing over between X and Y, giving rise to two kinds of J-shaped chromosomes, each consisting of an X with an attached arm of the Y ($X.Y^S$ or $X.Y^L$).

These experiments are an attempt to answer three

TABLE 11. RELATIVE FREQUENCY OF TYPES OF GAMETES PRODUCED BY MALES OF PARTICULAR CHROMOSOMAL CONSTITUTIONS

CHROMOSOME CONSTITUTION OF MALE	TYPES OF GAMETES							
	Y	YY	X	XY	$X5^Y$	5^Y	$Y5^Y$	5^Y5^Y
$XY Y^*$								
a^{**}	0.422	0.073	0.107	0.398				
b	1353	233	342	1275				
c	0.41	0.09	0.09	0.41				
$XY5_f^Y$								
a	0.178		(0.281)		0.219	0.184	0.138	
b	76		(108)		84	79	59	
c	0.20		0.13	0.17	0.20	0.17	0.13	
$X5_f^Y5_m^Y$								
a			0.227		0.273	0.267		0.233
b			222		267	222		194
c			0.23		0.27	0.27		0.23
$X5_f^Y5_f^Y$								
a			0.257		0.243	0.256		0.244
b			157		149	162		154
c			0.25		0.25	0.25		0.25
$XY5_f^Y5_f^Y$								
a^{***}				0.131	0.304		(0.555)	
b				40	93		(170)	
c				0.15	0.34		0.34	0.15

*Data taken from H. Kikkawa, *Cytologia* 6, 177-194 (1935).

**a = Actual frequency of observed types of gametes,

b = Number of testcrosses used to determine the frequency,

c = Calculated frequency.

***Only 2% of the segregations are of the 3-1 type.

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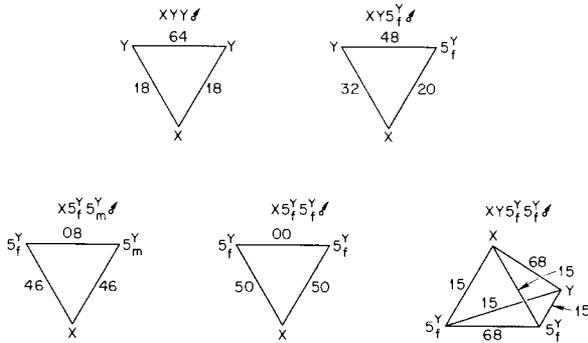


Fig. 7. Percentage Frequency of Pairing Determined from Segregation Data on the Assumption that Either a Bivalent and a Univalent or Two Bivalents are Formed.

questions relating to the induced separation of attached-X's. Is the frequency of induced separation of attached-X's in any way dependent on the presence or absence of a Y? Do these separations ever involve exchanges between attached-X's and Y, and if so, how often? Is there any dependence of the frequency of detachment on the wave length of radiation used?

The stocks used were $y^2 su-w^a w^a bb$ and $y v f car$, either as X.X or as X.X/sc⁸.Y. The sc⁸.Y is marked by having the normal alleles of yellow and achaete attached to the distal end of the long arm of the Y. X.X (no Y) females were obtained by crossing to $v f B$ males with both arms of the Y attached to the X, hence having no free Y. In all cases, females were placed individually in vials with two Muller-5 males each, and removed after four days. In tabulating the results, only attached-X females and females or males with a detached-X are included in the calculations. This is necessary as a correction, since only a very few Muller-5 X0 males survive, most dying during early pupal development. As a precaution, any culture producing Muller-5 males from attached-X no-Y females was discarded, since the possibility of accidental inclusion of results from X.X Y females could thereby be excluded.

The results in comparison of X.X with X.X/sc⁸.Y are listed in Table 12. The values in the case of the $y v f car$ attached-X are seen to be about half those of the $y^2 su-w^a w^a bb$ stock. It was found that spontaneous lethals had accumulated in the proximal region of the former so that most of these

detachments are lethal in the male. Hence these values should be approximately doubled to compensate for the difference in viability, making the results comparable in the two different stocks.

Although the numbers are small, it seems unlikely that the presence or absence of a Y chromosome has any large-scale effect on the rate of induced detachment. This experiment is being continued to see if any such difference may be demonstrated.

X chromosomes derived by detachment of attached-X chromosomes may be tested for the presence of an attached-X arm by crossing males with this X to attached-X females also having an incomplete Y ($y f = /Y^S$ or Y^L). Since both arms of the Y are necessary for male fertility, only the combinations of $X.Y^L/Y^S$ or $X.Y^S/Y^L$ will be fertile. The marked Y arms, sc.Y^L and sc.Y^S, were used in this test.

Should an X have an attached-X fragment without all the fertility factors of that arm, it might be possible in some cases to detect the presence of the fragment by the markers y^+ in the long arm of the sc⁸.Y, or bb^+ in the short arm.

All stocks derived from X.X/sc⁸.Y in which males with the detached X were viable were tested in this manner. These included 10 stocks listed in Table 12, and one from a previous experiment, not described here. The results of these tests are given in Table 13.

Four of the detachments are X.Y^L, four are X.Y^S, two are X.Y^L (incomplete), and one either does not involve the Y or involves a part which could not be detected. In this one case, since the stock employed was $y v f car$, it would be impossible to detect any fragment of Y^S which did not include all the necessary fertility factors.

It would seem that most of the induced detachments in X.X Y females involve exchanges with the Y chromosome; this result is not to be expected on the basis of the data in Table 12.

In order to find out if there is any dependence of frequency of detachment on the quality of radiation, the following treatments were employed: in the x-ray series either 80 kvp with 3 mm of aluminum filter, 27.5-cm target distance, giving a dose rate of approximately 100 r/min, or 250 kvp with 3.5 mm of copper filter, 33-cm target distance, with a dose rate of approximately 100 r/min. The flies were not covered with plastic, making possible a source of error, since secondary equilibrium would possibly not be reached in small objects. (The experi-

ments are being repeated, using a 1/4-in. covering of Lucite.) A distributed source of Co⁶⁰ was used as the gamma-ray source. The flies were surrounded by 1/4-in.-thick Lucite; the dose rate was approximately 1700 r/min. The results of this experiment are given in Table 14 and shown graphically in Fig. 8. In both the 80-kvp X-ray and the gamma series, the frequency increases approximately as the square of the dose; the X rays seem to be about twice as effective per unit of dose as the gamma rays.

X-Ray-Induced Crossing Over in Females of *Drosophila*

D. R. Parker

Earlier experiments by various workers on X-ray-induced crossing over in *Drosophila* females have involved comparison of egg-laying periods each of several days' duration, with the possibility that increases or decreases in frequency of crossing over might be missed if these changes in frequency did not correspond to the sampling periods used.

TABLE 12. DETACHMENT FREQUENCY OF ATTACHED-X CHROMOSOMES WITH AND WITHOUT A Y CHROMOSOME

Treatment: 1000 r, 80 kvp with 3-mm aluminum filter, 100 r/min

STOCK	X.X (no Y) FEMALES			X.X sc ⁸ Y FEMALES		
	Total Number Counted	Number With Detached-X Chromosome	Percentage Frequency	Total Number Counted	Number With Detached-X Chromosome	Percentage Frequency
<u>y² su-w^a w^a bb</u>						
Treated	1131	12	1.06	813	8	0.96
	2452*	18*	0.73*			
Control	3447*	0*	0*	788	0	0
Difference			0.84			0.96
<u>y v f car</u>						
Treated	1144	5	0.43	477	4	0.84
Control	512	0	0	656	2	0.30
Difference			0.43			0.54
Difference × 2			0.86			1.08

*See Table 14.

TABLE 13. FERTILITY TESTS OF MALES WITH DETACHED-X CHROMOSOMES

PHENOTYPE OF HOMOZYGOUS FEMALE	FERTILE WITH ADDED Y ^L	FERTILE WITH ADDED Y ^S	FERTILE WITH NEITHER Y ^S NOR Y ^L
y ⁺ (bb)		4	2
y (bb ⁺)	4		
y (?)			1*

*y v f car, hence cannot detect presence or absence of Y fragment containing bb⁺.

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TABLE 14. DETACHMENT FREQUENCY WITH 1000 r OF X OR GAMMA RADIATION

RADIATION	DOSE (r)								
	500			1000			2000		
	Total Number counted	Number with detached-X chromosome	Percentage frequency	Total Number counted	Number with detached-X chromosome	Percentage frequency	Total Number counted	Number with detached-X chromosome	Percentage frequency
80-kvp X ray	3615	5	0.14	2452	18	0.73	1070	29	2.71
250-kvp X ray				1374	4	0.30			
Co ⁶⁰ gamma	3949	4	0.10	3090	12	0.39	1017	11	1.08
Control	3447	0	0	3447	0	0	3447	0	0

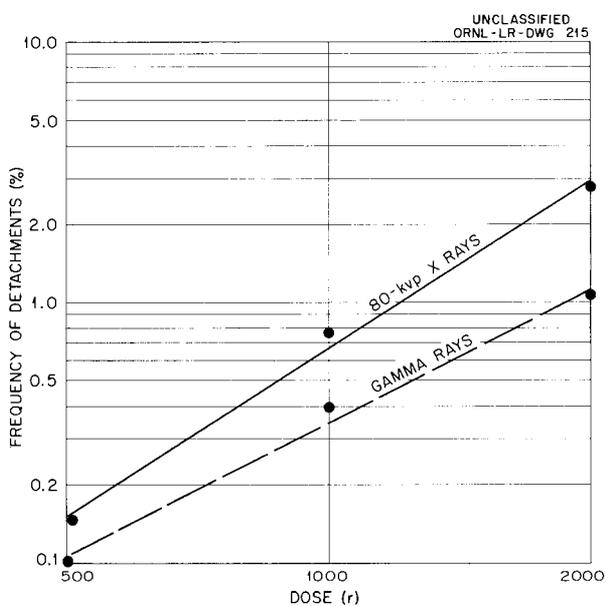


Fig. 8. Frequency-Dose Relation for Detachment of Attached-X Chromosomes.

Females heterozygous for the multiple recessive third chromosome stock "3ple" (*ru*, *roughoid*; *h*, *hairy*; *st*, *scarlet*; *p^p*, *pink-peach*; *ss*, *spineless*; *e^s*, *sooty*) were treated at an age of 0 to 12 hr after emergence. The treatment was 1000 r at 250 kvp, 30 ma, using a 2-mm aluminum filter. The dose rate was approximately 325 r/min. These females were mated immediately with three "3ple" males each, and placed in separate vials. Since very few eggs are laid on the first day after mating, transfers were begun after the second day and made daily until the end of the fourteenth day after treatment, when the adults were discarded. Egg counts were made for each female for each of these 13 days of

egg laying. After the fourth day of egg laying, there is only a slight difference in total number of eggs laid by treated and control females.

The percentage hatch by day is listed in Table 15. Hatchability in the treated series is markedly reduced on the first day of egg laying but increases rapidly to about 90% of the control value by the third day, varying around that value or slightly higher for the rest of the experiment.

The frequency of crossing over by day and by region is given for treated and for control series in Table 16. Significant increases are found in the following regions on the following days:

Region	Days
1	1, 2
2	3, 8, 9, 12
3	4, 7, 9, 10, 11, 12
4	3, 6, 8, 9
5	8, 10

Crossing over is induced in meiotic prophase, certainly in the first four days of egg laying; the induced crossing over detected in the later egg-laying periods may result from cells treated in premeiotic stages. The results over the first five days indicate some kind of terminalizing "competence" for induction of crossing over, since distal increases are found first and proximal increases later. This might be explained by a "hit" on a terminalizing chiasma resulting in induced crossing over. In this case, about half the increase should be expected to be in double crossing over in the same arm of the chromosome. The frequencies of the pertinent double crossover classes for the days on which increases in crossing over in distal regions were found (prior to the second cycle of

TABLE 15. PERCENTAGE OF EGGS GIVING ADULTS

DAY	CONTROL	EXPERIMENTAL	EXPERIMENTAL AS PERCENTAGE OF CONTROL
1	96.7	67.0	69.3
2	95.5	77.9	81.5
3	91.7	82.2	89.6
4	92.3	80.1	86.9
5	86.5	75.3	87.0
6	89.2	85.9	95.5
7	83.0	86.6	104.4
8	92.3	83.1	90.0
9	94.9	87.0	91.7
10	99.0	88.3	89.2
11	95.0	90.0	94.8
12	98.6	89.4	90.6
13	93.8	89.9	95.8

increase) are given in Table 17. Only in region 1 on the first day can the increase be accounted for as a "hit" on a terminalizing chiasma.

Table 16 likewise shows a significant decrease in crossing over in region 1 on days 9, 10, 11, and 13. This might be explained as resulting from induction of crossing over in either region 2 or 3 in a premeiotic stage, with the result that half the cells (following equational separation at the centromere) will be homozygous either for *ru* or for its normal allele, making detection of crossing over which may occur spontaneously at meiosis impossible in region 1 in such cells. Calculation of the expected reduction shows it to be only slightly more than one-fourth the actual reduction. It is suggested that the reduction effect may be a direct interference with spontaneous crossing over, perhaps an effect on pairing.

TABLE 16. PERCENTAGE OF CROSSING OVER BY REGION AND BY DAY

DAY	REGIONS									
	1 (<i>ru-h</i>)		2 (<i>h-st</i>)		3 (<i>st-p^P</i>)		4 (<i>p^P-ss</i>)		5 (<i>ss-e^S</i>)	
	Exptl.	Control	Exptl.	Control	Exptl.	Control	Exptl.	Control	Exptl.	Control
1	26.01*	19.26	22.27	23.70	8.19	7.35	15.23	14.00	14.66	13.12
2	23.01*	18.67	21.14	21.60	4.80	4.18	12.11	10.60	12.44	12.84
3	22.19	23.06	21.52*	17.32	3.88	2.76	10.83*	7.76	12.83	14.45
4	19.90	23.75	20.23	19.57	4.24*	1.67	8.16	8.19	13.38	11.04
5	23.19	23.75	17.28	19.79	3.12	2.64	7.80	7.65	12.49	14.78
6	22.99	21.44	17.31	17.02	3.22	1.86	8.89*	6.06	13.43	13.29
7	22.01	24.17	16.54	16.31	2.99*	0.60	8.78	7.25	12.34	11.18
8	19.51	22.91	22.24*	15.64	4.03	2.23	9.62*	5.59	13.65*	9.50
9	16.02*	20.98	25.22*	19.58	4.84*	2.10	10.68*	5.13	13.29	10.72
10	19.15*	25.46	20.99	17.51	5.92*	0.53	10.14	9.28	13.94*	9.81
11	17.31*	23.29	20.67	19.75	4.73*	1.52	8.72	8.35	13.82	13.41
12	20.59	24.02	22.14*	16.20	4.50*	1.96	6.05	6.15	10.68	13.41
13	18.07*	24.21	18.32	14.67	2.51	1.22	6.02	5.38	11.54	12.71

*Denotes significant difference (at 5% level).

BIOLOGY DIVISION PROGRESS REPORT**TABLE 17. PERCENTAGE OF DOUBLE CROSSING OVER**

TYPE OF DOUBLE CROSSOVER	PERCENTAGE OF DOUBLE CROSSEVERS ON INDICATED DAY					
	Day 1		Day 2		Day 3	
	Experimental	Control	Experimental	Control	Experimental	Control
1, 2	3.16	0.88	1.30	0.58	0.93	0.64
1, 3	1.43	1.18	0.57	0.87	0.80	0.43
2, 3	1.58	0.68	0.81	0.29	0.26	0
3, 4	1.15	0.98	0.89	0.49	0.80	0.21
3, 5	1.29	0.88	1.22	0.39	0.53	0.85
4, 5	0.57	0.39	0.16	0.25	0.27	0.53

RADIATION PROTECTION AND RECOVERY

PROTECTION AND RECOVERY IN BACTERIA

A. Hollaender
G. E. Stapleton
D. Billen

C. O. Doudney
A. J. Sbarra
R. W. Whittle

Modification of X-Ray Sensitivity of *Escherichia coli* B/r by Cysteamine and β -Mercaptoethanol

A. Hollaender
C. O. Doudney

A study of the mechanism of the protection of *Escherichia coli* strain B/r from X-ray damage by two thiol-containing compounds, cysteamine (β -mercaptoethylamine) and β -mercaptoethanol, has been initiated. Bacterial suspensions from 18-hr aerated cultures were exposed at ice-bath temperature for 30 min prior to irradiation. A balanced salt solution was used as the suspending medium since it has been found that the phosphate buffer previously used in similar studies markedly decreases the protective ability of cysteamine.

Cysteamine is highly effective in protection against radiation killing after treatment at 60,000 r or less. A maximum survival of about 50% at 60,000 r is afforded by a concentration of 0.006 M cysteamine, representing about a twelvefold increase in energy required to obtain an equivalent lethal effect. A higher concentration (0.1 M) of β -mercaptoethanol is required for a maximum survival of 30% at 60,000 r (representing an eightfold increase in energy). Both of these compounds are more effective than BAL (2,3-dimercaptopropanol) which affords a maximum survival of 16% at 0.02 M (representing a sixfold energy increase) (see Fig. 9). Above the 60,000-r energy level, there is a marked nonlinear decrease in the protective ability of cysteamine (but not of β -mercaptoethanol).

Release of Cellular Constituents by X-Irradiated *Escherichia coli*

D. Billen
R. W. Whittle

The release of adenosine triphosphate (ATP) by metabolizing *Escherichia coli* suspensions subsequent to X-ray exposure has been described.¹ In order to evaluate the significance of ATP depletion to the cell with regard to radiation-induced loss of viability, studies were initiated to identify other

¹D. Billen, B. L. Strehler, G. E. Stapleton, and E. Brigham, *Arch. Biochem. and Biophys.* 43, 1-10, (1953).

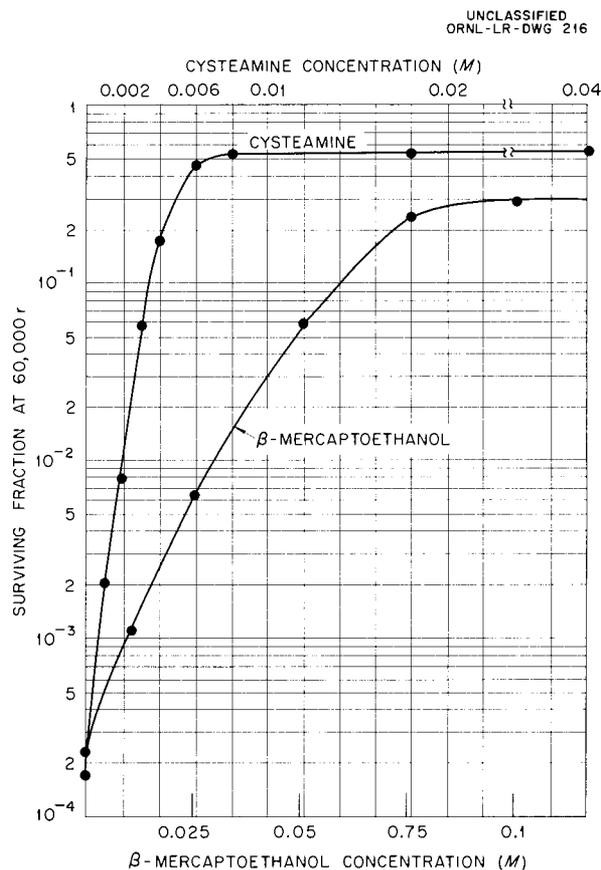


Fig. 9. Effect of Cysteamine and β -Mercaptoethanol on the Survival of *E. coli* B/r During Exposure to 60,000 r of 250-kvp X Rays.

cellular constituents which might be released from cells so treated and to determine what conditions of treatment would prevent or alter this loss of vital constituents. The methods used were essentially those already described.¹

Examination of the ultraviolet absorption spectra of the supernatant obtained from suspensions of X-irradiated (60,000 r) *E. coli* strain B/r, incubated in a phosphate-buffer-glucose medium showed a maximum at 260 μ as compared with relatively little absorption in this range by the supernatant obtained from unirradiated controls (Fig. 10). Quantitative analysis of ATP revealed that the absorption of light of wave length 260 μ caused by the presence of ATP could account for less than 10% of the total absorption found at this wave

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length. By use of ion-exchange chromatography on Dowex-1 columns, it was established that nucleic acid fragments varying in composition from free bases to nucleotides accounted for the remaining material absorbing in the ultraviolet region. It was also found that the quantity of ninhydrin-positive material present in the supernatants from exposed-incubated cells was similar to that found in the control supernatants. This was true in both acid-hydrolyzed and unhydrolyzed samples and indicated that X-ray exposure did not cause a subsequent loss of amino acids or peptides from the exposed cells greater than that found with the control

suspensions. Thus it appears that the release of cellular constituents is restricted to certain classes of compounds and is not due to general release of material from irradiated cells.

Conditions That Influence Release of Cellular Constituents. The data presented in Fig. 10 show that the presence of phosphate buffer is essential for development of the release of 260-m μ -absorbing material by X-irradiated cells since little absorption of ultraviolet light is noted in the supernatant obtained from irradiated cells incubated in distilled water plus glucose. The small absorption peak noted is probably due to an endogenous phosphate supply. The necessity of an added substrate that can be actively metabolized by the irradiated cells was found not to be so exacting for the development of cellular "leakage" as that for inorganic phosphate. In the many repeat experiments carried out, it was observed that the absence of added glucose in the phosphate-buffer solution, in which the irradiated cells were suspended, gave results that varied from essentially no release of 260-m μ -absorbing material (Fig. 11) to as much as 80% of that found when glucose was added. This variance might best be explained by assuming that the endogenous carbohydrate level of the cells differed from experiment to experiment.

Because of the implication of orthophosphate involvement in the events leading to release of cellular constituents from X-irradiated *E. coli*, it was thought feasible to determine if the addition of a chemical antagonist of phosphate utilization such as arsenate, which is known to disrupt oxidative phosphorylations, would alter the development of the leakage. It was found that the addition of sodium arsenate would effectively inhibit the release of 260-m μ -absorbing materials from exposed cells incubated in a phosphate-buffer-glucose medium (Fig. 11). The data presented in Table 18 show that the inhibitory effect is dependent upon the arsenate-phosphate ratio. Increasing the phosphate concentration results in an increased arsenate requirement. The possibility that either a pH or toxicity effect is responsible for the action of arsenate was ruled out in experiments in which the influence of various hydrogenous concentrations and also the influence of salts such as KCl or NaCl were studied for their effect on the development of leakage. It was also found that the concentrations of arsenate necessary to prevent leakage did not interfere with glucose oxi-

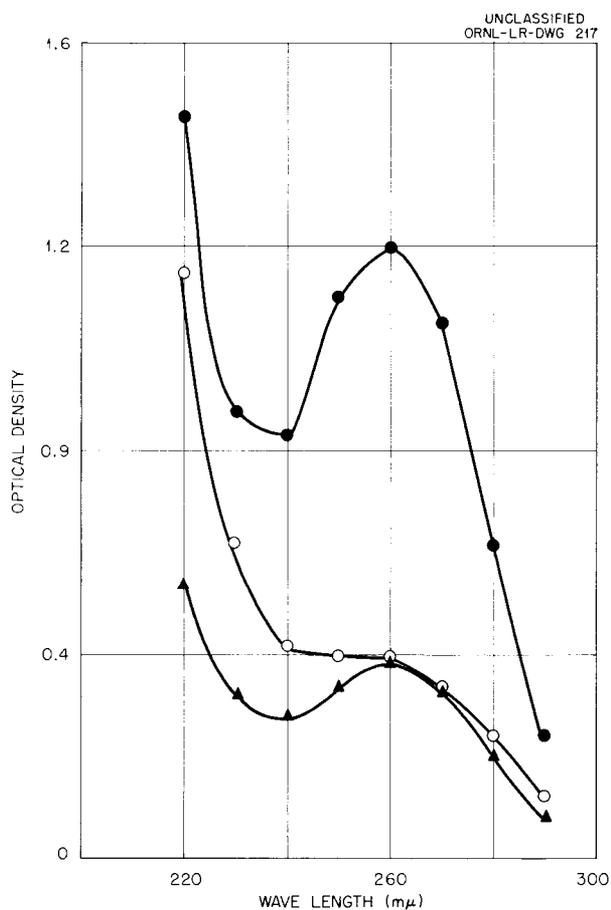


Fig. 10. Ultraviolet Spectra of Supernatant Fluids from *E. coli* Suspensions. The absorption of the supernatant fluids at 260 m μ was measured after 90 min incubation at 37°C of X-irradiated cells suspended in 0.067 M phosphate buffer (pH 6.8) and 1% glucose (●-●); 1% glucose (▲-▲); and of unirradiated cells suspended in 0.067 M phosphate buffer and 1% glucose (○-○).

dation or glycolysis; on the contrary, stimulation was observed. To lend further support to the evidence that an active metabolizing state is necessary for leakage development, the influence of temperature on this release of constituents by X-irradiated cells was investigated. It was found that if the exposed cells were suspended in phosphate-buffer-glucose medium and incubated at ice-bath temperatures (0 to 2°C) there was no release of ultraviolet-absorbing material into the

medium. The appearance of these nucleic acid fragments (as well as ATP) in the suspending fluid coincides with the reported breakdown of nucleic acid-containing components found in such cells.²

On the basis of the evidence accumulated to date, it would appear that a phosphorylation of some compound or compounds is necessary before the release of cellular constituents absorbing in the ultraviolet region is brought about in X-irradiated cells. The need for phosphate, the antagonistic action of arsenate, and the necessity of conditions favoring an active metabolic state (involving phosphate utilization) support such a conclusion.

Postirradiation Recovery of *Escherichia coli* B/r

G. E. Stapleton

A. J. Sbarra

C. W. Edington

The spread plate assay for recovery of *E. coli* B/r has been described.³ This technique has been used exclusively during this period for assessing the activity of separated fractions from tissue extract and available pure chemical compounds. Paper chromatography of yeast extract and other extracts showed several active fractions which appeared to act in a synergistic manner when used simultaneously. This finding suggested a multiple requirement for increased viability of irradiated bacteria. It was decided to investigate the role of amino acids which were present in the extracts used. A large number of known amino acids were tested individually and in all possible combinations. Since a tabulation of the data obtained would offer laborious reading, a summary of the findings is presented in Table 19. It is of interest to note that a combination of four amino acids, namely, glutamic acid, serine, methionine, and tryptophane, assayed together at the 1-mg level gave increases in survival essentially similar to that obtained by pooling thirteen of the amino acids known to be present in the active extracts of natural materials.

Quantitative estimates of the relative activities of these amino acids, based on survival curves for bacteria as a function of gamma-ray dose, showed that 40 to 50% of the recovery effect obtained with yeast extract could be brought about by these four

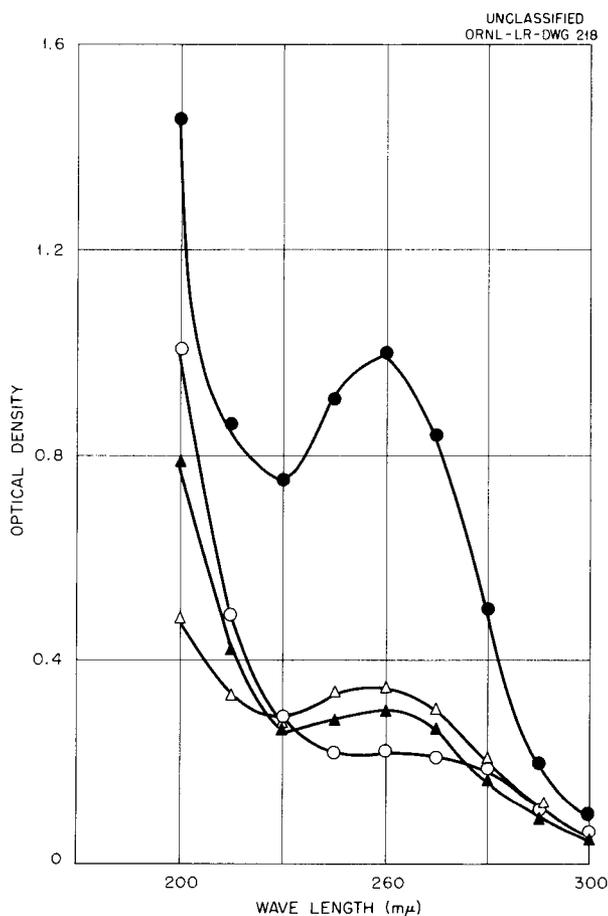


Fig. 11. Effect of Arsenate Addition or Metabolite Deletion on the Release of 260 mμ Absorbing Material from X-Irradiated *E. coli* Suspensions. The absorption of the supernatants was measured after 90 min incubation at 37°C of X-irradiated cells suspended in 0.0083 M phosphate buffer (pH 6.8) and 1% glucose (●—●); phosphate, glucose, and 0.01 M sodium arsenate (▲—▲); or phosphate buffer alone (Δ—Δ). The absorption spectra of the supernatant from unirradiated *E. coli* suspended in phosphate-glucose solution is indicated (O—O).

²D. Billen and E. Volkin, *J. Bacteriol.* (1954) (in press).

³G. E. Stapleton, C. W. Edington, and P. W. Rueff, Jr., *Biol. Semiann. Prog. Rep.* Aug. 15, 1953, p 37.

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TABLE 18. ARSENATE INHIBITION OF THE RELEASE OF 260 m μ -ABSORBING MATERIAL FROM X-IRRADIATED *ESCHERICHIA COLI*

ARSENATE CONCENTRATION (M)	OPTICAL DENSITY* AT 260 m μ		
	Experiment 1	Experiment 2	
	0.067 M phosphate	0.067 M phosphate	0.0083 M phosphate
0	0.92	0.74	0.60
0.005	0.77		
0.01	0.72	0.60	0.11
0.05	0.17		0.02
0.1	0.05		

*Corrected for controls.

TABLE 19. EFFECT OF COMBINATIONS OF AMINO ACIDS ON RECOVERY

AMINO ACIDS	RELATIVE SURVIVAL — BASAL + ADDITIONS: BASAL
(1) Glutamate	10
(2) Serine	5
(3) Methionine	15
(4) Tryptophane	11
1 + 2	46
1 + 3	24
1 + 4	28
2 + 3	30
2 + 4	25
3 + 4	25
1 + 2 + 3	35
1 + 3 + 4	40
1 + 2 + 4	60
2 + 3 + 4	40
1 + 2 + 3 + 4	100
Pool of 13 amino acids	60

amino acids. A search is now being made to isolate the additional components in tissue and yeast extracts which bring about the remaining recovery. Routine assays now include these amino acids as an integral part of the system. The activity of the amino acids in stimulating recovery suggested that polypeptides might be active in the recovery process. A number of dipeptides and polypeptides have now been tested for recovery activity, but none appear to be any more active than their component amino acids.

The large-scale production of purified spleen extracts mentioned in the last report³ has been curtailed since such fractions were found to be unstable in solution at refrigerator or deep-freeze temperatures. Purified fractions will be accumulated in future preparations only in the dried state. The fractions which became inactive on holding at low temperatures have been hydrolyzed and/or treated with reducing agents in an unsuccessful attempt to regenerate the lost activity. Some attempt will be made in the future to stabilize the activity of purified fractions and to learn the nature of the process resulting in decay of the stimulatory activity of these fractions.

ENZYMOLOGY AND PHOTOSYNTHESIS

ENZYMOLOGY

J. R. Totter A. N. Best
 W. T. Burnett, Jr. M. J. Cormier
 J. V. Passonneau¹

**Metabolism of Formate-C¹⁴ and Adenine-8-C¹⁴ by
 Chick Embryos**

J. V. Passonneau J. R. Totter

Chick embryos from eggs which had been incubated for 50 to 60 hr were used to study formate and adenine metabolism under various conditions. The surviving embryos when suspended in saline solution were found to incorporate formate at a rate of about 0.015 μ mole per hour per embryo. Adenine was incorporated into the nucleic acids of the embryos at a rate comparable to formate fixation into these substances. The dry weights of the embryos were 10 to 20 mg.

The effects of treatment of the embryo with the antifolic acid compound, aminopterin, have been investigated with the two tracers, and alterations in their metabolism brought about by gamma irradiation of the embryos are being studied.

The embryos, after removal from the egg, were suspended in saline. Irradiation was carried out under air at room temperature (20°C) in a Co⁶⁰ irradiator delivering 2000 r/min. Controls were treated in the same manner except for irradiation. When aminopterin was used, it was added to the saline suspensions 15 min prior to addition of tracer substrate. After a 2-hr incubation period with added tracer (38°C under air, with shaking), the embryos were centrifuged from the suspension and lipids extracted with alcohol, alcohol-ether (3:1), and ether. The acid-soluble fraction was then removed by three cold 5% trichloroacetic acid (TCA) washes. Ribonucleic acid was extracted with cold 1 N HClO₄ and deoxyribonucleic acid with hot TCA.

The effects of the experimental treatments on the radioactivity of the ribo- and deoxyribonucleic acid fractions are shown in Table 20. Either gamma irradiation or treatment with aminopterin was found to inhibit formate carbon incorporation into the nucleic acids. In contrast, little depression in radioactivity was found when adenine-8-C¹⁴ was employed as a tracer.

¹Consultant.

Interpretation of these results depends on a more complete knowledge of the precursors of the nucleic acid purines and pyrimidines. The related purines in the acid-soluble fractions from aminopterin-treated and control embryos have been separated by ion-exchange and paper chromatographic methods. The specific activities of the isolated and purified compounds are given in Table 21. The very striking difference in specific activities of the hypoxanthines was an unexpected finding. Sufficient data to permit a satisfactory interpretation of this result are not yet available. Further data will be obtained.

Two possible interpretations for the adenine results (Table 20) suggest themselves: (1) there may be a radiosensitive reaction involved in the incorporation of formate into adenylic acid, or (2) free adenine itself may enter into preformed nucleic acids by exchange, even though the cell is so damaged that new synthesis of nucleic acid is impaired.

Investigations on Ergothioneine

W. T. Burnett, Jr.

It would appear a priori that ergothioneine, a naturally occurring sulfhydryl compound associated with the mammalian red cell, might be one determinant of the sensitivity of mammals to ionizing radiations. This possibility now seems more plausible since evidence has recently been reported that sulfhydryl compounds are essential for the integrity of the red cell. The results of work now in progress suggest that ergothioneine is partially destroyed or that it leaks from the red cells of rats following exposure to 400 r of X rays. If it can be shown that the decrease in the ergothioneine content of the cells is related to the radiation dose, the determination of this compound might serve as a "radiation dosimeter." Preliminary tests of ergothioneine as a protective agent for mice are encouraging, although not yet conclusive. Ergothioneine is biologically stable. The body stores of ergothioneine can be increased by supplementing the ration of an animal with the pure compound or with certain foods. This attractive possibility of thus increasing radiation resistance in mice is being examined.

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TABLE 20. RELATIVE RADIOACTIVITY IN RIBO- (RNA) AND DEOXYRIBONUCLEIC (DNA) ACID FRACTIONS FROM CHICK EMBRYOS AFTER VARIOUS TREATMENTS

	RELATIVE RADIOACTIVITY			
	Potassium Formate-C ¹⁴ ^(a)		Adenine-8-C ¹⁴ ^(b)	
	RNA	DNA	RNA	DNA
Aminopterin ($\mu\text{g/ml}$)				
0	100	100	100	100
0.2	111	92		
0.4	104	89		
1.0	101	72		
2.0	62	63		
5.0	70	57		
10.0	49	34		
50.0	24	20	95	65
50.0			100 ^(c)	133 ^(c)
Irradiation (r)				
0	100	100	100	100
1,000	109	110	115	100
2,000	91	90		
5,000	80	79	81	75
8,000	67	67		
10,000	57	54	107	90
11,000	49	43		
20,000	42	38	148	123

(a) Two embryos incubated 2 hr with 3.6 μc (0.4 μmole) of potassium formate-C¹⁴ in 1 ml saline.

(b) As in (a) with 0.1 μc (0.1 μmole) of adenine-8-C¹⁴ instead of potassium formate.

(c) As in (a) with 0.4 μc (0.4 μmole) of adenine-8-C¹⁴ instead of potassium formate.

TABLE 21. PURINES FROM ACID-SOLUBLE FRACTIONS OF CHICK EMBRYOS LABELED WITH POTASSIUM FORMATE-C¹⁴

	SPECIFIC ACTIVITY (counts/sec μmole)	
	Control	Aminopterin Treated
Hypoxanthine	324	11,500
Xanthine	<0.3	3,840
Guanine	<0.3	235
Adenine	1120	1,120

PHOTOSYNTHESIS

W. A. Arnold

E. S. Meek

Further Studies on the Delayed Light Emission of Green Plants and Evidence That Light Energy Is Transferred Long Distances Within the Chloroplasts

W. A. Arnold

E. S. Meek

Chlorella plants that had been kept in the dark for some minutes were illuminated with a single flash of light; the intensity (S) of the delayed light was measured. It was found that if the duration of the flash (T) was less than a few hundredths of a second, S depended only on the product IT , where I is the intensity of the flash.

The relation between S and IT is approximately

$$S = S_0 (1 - e^{-\sigma IT}) ,$$

where S_0 is the saturation value of the delayed-light intensity and σ is a constant.

If it is assumed that the reaction which stores energy for the delayed-light production is



that the intensity of the delayed light is proportional to the amount of B , and that saturation implies starting the flash with a definite amount of A , then the experimental relation can be derived.

Let X be the amount of A at any time and X_0 the amount at the beginning of a flash. The rate at which A is changed to B is

$$\frac{dX}{dT} = -\sigma IX ,$$

where σ is the cross section in square centimeters for the reaction. The amount of A at the end of a flash T seconds long will be

$$X = X_0 e^{-\sigma IT}$$

and the amount of B will be

$$X_0 - X_0 e^{-\sigma IT} .$$

If it is assumed that the delayed light is proportional to the amount of B ,

$$S = S_0 (1 - e^{-\sigma IT}) .$$

Since the slope will be $S_0 \sigma$ for small values of IT , it is clear that the absolute value of σ can be determined by dividing the initial slope of the S against the IT curve by the saturation value. I must be expressed in terms of light quanta per square centimeter per second, and T must be expressed in seconds; however, the amounts of A and B do not have to be known.

Determinations of σ have been made using red light at wave lengths of 6000 to 7000 Å giving

$$\sigma = 3.1 \times 10^{-14} \text{ sq cm} .$$

The action spectrum for the production of delayed light given by Strehler and Arnold² shows that chlorophyll absorbs the light; however, the cross section of a chlorophyll molecule for red light is

$$\sigma_{ch} = 0.5 - 1.4 \times 10^{-16} \text{ sq cm} .$$

Thus it is believed that any one of several hundred chlorophyll molecules can absorb the energy that makes one A go to B and that the energy can be transferred through the chloroplasts for a distance comparable to the space occupied by several hundred chlorophyll molecules.

²B. L. Strehler and W. A. Arnold, *J. Gen. Physiol.* 34, 809-820 (1951).

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell – Section Chief

E. F. Oakberg	M. K. Freeman
L. B. Russell	J. C. Furney
J. S. Gower	E. M. Kelly
J. C. Kile, Jr.	M. H. Major
L. Wickham	L. H. Millemann
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Relative Effectiveness of Neutrons from a Nuclear Detonation and from a Cyclotron in Inducing Dominant Lethals in the Mouse¹

W. L. Russell L. B. Russell
A. W. Kimball²

The main purpose of this investigation was to attempt to discover, for the induction of dominant lethals in mice, whether or not the high intensity, and the energy spectra under the experimental conditions at various distances, of the neutron radiation from a particular nuclear detonation would show a biological effectiveness significantly different from that obtained in an earlier experiment with neutrons from a cyclotron, or from that to be obtained in anticipated experiments with neutrons from other laboratory sources. Dominant lethals were chosen for study because it was felt that, with the limited number of animals that could be exposed, this particular effect would yield the most rapid and reliable answer to the above question so far as genetic damage in a mammal is concerned.

The experimental material consisted of 144 young adult hybrid males obtained by crossing inbred 101 strain females with inbred C3H strain males. Twelve males were exposed in each of 10 lead hemispheres of 7-in. wall thickness and 14-in. inside diameter placed at various distances from the detonation. The remaining 24 animals, used as controls, were placed in hemispheres two days before the detonation and for a length of time approximately the same as that required for the exposed animals. One day and a half after the detonation each male was placed with four adult untreated females of the same hybrid strain. Matings were

detected by daily examination of all females for vaginal plugs. The uteri of all females that were pregnant from matings made from two to six days after irradiation of the male were removed at a late stage in pregnancy, and the uterine contents were examined under a dissecting microscope. At the same time, the number of corpora lutea in the ovaries of each of these females was recorded. Eighteen days and a half after irradiation each surviving male was placed with a new group of four females. Pregnancies obtained from these later matings were examined by the procedure used for the earlier pregnancies.

The results obtained from early matings of the exposed males are given in Table 22 and Fig. 12. The results obtained with neutrons from a comparable cyclotron experiment, reported earlier,³ in which gamma-ray contamination was estimated to be approximately 10% of the total dose in rep are shown in Fig. 13.

It is apparent from Figs. 12 and 13 that, although the distribution of deaths with respect to age of embryos varies with dose, there is no significant difference between results from the detonation and the cyclotron for comparable levels of total effect.

A comparison of the results of early and late matings for similar levels of effect in the cyclotron and detonation experiments is shown in Table 23. It is clear that for this effect, also, there is no significant difference between the results of the two experiments.

Turning to the quantitative response to neutron dose, it might appear that, with the large uncertainty about the gamma component of the radiation in the detonation experiment (see Table 22, cols. 3 and 4), little could be said about the relative biological effectiveness of neutrons from the detonation as compared with neutrons from the cyclotron. However, it will be shown that when both sets of gamma-radiation figures are used to calculate minimum and maximum estimates of the ratio of biological effectiveness, these estimates turn out to be not very far apart. For statistical treatment the biological effect is expressed as percentage survival through day 10½ of gestation, since there is no statistically significant effect of radiation on

¹This is a condensed version of a paper that is in press.

²Mathematics Panel.

³W. L. Russell, L. B. Russell, and J. S. Gower, *Biol. Semiann. Prog. Rep. Aug. 15, 1953*, ORNL-1614, p 45.

TABLE 22. DOMINANT LETHALITY IN OFFSPRING OF MALE MICE MATED FROM TWO TO SIX DAYS AFTER EXPOSURE TO NUCLEAR DETONATION

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Exposure station	Total, neutron + gamma, dose (lan chamber readings) (rep)	Minimum estimate of dose of gamma radiation (r)	Maximum estimate of dose of gamma radiation (r)	Number of pregnancies	Number of corpora lutea	Percentage of Corpora Lutea Represented by				
						Living fetuses	Embryos or fetuses dying after day 10½	Embryos dying between implantation and day 10½	Eggs or embryos dying before implantation (100 minus sum of columns 7, 8, and 9)	"Survivors" (sum of columns 7 and 8)
Control	0	0	0	48	446	84.30	0.45	3.59	11.66	84.75
Various stations arranged in order of decreasing distance from detonation	22	0.8	11	21	201	73.13	2.49	7.46	16.92	75.62
	50	1.5	25	22	208	60.58	0.48	19.71	19.23	61.06
	83 ^a	2	33	22	214	50.47	0.47	21.03	28.04	50.93
	96	2.5	47	25	249	42.57	0.00	26.10	31.33	42.57
	131	3	62	21	198	34.85	0.00	27.78	37.37	34.85
	157 ^a	4	67	24	232 ^b	32.82	1.30	25.91	39.97	34.12
	266.5 ^a	5	83	17	155 ^b	23.22	0.00	24.51	52.28	23.22
		6	120	8	80 ^b	8.74	2.50	21.23	67.53	11.24
		9	266	6	57 ^c	0.00	0.00	13.99	86.01	0.00
	11	426	2	19 ^c	0.00	0.00	10.49	89.51	0.00	

^aMean of two readings.

^bIncludes one or two pregnancies in which, because of early death of all embryos, there were no corpora lutea. In these cases the number of ovulated eggs was taken as 9.53, which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

^cThere were no corpora lutea in any of these pregnancies. The number of ovulated eggs was estimated in the way described in footnote b.

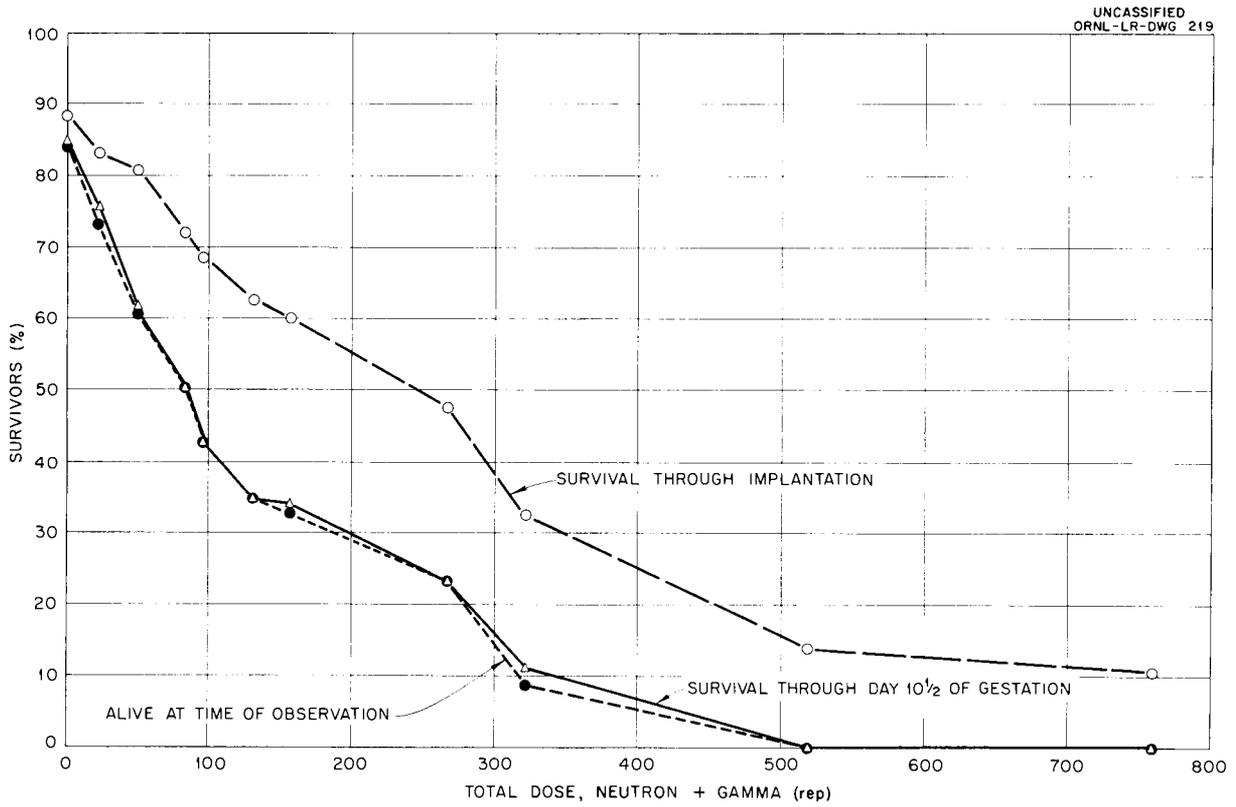


Fig. 12. Dominant Lethality in Offspring of Male Mice Mated from Two to Six Days After Exposure to a Nuclear Detonation. Data from Table 22.

mortality between this time and the time of observation. It is assumed that the logarithm of survival is linearly related to dose. Both the cyclotron and the detonation data give good fits on this interpretation. The relation of biological effect to dose can then be expressed in the following way.

Let

- u_c = biological effect, cyclotron,
- u_d = biological effect, detonation,
- R_c = total dose (rep), cyclotron,
- R_d = total dose (rep), detonation,
- G = gamma dose (r), detonation,
- E_c = relative biological effectiveness (RBE) of cyclotron neutrons compared with gamma,
- E_d = RBE of detonation neutrons compared with gamma.

Then, assuming additive effects of neutrons and gamma,

$$\log u_c = a_c + \beta[0.9R_c E_c + 0.1R_c] ,$$

since 10% of the total dose in the cyclotron was found to be gamma radiation, and

$$\log u_d = a_d + \beta[(R_d - G)E_d + G] .$$

These equations can be rewritten as

$$y = a_c + \beta x_1 ,$$

$$z = a_d + \beta' x_2 + \beta x_3 ,$$

where

- $y = \log u_c$,
- $x_1 = 0.9R_c E_c + 0.1R_c$,
- $z = \log u_d$,
- $\beta' = \beta E_d$,
- $x_2 = R_d - G$,
- $x_3 = G$.

By the method of weighted least squares, the two equations were fitted simultaneously to the values given in Tables 22 and 23. Data from the three stations closest to the detonation were excluded. Two separate fits were made, one using the minimum and the other the maximum estimates

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of gamma dose. E_c was taken as 8.0. This is the best estimate available, but it is based on X rays rather than gamma rays. It is the figure obtained by comparing the cyclotron results with data from an 800-r experiment with 250-kvp X rays. An estimate of E_c determined from a gamma-radiation experiment might turn out to be slightly higher, and E_c might vary somewhat with dose. However, even considerable error in the estimate of E_c would have little effect on the estimate of E_d/E_c . Minimum and maximum estimates of E_d were obtained from the ratio of the estimates of β' and β

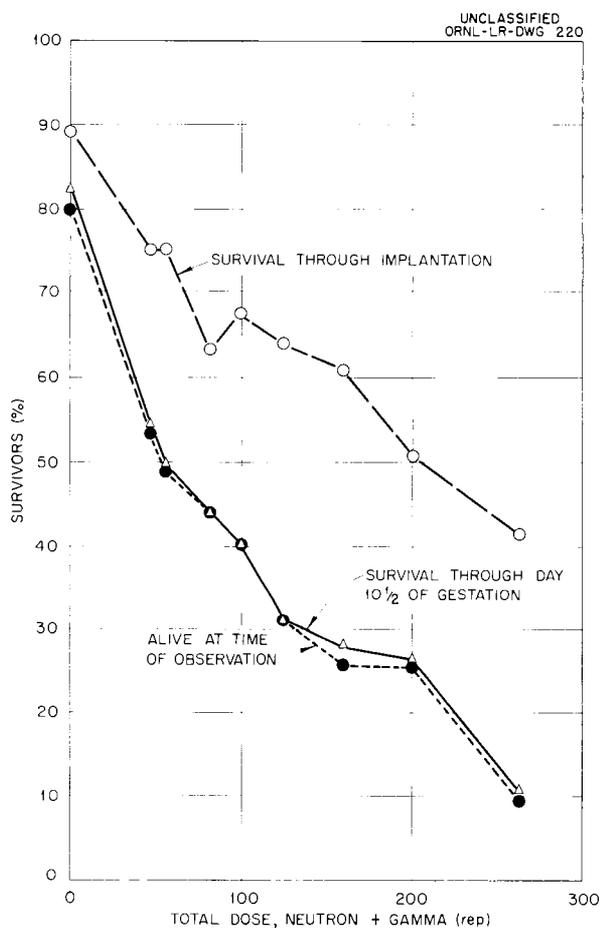


Fig. 13. Dominant Lethality in Offspring of Male Mice Mated from Two to Six Days After Exposure in a Cyclotron. Data from Table 19, ORNL-1614. At some of the higher doses, where the number of pregnancies is small, the results for similar doses have been combined and plotted at the weighted mean dose.

for each of the two fits. The corresponding minimum and maximum estimates of E_d/E_c , together with their 95% confidence limits, are given in Table 24.

An alternative statistical treatment, which assumes that the proportion of the gamma radiation in the total dose was the same in each of the hemispheres used, gives the same minimum and maximum estimates.

From both statistical treatments, it is clear that, under the conditions of this test, the biological effectiveness of the detonation neutrons is not significantly different from that of the neutrons in the cyclotron experiment.

With regard to the practical problem of human hazards, it is of first importance to consider an upper probability limit of the biological effect of the detonation neutrons relative to the cyclotron neutrons. Since the upper 95% confidence limit of the maximum estimate of the ratio of effects is 1.55, it seems safe to assume, as a starting point in the extrapolation of mouse data to man, that it is unlikely that the hazard, from the intensity and the spectrum of energies, of the neutrons encountered in the detonation experiment is more than one and a half times that of the neutrons in the cyclotron experiment. It should be emphasized that this is an upper limit; the hazard may actually be no greater.

It must be kept in mind that this comparison between the effects of laboratory and detonation neutrons is based on physical methods of dose estimation which, although they appear to be satisfactory, have not as yet been subjected to sufficiently vigorous tests. It is hoped that it will soon be possible to improve the laboratory side of the comparison by using a neutron facility designed to permit simpler and more precise radiobiological experiments.

Dominant Lethals in Mouse Oocytes Induced by X Rays in Air and in 5% Oxygen

L. B. Russell

M. H. Major

Mature (101xC3H) F_1 females, after 10 min in a Lucite chamber flushed with air or (5% O_2 + 95% He), were irradiated with 400 r of X rays in the same gas or left an equivalent length of time without irradiation. The body anterior to the ovaries was shielded. Females were placed with males immediately after exposure. All copulated within five days, and there was no significant difference

TABLE 23. COMPARISON OF EFFECT OF TIME OF MATING ON DOMINANT LETHALITY IN CYCLOTRON AND DETONATION EXPERIMENTS

	TOTAL DOSE ^a (rep)	MATINGS MADE 2 TO 6 DAYS AFTER IRRADIATION	MATINGS MADE 19 TO 28 DAYS AFTER IRRADIATION		
		"Survivors" ^b (%)	Number of Pregnancies	Number of Corpora Lutea	"Survivors" ^b (%)
Cyclotron	82	44	11	106 ^c	25
	99	40	9	83 ^d	10
	116	36	2	20	15
	141 and 160	25	2	20	10
Detonation	83	51	22	201 ^c	26
	96	43	24	217 ^d	24
	131	35	21	187 ^d	18
	157	34	18	161 ^d	9

^aGamma-radiation component may be different in the two experiments.

^bCyclotron data from Table 19, ORNL-1614, and detonation data from Table 22, this report.

^{c,d}Includes respectively 1 to 2 and 3 to 5 pregnancies in which, because of early death of all embryos, there were no corpora lutea. In these cases the number of ovulated eggs was taken as the mean number of corpora lutea per pregnancy for all pregnancies, within that experiment, in which the corpora lutea were counted.

TABLE 24. MINIMUM AND MAXIMUM ESTIMATES OF E_d/E_c , THE BIOLOGICAL EFFECTIVENESS OF DETONATION NEUTRONS RELATIVE TO CYCLOTRON NEUTRONS IN INDUCING DOMINANT LETHALS IN MICE

ESTIMATE	$\frac{E_d}{E_c}$	
	Point Estimate	95% Confidence Limits
Minimum	0.80	0.67 and 0.96
Maximum	1.18	0.91 and 1.55

between controls and experimentals in the proportions copulating each day. The uterine contents of 76 were examined 13½ to 17½ days after conception, and a total of 869 corpora lutea counted.

In the absence of radiation, hypoxia had no effect on any factor studied. An unexpected result was the highly significant increase in corpora lutea from 10.5 per female in controls to 14.2 and 12.9 in air- and hypoxia-irradiated groups, respectively. Within the five-day mating period, the excess increases with the interval between irradiation and copulation. Assuming the proportion of the corpora lutea count represented by fertilized eggs to be equal in all groups (assumption being tested), and assuming indirect effects of maternal irradiation on litter size to be negligible because of shielding, the data indicate that, with 400 r (in air), dominant lethals are induced in oocytes with a frequency approximately equal to that induced in sperm by the same dose. In contrast to sperm results,⁴ hypoxia probably provides some protection to oocytes.

⁴W. L. Russell, J. C. Kile, Jr., and L. B. Russell, *Genetics* 36, 574 (1951).

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Survival of Spermatogonia Following Exposure of Male Mice to 600 r of X Rays

E. F. Oakberg

J. C. Furney

Histological studies of testes of mice given sufficient irradiation to induce a period of temporary sterility have indicated disappearance of most spermatogonia, but a few cells with dusty nuclei (type A) survive and eventually repopulate the seminiferous tubules. Cell death has been indicated as the primary factor in reduction in number of spermatogonia. However, as emphasized in the last semiannual report,⁵ rapid lysis of necrotic cells results in an underestimate of total cell destruction when degenerative changes are the basis of scoring.

In spermatogenesis of the mouse, development of germinal cells proceeds along the tubule in a series of waves or cycles. Accordingly, cross sections taken at random through the testis represent tubules in various stages of development. Several attempts have been made to classify these random cross sections into an orderly series, and recently LeBlond and Clermont⁶ have been able to divide the spermatogenic cycle into 14 stages on the basis of acrosome development. Progression of type A spermatogonia to sperm follows a very regular pattern, and only certain associations of cell types are present at any given time. Likewise, the number of each type of cell represented is characteristic for each stage in the cycle.

General procedures of irradiating the mice and times at which tissues were taken were described in the last semiannual report.⁵ Testes fixed in Zenker-formol were sectioned at 7μ and stained by the periodic acid-fuchsin-sulfurous acid technique. Tubule cross sections were classified according to the method of LeBlond and Clermont, and the number of spermatogonia counted. Thirteen control mice and 64 irradiated animals distributed evenly among the 16 intervals after exposure were used. One section was scored for each mouse. Development of the acrosome appeared to be unaffected by the dose of 600 r. Therefore classification of the

tubules was unbiased, and the comparison with tubules of control animals should be valid. The mean number of type A spermatogonia was computed for each of the 14 tubule stages of controls, and the mean number of type B spermatogonia was determined for stages I-VI. Expected numbers for the irradiated series were estimated from control means multiplied by the frequency distribution of tubules sampled for each experimental group of four mice.

The previous observation that the 12-hr interval shows the highest number of necrotic spermatogonia is substantiated by these data (Table 25). Also, it is apparent that this is true for both type A and type B cells. However, degenerative changes observed in spermatogonia after the first 24 hr are limited to cells of type A, because all cells of type B have disappeared within two days.

The generally accepted observation that a few type A spermatogonia survive and repopulate the germinal epithelium is substantiated, for, while they are reduced to only 0.9% of normal at five days, rapid proliferation occurs and results in nearly normal numbers by four weeks. Ninety-five per cent confidence intervals indicate significant increase in percentage of necrotic cells for only the 6-hr through three-day intervals. Additional data will be necessary to determine if the higher percentage of degeneration observed at the later time intervals is statistically significant.

Normally, type B spermatogonia show a very low incidence of degenerative changes. However, rapid necrosis occurs after exposure to 600 r of X rays. Ninety-five per cent confidence intervals indicate significantly increased rates of necrosis for the 3- to 24-hr interval. Type B spermatogonia are reduced to three per cent of normal within 24 hr after irradiation with 600 r. Therefore degeneration must occur the first time cell division is attempted, for the two divisions of type B spermatogonia normally are separated by an interval of about 45 hr. Additional observations on the relation of cell death to mitotic division of these cells are being made.

Experiments on the effect of low doses of gamma rays on spermatogonia are in progress, and preliminary results indicate that type B spermatogonia may have the highest sensitivity to radiation of any mammalian cell.

⁵E. F. Oakberg and J. E. Crowell, *Biol. Semiann. Prog. Rep.* Aug. 15, 1953, ORNL-1614, p 48.

⁶C. P. LeBlond and Y. Clermont, *Am. J. Anat.* 90, 167-215 (1952).

TABLE 25. SURVIVAL OF SPERMATOGONIA OF THE MOUSE FOLLOWING EXPOSURE TO 600 r OF X RAYS

TIME AFTER IRRADIATION	SPERMATOGONIA TYPE A					SPERMATOGONIA TYPE B				
	Number Expected	Number Observed	Number Necrotic	$\frac{\text{Observed}}{\text{Expected}} \times 100$	Percentage Necrotic	Number Expected	Number Observed	Number Necrotic	$\frac{\text{Observed}}{\text{Expected}} \times 100$	Percentage Necrotic
Control		2238	43		1.92		3176	0		
1 hr	577	482	15	83.5	3.11	856	838	7	97.9	0.84
2 hr	790	626	15	79.2	2.40	837	823	2	98.3	0.24
3 hr	537	444	14	82.9	3.15	706	712	40	100.9	5.62
6 hr	576	511	85	75.7	16.63	686	635	53	92.6	8.35
12 hr	440	323	110	73.4	34.06	713	391	194	54.8	49.62
18 hr	594	270	55	45.5	20.37	609	110	93	18.1	84.55
1 day	573	254	28	44.3	11.02	1076	33	30	3.1	90.91
2 days	521	87	8	16.7	9.20	708	0			
3 days	623	41	6	6.6	14.63	762	0			
4 days	553	14	1	2.5	7.14	931	0			
5 days	645	6	1	0.9	16.67	855	0			
7 days	874	16	4	1.8	25.00	881	0			
10 days	866	82	6	9.5	7.32	1108	0			
15 days	770	192	7	24.9	3.65	922	0			
21 days	1023	744	10	72.7	1.34	951	704	0	74.0	
28 days	597	544	20	91.1	3.68	1012	787	0	77.8	

PERIOD ENDING FEBRUARY 15, 1954



PATHOLOGY AND PHYSIOLOGY

PATHOLOGY AND PHYSIOLOGY

A. C. Upton - Section Chief

T. T. Odell, Jr.	F. G. Tausche
W. H. Benedict ¹	B. Anderson
K. W. Christenberry ¹	P. Ledford
F. P. Conte ²	F. F. Farbstein
W. D. Gude	

Operation Greenhouse

K. W. Christenberry	P. Ledford
W. H. Benedict	F. F. Farbstein
W. D. Gude	B. Anderson

A preliminary survey, "Late Effects in Mice of Ionizing Radiation from an Experimental Nuclear Detonation,"³ presented at the meeting of the Radiological Society of North America in December 1953, is summarized in the following report.

This analysis was made 30 months after irradiation, when most of the exposed animals and about

two-thirds of the controls were dead. It does not include the groups of animals exposed to neutrons (lead hemispheres) and X rays, and the figures given are subject to minor revision.

Mortality. The LD₅₀ (30 days) was approximately 755 r for both males and females. There were a few delayed deaths among the massively irradiated animals during the second month postirradiation, followed by a period of apparent well-being of the survivors. Delayed mortality and longevity are related to the dose of radiation, as indicated in Fig. 14. A significant shortening of life resulted from doses well beneath the threshold for acute lethality. This reduction of longevity was caused mainly by degenerative and neoplastic diseases induced or accentuated by irradiation.

Cataracts. Opacities of the lens made their appearance during the third month postirradiation; within 90 days after exposure almost all irradiated mice had opacities detectable with a slit lamp. Their rate of progression varied directly with the dose (Fig. 15). The opacities induced by small doses, up to about 300 r, did not progress markedly, whereas those caused by larger doses progressed

¹Consultant.
²U.S. Air Force.
³J. Furth, A. C. Upton, K. W. Christenberry, W. H. Benedict, and J. Moshman (in press).

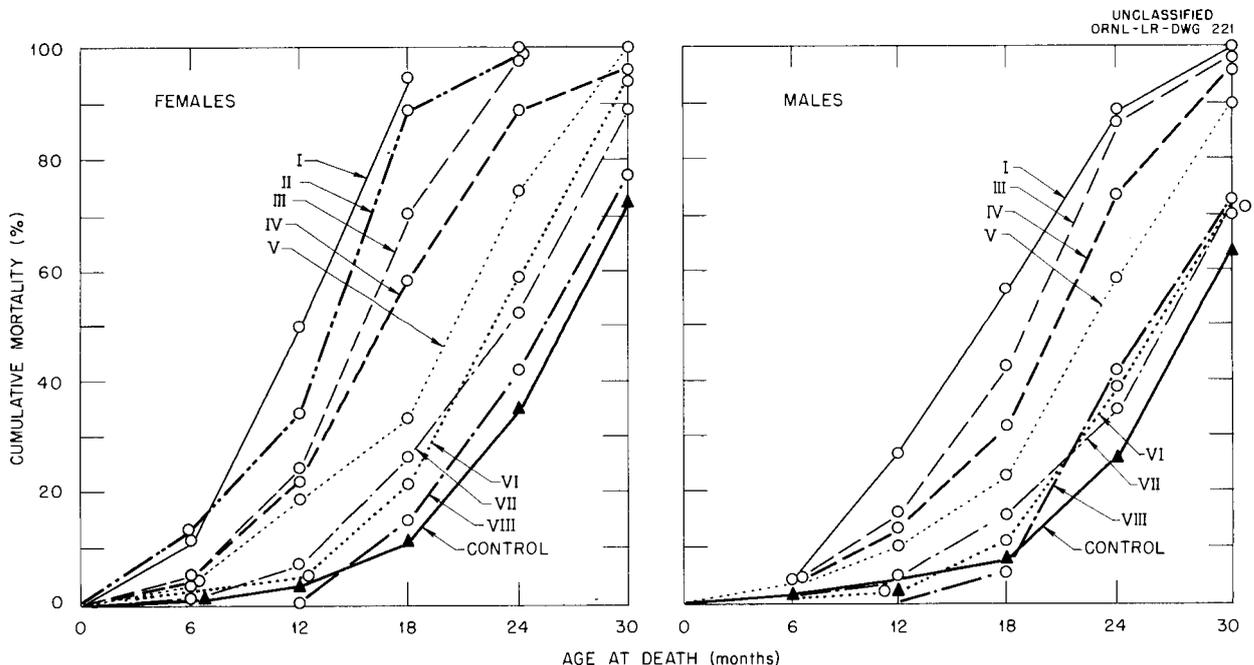


Fig. 14. Cumulative Mortality of Mice Exposed to Ionizing Radiation from a Nuclear Detonation. Dose (r): curve I, 812-841; curve II, 759-785; curve III, 711-733; curve IV, 631-687; curve V, 491-556; curve VI, 287-318; curve VII, 192; curve VIII, 367-424.

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to complete cataract. The system of grading the opacities is described in an earlier publication.⁴

Iris Defects. Studies of the eye disclosed a progressive loss of iris tissue, beginning with fenestration, which terminated in nearly complete

⁴K. W. Christenberry and J. Furth, *Proc. Soc. Exptl. Biol. Med.* 77, 556-560 (1951).

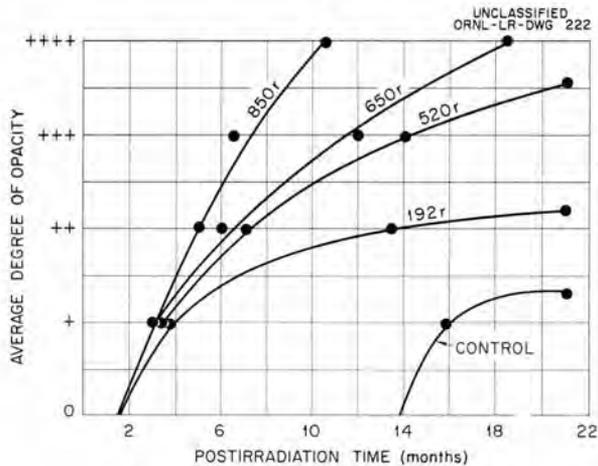


Fig. 15. Opacities of the Lens of Mice Exposed to a Nuclear Detonation.

loss of iris parenchyma, illustrated in Fig. 16. This appears to be an abiotrophy that is apparently hereditary, mild atrophy of the iris occurring in aging nonirradiated controls. However, atrophy of the iris appeared sooner and was more severe following irradiation, in proportion to the dose.

Graying. As early as the third month postirradiation, graying of the fur began to appear and progressed with time as a function of the dose (Fig. 17). It varied with different anatomical regions, but on the top of the head it was sufficiently well correlated with the dose to constitute a simple, though only approximate, biological dosimeter.⁵

Leukemia. The incidence of thymic lymphoma was greatly elevated by irradiation, in proportion to the dose (Fig. 18). The figure indicates that about 30% of the controls and a smaller number of animals exposed to sublethal doses are still alive, but it is unlikely that many of the mice still living will develop this type of lymphoma, which commonly occurs in young mice. In the groups exposed to more than 700 r, thymic lymphoma occurred with greater frequency among males, but in females the

⁵J. Moshman and A. C. Upton, *Science* 119, 186-187 (1954).

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ORNL-LR-DWG 223



Fig. 16. Severely Fenestrated Iris of an Irradiated Mouse (A), As Compared with the Normal Iris (B).

threshold dose appeared to be lower, even the 192-r group showing a distinct increase. The earliest cases appeared in the fourth month post-irradiation, and the peak incidence occurred 7 to 12 months after exposure. The greater incidence of thymic lymphomas in males is contrary to previous experience with mice of most other strains exposed to sublethal doses of radiation.

Other forms of leukemia, including nonthymic lymphoma, reticulum cell sarcoma, and rarely myeloid leukemia, occurred relatively late in life and therefore were less numerous in the irradiated mice than in the controls (Fig. 19). Corrected for longevity, however, the incidence of these types of leukemia was slightly higher among exposed animals. Analysis according to hematologic type is in progress.

Tumors. Ovarian tumors were common after irradiation at all dose levels but rare among controls (Fig. 20). The lower incidence of ovarian tumors after high doses is related to reduced longevity, since the latent period of these neoplasms is relatively long (12 to 15 months). These include luteomas, granulosa cell tumors, tubular adenomas of germinal epithelium, cystadenomas, hemangiomas, and mixtures of these types.

The induction of pituitary tumors was an unanticipated finding;⁶ these tumors were more common in females (Table 26). Their absence in the most

heavily irradiated groups is explained by reduced longevity. Of the radiation-induced neoplasms observed in these mice, pituitary tumors have the longest latent period, and if correction is made for survival (Table 27), a correlation is found between dose of radiation and tumor induction. Those studied so far have been chromophobe adenomas. Transplantation studies of these pituitary tumors indicate that most of them secrete ACTH.⁷

Another unexpected neoplasm was adenocarcinoma of the Harderian gland of the orbit. Locally invasive, it often obliterated the orbit infiltrating the skull and adjacent soft tissues. It was more common after relatively low doses (Table 28), which is attributable to its long latent period (15 to 17

⁶A. C. Upton and J. Furth, *Proc. Soc. Exptl. Biol. Med.* 84, 255-257 (1953).

⁷J. Furth, E. L. Gadsden, and A. C. Upton, *Proc. Soc. Exptl. Biol. Med.* 84, 253-254 (1953).

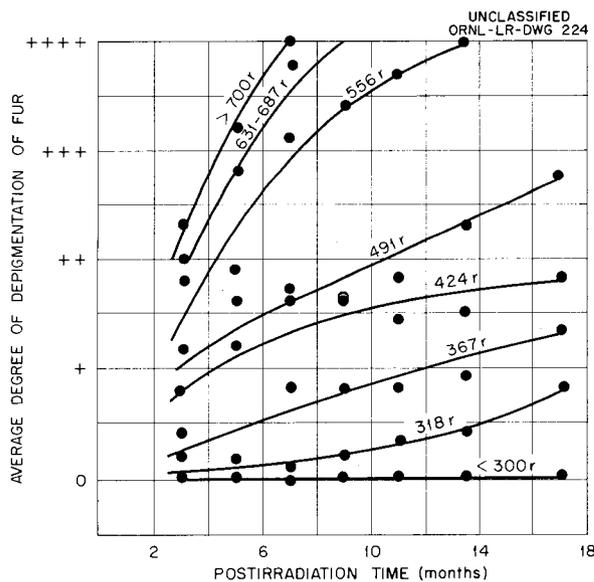


Fig. 17. Depigmentation of Fur of Mice Exposed to Nuclear Detonation.

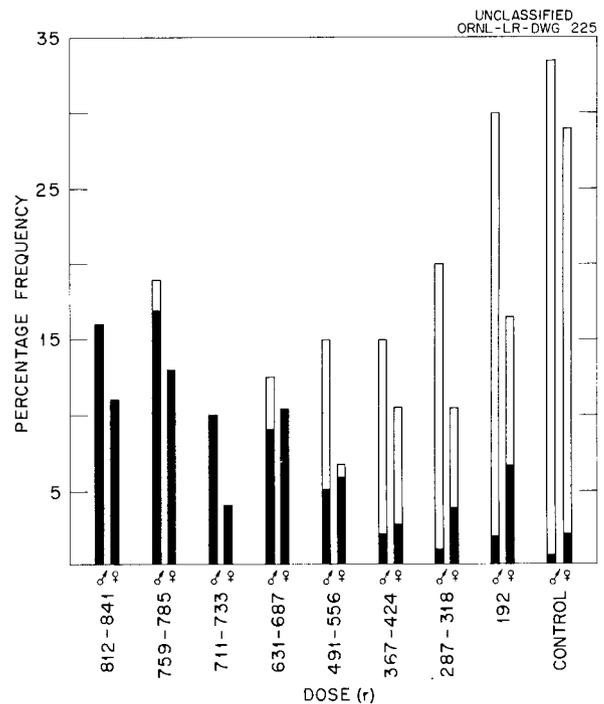


Fig. 18. Incidence of Thymic Lymphoma 30 Months After Exposure to Ionizing Radiation from a Nuclear Detonation. The chart indicates that about 30% of the controls and a smaller number of animals exposed to sublethal doses are still alive, but it is unlikely that many of the mice still living will develop this type of lymphoma, which commonly occurs in young mice.

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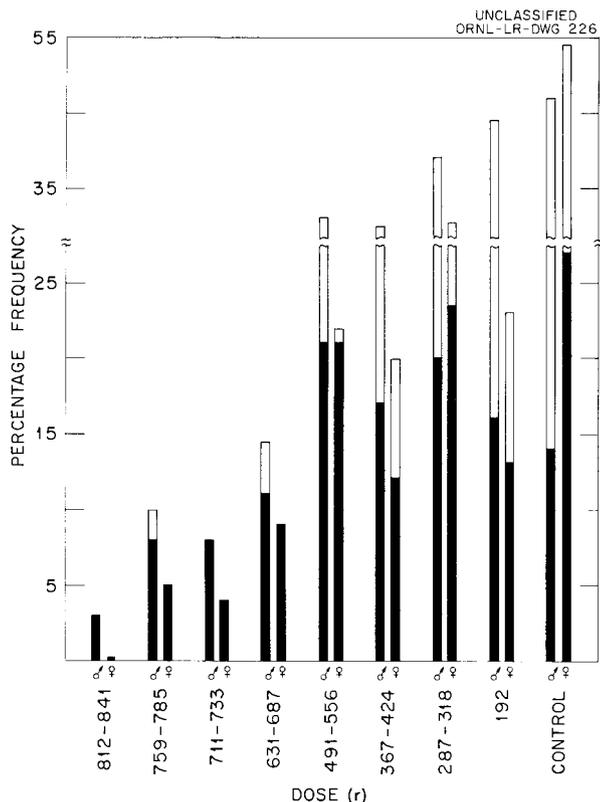


Fig. 19. Incidence of All Nonthymic Leukemias 30 Months After Exposure to Ionizing Radiation from a Nuclear Detonation.

months) and the greater longevity of mice at low dose levels, the induction rate of this tumor being low and apparently not dose dependent. No such neoplasms have been observed in the controls so far, and, to the knowledge of this group, none have

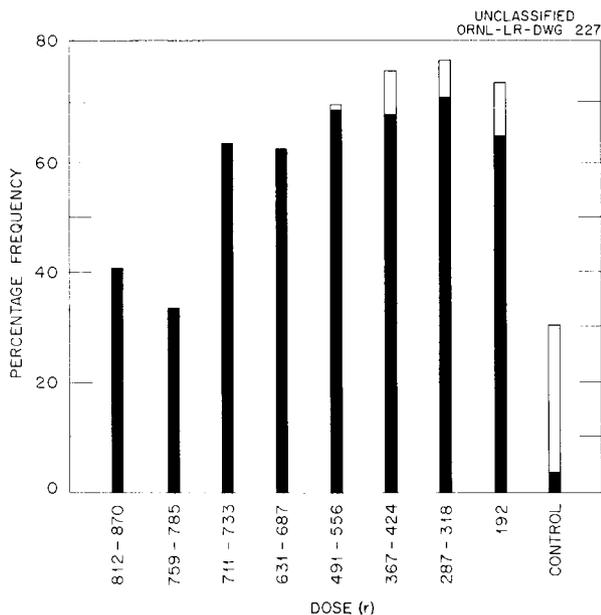


Fig. 20. Incidence of Ovarian Tumors 30 Months After Exposure to Ionizing Radiation from a Nuclear Detonation.

TABLE 26. INCIDENCE OF PITUITARY TUMORS 30 MONTHS AFTER EXPOSURE

DOSE (r)	PITUITARY TUMORS			
	Female		Male	
	Number	Percentage*	Number	Percentage*
812-932	0	0	0	0
759-785	0	0	3	5.2
711-733	14	11.9	2	1.5
631-687	18	12.3	8	4.5
491-556	29	16.2	4	2.2
367-424	10	15.2	0	0
287-318	17	8.7	5	2.5
192	8	7.8	0	0
0	5	1.7	0	0

*Of those surviving 12 months.

TABLE 27. PITUITARY TUMORS IN FEMALES - INCIDENCE CORRECTED FOR SURVIVAL

DOSE (r)	7 TO 12 MONTHS		13 TO 18 MONTHS		19 TO 24 MONTHS		25 TO 30 MONTHS	
	Dead, with Tumor	Tumor Incidence (%)						
	Alive, Healthy*		Alive, Healthy*		Alive, Healthy*		Alive, Healthy*	
711-733	0/152	0	4/120	3.3	10/47	21.3	0/1	0
631-687	1/187	0.5	5/149	3.3	9/80	11.2	4/20	20.0
491-556	2/206	1.0	2/179	1.1	17/146	11.6	10/56	17.8
367-424	0/78	0	2/78	2.6	4/66	6.1	4/45	9.1
287-318	0/210	0	1/213	0.5	8/170	4.7	8/89	9.0
192	0/106	0	0/106	0	3/79	3.8	5/51	9.8
0	0/299	0	0/294	0	0/270	0	5/188	2.6

*Surviving at beginning of indicated period.

TABLE 28. INCIDENCE OF HARDERIAN GLAND TUMORS 30 MONTHS AFTER EXPOSURE

DOSE (r)	PERCENTAGE*	
	Female	Male
812-932	0	0
759-785	0	0
711-733	1.7	3.1
631-687	3.3	1.1
491-556	1.7	2.7
287-318	2.9	1.9
192	3.0	1.9
0	0	0

*Of those surviving 12 months postirradiation.

been reported in mice previously.

Mammary-gland tumors were rare in males; their incidence in females was moderately increased after doses of 192 to 687 r (Fig. 21). The absence of an increased frequency at high dose levels is probably related to reduced longevity. The neoplasms occurring early in life were predominantly adenomas and adenocarcinomas, whereas those developing later were sarcomas, as described by Lorenz *et al.*⁸

Adenomas of the lung, liver, adrenal, and kidney, and carcinomas and sarcomas of these and other organs have been encountered, but their incidence has not yet been analyzed.

Nephrosclerosis. Renal degeneration, often fatal, occurred frequently at high dose levels, rarely at low dose levels, and not thus far in controls (Fig. 22). The characteristic lesion is most conspicuous in the glomerulus and consists of deposition of homogeneous material in the glomerular tufts, ultimately resulting in their obliteration. Associated with it there is sclerosis of arterioles and interstitial fibrosis and atrophy of the kidney. Inflammatory cells are absent. The lesion is bilateral and frequently accompanied by generalized edema. The pathogenesis of this disease remains to be determined. The lesions are unlike those of glomerulonephritis and pyelonephritis but could be the consequence of delayed vascular damage produced by irradiation.⁹

Cataract Induction by Fast Neutrons

K. W. Christenberry

P. Ledford

Studies of cataract induction by fast neutrons have been described in earlier progress reports

⁸E. Lorenz, A. B. Eschenbrenner, W. E. Heston, and D. Uphoff, *J. Natl. Cancer Inst.* 11, 947-965 (1951).

⁹J. Furth and A. C. Upton, *Ann. Rev. Nuclear Sci.* 3, 303-338 (1953).

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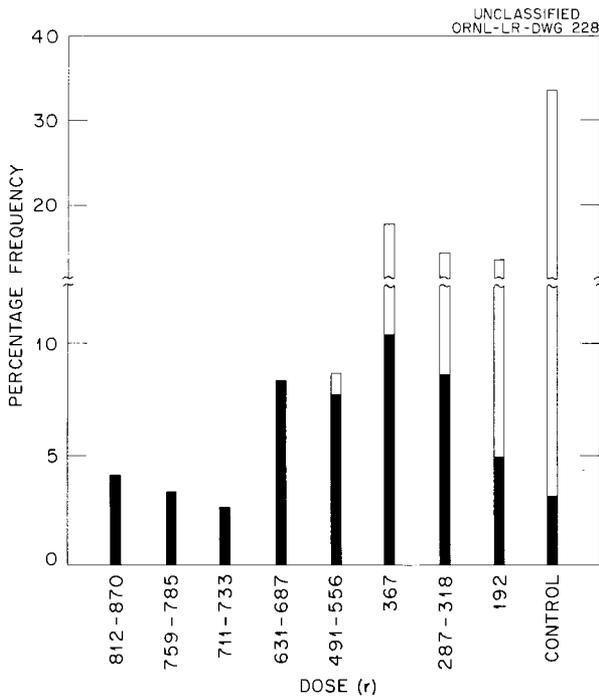


Fig. 21. Incidence of Mammary-Gland Tumors in Females 30 Months After Exposure to Ionizing Radiation from a Nuclear Detonation.

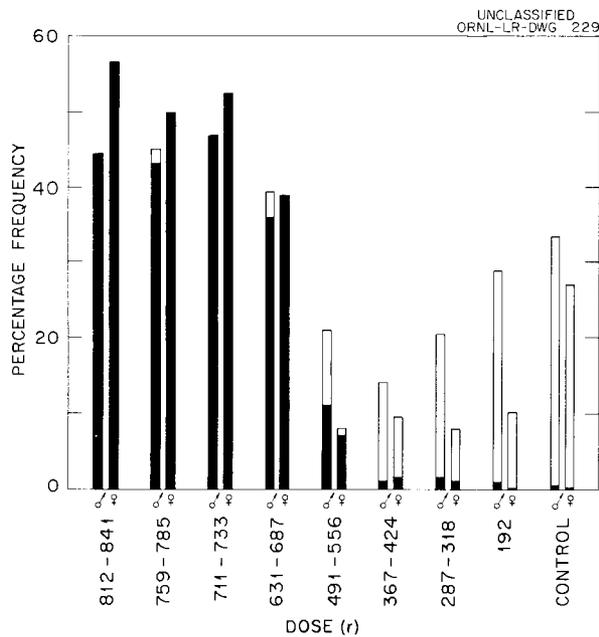


Fig. 22. Incidence of Nephrosclerosis 30 Months After Exposure to Ionizing Radiation from a Nuclear Detonation.

from this laboratory. Rats, mice, and rabbits have been studied systematically with a slit lamp after exposure to graded doses of 250-kvp X rays, Co^{60} gamma rays, and fast neutrons of approximately 2.5-Mev (polonium-boron)¹⁰ and 14-Mev (Cockcroft-Walton)¹¹ energies.

Preliminary data indicate that a dose of 1×10^8 neutrons/sq cm causes the appearance of mild opacities in mice within eight months, significantly earlier than the development of similar senile cataractous changes in nonirradiated controls. However, opacities of moderate or advanced severity have not occurred thus far with doses below 1×10^9 neutrons/sq cm. It appears significant that under the conditions of this study a dose of 1×10^{10} neutrons/sq cm appears to be equally damaging to the lens within 14 months postirradiation if given in a single exposure or fractionated in 10 doses of 1×10^9 neutrons/sq cm at intervals of 1, 3, or 7 days.

The rat and the rabbit appear much less susceptible to cataract induction than the mouse. However, since the latent periods are longer in the larger mammals, it is not yet possible to establish precisely any species differences in relative radiosensitivity of the lens. Analyzed 20 months postirradiation, the threshold cataract-inducing dose in rats appears to be slightly above 1×10^9 neutrons/sq cm.

Rabbits remain negative for opacities 20 months after receiving doses of 3.3×10^9 neutrons/sq cm and 14 months after receiving 1×10^{10} neutrons/sq cm.

Comparative data of animals exposed to neutrons and X and gamma rays, respectively, do not yet enable precise estimation of the relative biological effectiveness (RBE) of fast neutrons for cataract formation but indicate that this RBE is relatively high.

Platelet Life-Span Studies

T. T. Odell, Jr.

F. G. Tausche

Additional data have been obtained on the survival of labeled platelets transfused into non-labeled rats.

¹⁰The neutron source and guidance in the dosimetry and physical design of these experiments were supplied by K. Z. Morgan and G. S. Hurst, Health Physics Division, ORNL.

¹¹Use of the Cockcroft-Walton accelerator was provided by J. L. Meem and R. G. Cochran, Physics Division, ORNL.

In vivo labeling of platelets was accomplished by injecting intravenously methionine- S^{35} (225 μc) or formate- C^{14} (200 μc) into adult Sprague-Dawley rats. The rats were exsanguinated 22 hr later, and the platelets were separated from the blood by differential centrifugation, as described previously.¹² Suspensions of these platelets were transfused into normal rats of the same inbred strain, which were exsanguinated at intervals thereafter, and the radioactivity of their platelets was measured. The total radioactivity of circulating platelets was estimated on the basis of the samples measured and was expressed as percentage of the activity injected. The activity curve from such an experiment is presented in Fig. 23. Similar curves have been obtained in other experiments in which either formate- C^{14} or methionine- S^{35} was used for labeling the platelets. The percentage of the injected activity observed 30 min or one day after injection of labeled platelets was always considerably lower than the theoretical 100%. It seems likely that platelets which have been damaged by handling are rapidly removed from the circulation. The radioactivity declines during the 24 hr following injection, then appears to level out between the first and second days, but falls off again thereafter at a fairly regular rate. Minimal activity was detected six days following transfusion. The shape of the

curve probably reflects the age composition of the population of platelets which were injected. The detection of activity five and six days post transfusion demonstrates that some of the platelets live at least that long. The average life span is probably somewhat shorter. It should be pointed out that no activity was found in plasma or in the rinse solution after washing platelets at any time following platelet transfusion, and therefore the labeled platelets were not exchanging activity with other blood components.

Platelets were also labeled *in vitro* by incubating them for 3 hr at 37°C ($\pm 2^{\circ}\text{C}$) in a plasma medium to which formate- C^{14} (1.43 $\mu\text{c}/\text{ml}$) had been added. The platelets were subsequently separated by centrifugation from the incubation medium, washed three times, and transfused into nonlabeled rats. The specific activity of these *in vitro*-labeled platelets was higher than that the platelets labeled *in vivo* with formate- C^{14} . Loss of platelets during the incubation procedure was, however, considerable, ranging from 54 to 77%. The circulation time varied markedly among three different experiments, from less than one day to more than three days (about $4\frac{1}{2}$ days by extrapolation) but was at best less than that found for the *in vivo*-labeled platelets. The large amount of variation in circulation time of transfused platelets among the three experiments is believed to be related to the amount of injury sustained by the platelets prior to transfusion, even though the same procedure was followed in preparing the platelets in all three experiments. It is believed that the shorter circulation time of *in vitro*-labeled platelets following transfusion, as compared with *in vivo*-labeled platelets, is a result of greater injury to the platelets during their preparation.

¹²M. C. Woods, F. N. Gamble, J. Furth, and R. R. Bigelow, *Blood* 8, 545-553 (1953).

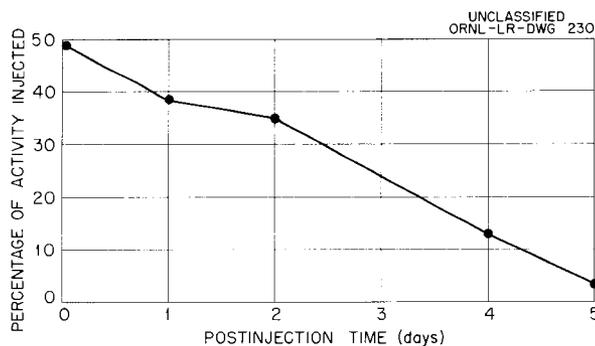


Fig. 23. C^{14} Activity in Platelets at Intervals After Transfusion of *In Vivo*-Labeled Platelets into Normal Rats. The total estimated circulating activity at a specific time, based on the platelet samples counted, is expressed as a percentage of the total activity of the labeled platelets injected.

Cooperative Projects with the U.S. Air Force and ANP

F. P. Conte

Orientation studies of the RBE of fast neutrons for acute lethality of mice, rats, and guinea pigs were carried out in the 86-in. cyclotron, as described previously. The surviving animals have been systematically examined with a slit lamp for cataract. The average degree of opacities of the lens plotted against the time (months) postirradiation is shown in Fig. 24 for mice, guinea pigs, and rats exposed in the cyclotron to a single dose of 240 rep. This dose, above the LD_{50} for mice

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and guinea pigs, produced complete opacities of the lens in both mice and rats within six and nine months, respectively. Guinea pigs, however, developed only moderately severe cataracts (grade

II) during the same interval. X irradiation at the LD_{50} level has resulted thus far in lesions considerably less severe – in no instance greater than grade II. Thus the preliminary data, although not yet permitting precise estimation of the RBE, indicate the relatively high effectiveness of fast neutrons for cataract induction in each species.

A technique for measuring the uptake of Fe^{59} by mouse erythrocytes has been perfected. With the use of this method, attempts are being made to determine the RBE of acute fast-neutron (cyclotron) irradiation for depression of erythropoiesis in the mouse.

Dosimetry studies have been carried out in collaboration with C. W. Sheppard and E. B. Darden of the Biology Division, G. S. Hurst and W. A. Mills of the Health Physics Division, and H. H. Rossi of Columbia University in an effort to monitor the mixed neutron-gamma radiations in the mammalian exposure chamber at the 86-in. cyclotron. A pulse counter constructed by Hurst and associates and Victoreen and Rossi-Failla tissue-equivalent chambers are being intercalibrated. Depth dose studies are being carried out in paraffin phantoms.

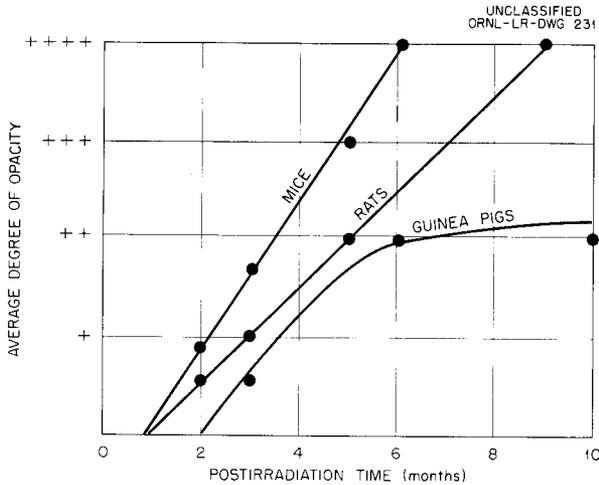


Fig. 24. Opacities of the Lens of Animals Receiving 240 rep from a Cyclotron.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY
METABOLISM

S. F. Carson	M. V. Long
E. F. Phares	H. Sherwood
M. I. Dolin	J. M. Adams ¹

Peroxidation Enzymes

M. I. Dolin

A cyanide- and azide-resistant peroxidation of DPNH₂ has been demonstrated² in extracts of *Streptococcus faecalis*, an organism which contains no catalase or demonstrable cytochrome components. Further properties of the purified peroxidase have been investigated. The enzyme does not utilize TPNH₂. The Michaelis constants for H₂O₂ and DPNH₂ are 2.4×10^{-4} and 5×10^{-6} M, respectively. Other oxidizing agents, including organic and inorganic sulfhydryl-oxidizing reagents or cytochrome c, will not substitute for H₂O₂. However, there is present, possibly as a contaminant, an enzyme which oxidizes DPNH₂ with 2,6-dichlorophenolindophenol or ferricyanide, but not with methylene blue, brilliant cresyl blue, or oxygen. Typical substrates for hematin peroxidases, including reduced cytochrome c, are not attacked. Incubation of the enzyme with DPNH₂ leads to an inactivation which is not reversible by dialysis or by incubation with known cofactors or crude Kochsafts. The purified enzyme contains flavin-adenine dinucleotide (FAD) and small amounts of lipoic acid; however, there is no conclusive evidence for the function of these compounds in the reaction. There is no inhibition by versene or 8-hydroxyquinoline or by exposure of the enzyme to strong ultraviolet light (365 mμ). In a search for other peroxidation systems, it was found that cytochrome c catalyzed the peroxidation of DPNH₂. The latter reaction has the same pH optimum as the *S. faecalis* enzyme. However, it is heat stable, whereas the bacterial enzyme is extremely heat labile. DPNH₂ peroxidation is catalyzed to some extent by crystalline hemin, but not by ferric iron or by ferrous iron at a neutral pH.

¹ORINS Predoctoral Fellow.²M. I. Dolin, *Arch. Biochem. and Biophys.* 46, 483 (1953).Fermentation of Xylose by *Escherichia coli* –
Mechanisms of Succinic Acid Biosynthesis

J. M. Adams

In a study of lactic acid biosynthesis during the fermentation of xylose by *Escherichia coli* K-12, Nutting and Carson³ observed that succinic acid, produced in the presence of acetate-2-C¹⁴ was labeled almost exclusively in the methylene carbons. In addition, the C₂ fragment, necessarily produced in the aldolase-like cleavage of xylose supported by the authors just mentioned, was labeled by equilibrium with the tracer and appeared to be involved in the net synthesis of lactic acid.

These results implied that the C₂ fragment might also be involved in the net synthesis of succinic acid and that this reaction could be followed isotopically. The present investigation was undertaken in an effort to determine the mechanisms responsible for the biosynthesis of succinic acid by *Escherichia coli* during the fermentation of xylose.

Three metabolic pathways which could have accounted for the incorporation of acetate (C₂) activity into succinic acid were examined with the aid of tracer techniques and enzyme inhibitors: (1) a double fixation of carbon dioxide onto acetic acid, (2) the tricarboxylic acid cycle, and (3) the Thunberg (C₂) condensation and the dicarboxylic acid cycle.

The double fixation of carbon dioxide onto acetic acid appeared to represent an unimportant mechanism for the synthesis of succinic acid based on evidence obtained from fermentations carried out in the presence of arsenite and labeled acetate or carbon dioxide. However, the fixation of C¹⁴O₂ in the carboxyl carbons of succinic acid was observed, and a synthetic mechanism involving carbon dioxide may play a significant role in the uninhibited fermentation.

The tricarboxylic acid cycle appears to be present in this organism and thus would incorporate acetate activity into succinic acid, particularly under oxidative conditions. This conclusion is based primarily on the recovery of appropriately labeled alpha-ketoglutaric acid from fermentations carried out in the presence of labeled acetate.

³L. A. Nutting and S. F. Carson, *J. Bacteriol.* 63, 575-580, 581-589 (1952).

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The Thunberg (C_2) condensation, and thus the dicarboxylic acid cycle, on the basis of the present studies, is believed to be present in this organism and to be the major mechanism for the incorporation of acetate activity into succinic acid in the presence of arsenite. This conclusion is based primarily on the following observations:

1. The presence of 0.3 M monofluoroacetic acid, a specific inhibitor of the tricarboxylic acid cycle, fails to inhibit the cyclic mechanism in operation, despite a 75 to 85% inhibition of the mechanism which incorporates acetate activity.

2. The presence of 0.07 M arsenite inhibits the cyclic mechanism almost completely without inhibiting the mechanism which incorporates acetate activity.

3. Despite the almost complete inhibition of the cyclic mechanism by 0.07 M arsenite, the acetate activity incorporated into alpha-ketoglutaric acid (which varies directly with the presence of oxygen) represents only 5 to 25% of the activity incorporated into succinic acid.

In the course of this investigation it has further been observed that arsenite (0.07 M) exhibits a striking effect on the fermentation of xylose, presumably by inhibiting the decarboxylation of pyruvic acid (an integral reaction in the dicarboxylic acid cycle) and which results in the production of theoretical amounts of lactic acid (1.66 moles per mole of xylose fermented). Since this result requires the fixation of carbon dioxide at some stage during the dissimilation of the substrate, the use of this system is advocated for future studies of lactate biosynthesis and carbon dioxide fixation.

Succinate Decarboxylation Systems in *Propionibacterium* and *Veillonella*

E. F. Phares

S. F. Carson

Cell-free extracts of *Propionibacterium pento-*

saceum and *Veillonella gazogenes* activate (hydroxamate formation) succinate, propionate, and acetate. At a pH of 5.6, *P. pentosaceum* extracts do not produce CO_2 from succinate. Exchange experiments with propionate-2- C^{14} and $C^{14}O_2$ show very little exchange of CO_2 into succinate compared with propionate exchange with succinate. Contrariwise, fresh *V. gazogenes* extracts produce CO_2 and propionate stoichiometrically from succinate, and propionate-2- C^{14} and $C^{14}O_2$ exchange with succinate. However, treated *V. gazogenes* extracts (aged, frozen and thawed, ammonium sulfate precipitated, or dialyzed) do not produce CO_2 from succinate, nor does propionate-2- C^{14} or $C^{14}O_2$ exchange with succinate. Nevertheless, when "inactive" *P. pentosaceum* and *V. gazogenes* preparations are mixed, CO_2 is produced from succinate. All these reactions have an optimum pH of about 7.5; however, at pH 5.6 the different functions of the two extracts can be separated. It has been demonstrated that the following reactions occur:

1. *P. pentosaceum* preparations (and an ammonium sulfate fraction therefrom) decarboxylate succinate \longrightarrow " C_1 " + propionate, but do not convert " C_1 " \longrightarrow CO_2 .

2. An ammonium sulfate fraction from *P. pentosaceum* plus a "particulate fraction" from *V. gazogenes* produces CO_2 from succinate.

3. Succinyl-coenzyme A acts as a substrate from the decarboxylation reaction; succinyl-CoA, propionyl-CoA, and other acyl-CoA compounds "spark" succinate decarboxylation reaction via the CoA transphosphorase system.

The data indicate that there are two separate types of reactions: (1) succinate \longrightarrow propionate + " C_1 ", and (2) " C_1 " \longrightarrow CO_2 . A "malic enzyme" is not responsible for the " C_1 " \longrightarrow CO_2 conversion.

BIOCHEMISTRY

STUDIES ON NUCLEIC ACIDS, ENZYMES, AND ENERGY-TRANSFER SYSTEMS

W. E. Cohn	M. J. Cormier ¹
D. G. Doherty	E. Eavenson
J. X. Khym	M. H. Jones
B. L. Strehler ¹	A. B. Ottinger
E. Volkin	L. A. Thomas

Nucleic Acid Structure

W. E. Cohn	J. X. Khym
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Since it was supposedly established that Levene and Harris,² in 1933, had prepared ribose-3-phosphate from yeast adenylic and guanylic acids, there has been no interest evident in the possible existence of ribose-2-phosphate. As a result of our studies on the structure of the isomeric adenylic acids (*a* and *b*, now known to be 2' and 3', respectively), it was found that ribose-2-phosphate could be prepared and characterized. Following this discovery, it became important not only to determine the properties of this new compound but also to determine whether Levene and Harris had really isolated the ribose-3-phosphate as claimed, or whether they had prepared a mixture of the two ribose phosphates. The obvious method for solving the problem was to compare the properties of both ribose phosphates (2 and 3) with the data of Levene and Harris, who reported only the rotational properties of their preparations. Such a comparison involved the preparation of both the

¹Inasmuch as B. L. Strehler and M. J. Cormier have left the group, the work on Energy-Transfer Systems is concluded.

²P. A. Levene and S. A. Harris, *J. Biol. Chem.* 101, 419 (1933).

brucine salts as well as the sodium salts of both compounds.

The possibility of a large-scale separation of the isomers by crystallization procedures, using the brucine salts, was first explored. While pure ribose-3-phosphate can be obtained in this fashion in good yield, pure ribose-2-phosphate cannot. This was finally prepared by large-scale ion-exchange chromatography. Both the brucine and the barium salts have been prepared, the latter serving as a source of the sodium salt (by exchanging Na⁺ for Ba⁺⁺ on Dowex-50-Na⁺).

The rotations, determined by D. G. Doherty, are shown in Table 29.

When adenosine-3'-phosphate is completely hydrolyzed to ribose phosphates, the mixture is about 80% in the 3 form. When adenosine-2'-phosphate is the starting substance, about 70% of the ribose phosphate is in the 3 form. Since the normal ratio 2':3' in adenylic acid from yeast nucleic acid is 40:60, the ribose phosphates produced from such a mixture might be about 76% in the 3 form. A slight enrichment in the less soluble ribose-3-phosphate in the course of working these up would give the 77 and 83% figures which are now calculated as the purity of the Levene and Harris preparations. It would thus seem that they did not have a pure preparation of ribose-3-phosphate and the deduction that their starting nucleotides were of the 3' variety was actually based on an erroneous identification of the ribose phosphate.

Two papers, one dealing with the identification of the adenylic acids through the ribose phosphates and the second dealing with the preparation and characterization of ribose-2- and -3-phosphates, are in press.

TABLE 29. OPTICAL ROTATIONS ($\alpha_D^{22} - \alpha_D^{23}$)

	RIBOSE-2-PHOSPHATE	RIBOSE-3-PHOSPHATE	DATA FROM LEVENE AND HARRIS	PERCENTAGE OF RIBOSE-3-PHOSPHATE*
Brucine salt (P _y -H ₂ O)	-27.5	-35.0	-33.3	77
Na salt	-10.7	-10.2	-9.7	
Na salt + borate	-14.6	+50	+39	83

*Calculated from our figures for the Levene and Harris preparation.

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Energy-Transfer Systems

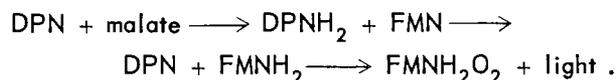
B. L. Strehler³ M. J. Cormier

In order to determine, if possible, the function of the various components necessary for the bacterial extract luminescence recently discovered in this laboratory,⁴ a number of kinetic measurements have been made. These include (1) measurements of apparent activation energies, (2) determination of the time required for luminescence to become half maximal after the final component is added ("half-rise time"), and (3) determination of Michaelis constants for the various components and the effect of long chain aldehydes on the apparent Michaelis constant for oxygen.

It was found that the activation energy for the over-all reaction depends upon which factor is rate-limiting. The half-rise times are as follows:

Substance Added Last	Half-rise Time (seconds)
DPN	108
Malate	108
DPNH ₂	2.8
FMN	2.8
KCF	0.1
O ₂	0.08
FMNH ₂	0.07

They are greater for reactions farther from the final luminescent reaction and similar for substances which might be expected to react with each other in the following sequence:



Varying the concentrations of various intermediates causes changes in luminescence that are linear on Lineweaver-Burk plots. Of particular interest is that long chain aldehydes affect the apparent binding of oxygen both for luminescence and for respiration by a factor of about 4. Together with the rise-time data, this suggests that reduced flavin, the aldehyde (KCF, identified as palmitaldehyde), oxygen, and enzyme are the active light-producing system.

³Now at University of Chicago.

⁴B. L. Strehler and M. J. Cormier, *Biol. Semiann. Prog. Rep.* Aug. 15, 1953, ORNL-1614, p. 68.

A direct demonstration of this was carried out with E. N. Harvey and J. J. Chang at Princeton University. It was shown that chemically produced FMNH₂ or reduced riboflavin could supplant the requirement for reduced DPN and that the rate of flavin oxidation as well as the rise time of luminescence depends on the presence of aldehyde. Further experiments have essentially eliminated the possibility that factors other than those enumerated are necessary for the extract luminescence, thus giving a high degree of probability to the identification of FMN as bacterial luciferin.

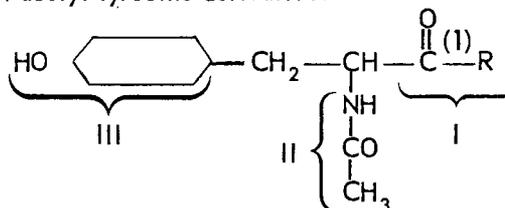
With F. H. Johnson, also at Princeton, the pressure-sensitive (inhibited) step in bacterial luminescence has been identified as the reduction of FMN by DPNH₂. It was also possible to reproduce all the luminescent phenomena that intact bacteria show under pressure with the cell-free system. The actual luminescent reaction proceeds with an apparent volume decrease on activation, the net effect of pressure being a balance between the inhibitory effect on prior steps and the acceleration of the luminescent reaction.

The factors found necessary for *Achromobacter fischeri* luminescence have been found to be necessary for 10 other strains of luminous bacteria. Michaelis constants, however, vary from strain to strain.

Studies on Enzyme-Substrate Binding

D. G. Doherty L. A. Thomas
E. Eavenson

The previously reported studies^{5,6} on the binding of various substrates and inhibitors to α -chymotrypsin established the structural requirements necessary for binding on the active center of the enzyme and for consequent enzymic activity (or inhibitory activity) to be essentially three in number as indicated in the structural formula of an N-acetyl tyrosine derivative:



An N-Acetyl Tyrosine Derivative.

⁵D. G. Doherty and F. Vaslow, *J. Am. Chem. Soc.* 74, 931-936 (1952).

⁶F. Vaslow and D. G. Doherty, *J. Am. Chem. Soc.* 75, 928-931 (1953).

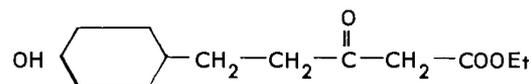
Only I is essential; the acetoamino group and the *p*-hydroxy group assist but are not absolute requirements. When bond (I) was to N, O, or S, hydrolysis was effected by the enzyme (the compound was a "substrate"); when it was to a carbon atom (e.g., the methyl ketone), the substance was bound but not hydrolyzed (it was a "competitive inhibitor").

The quantities obtained in these studies for the enzyme-substrate binding forces were of a magnitude which indicated that the failure to hydrolyze a carbon-carbon bond was only a matter of the strength of this bond. Such bonds are indeed split by various other enzymes but only as a result of oxidative processes (e.g., successive dehydrogenation, hydration, and oxidative cleavage). It became of interest to see whether or not α -chymotrypsin might cleave, nonoxidatively, a carbon-carbon bond suitably activated (bond strength weakened) by adjacent groups. Such cleavage would serve as additional proof for the hypothesis that there is no sharp line of demarcation between substrates and competitive inhibitors and that enzymic action or inaction is a competition between the enzyme-substrate binding energy and the energy of the susceptible bond, structural considerations being met.

Suitable compounds for testing this hypothesis would be the β -keto ester or β -keto nitrile derivatives of *N*-acetyl tyrosine ($R = CH_2-COOEt$ or CH_2CN). Attempts to synthesize these were unsuccessful in that the products could not be well characterized. However, it was possible to synthesize and characterize ethyl-3-keto-5-phenyl valerate and ethyl-3-keto-5-(*p*-hydroxyphenyl) valerate, which differ from the desired substances only in the absence of the acetamino group, group II in the structural formula, or the *p*-hydroxy group, neither of which are absolute necessities for α -chymotrypsin action. These compounds were obtained by a C-acylation of acetoacetic ester with the appropriate acid chloride followed by deacetylation and removal of protecting groups by hydrolysis.

When ethyl-3-keto-5-phenyl valerate was subjected to the action of α -chymotrypsin, a slow hydrolysis was observed, presumably giving phenylpropionic acid and ethyl acetate. However, the *p*-hydroxy analog (more similar to tyrosine in structure) was rapidly hydrolyzed to ethyl acetate and *p*-hydroxyphenyl propionic acid. The latter was isolated

from a 70% hydrolyzed mixture and characterized to prove that the point of rupture was indeed between the carbonyl and methylene ester carbons, as shown below:



Thus a proteolytic enzyme which "normally" hydrolyzes C-O, C-N, or C-S bonds can, under suitable conditions, rupture a C-C bond.

Nucleic Acid Enzymology

E. Volkin M. H. Jones

In the preceding semiannual report⁷ a description was given of the action of a bacterial deoxyribonuclease (DNAase) isolated in crude form from bacteriophage lysates. The enzyme has now been purified and obtained essentially free of other interfering enzymes (monoesterases, deaminases, etc.), and its characteristics have been found to be quite similar to those of the familiar (crystalline) pancreatic DNAase. An ion-exchange analysis of the products resulting from its action on thymus deoxyribonucleic acid (DNA) reveals that the very-low-molecular-weight fragments are continuously liberated over the time course of the hydrolysis rather than being produced in bulk as the initial reaction products. In order to study the action of the bacterial DNAase on bacteriophage DNA, a mild procedure was sought for the preparation of the latter.

In the course of these studies, it appeared that some bacteriophage protein which is not a part of the membrane was associated with the DNA. Since this finding seemingly contradicted the present concepts of bacteriophage structure, an attempt has been made to describe more definitively this protein moiety. Some of the evidence for its existence and some of its properties are summarized:

1. When highly purified bacteriophage T4r+ is osmotically lysed⁸ and the remaining intact phage and phage "ghosts" are removed by high-speed centrifugation, the supernatant fraction contains most of the phage DNA, some soluble protein, and small amounts of dialyzable amino acids and peptides.

⁷E. Volkin and M. H. Jones, *Biol. Semiann. Prog. Rep. Aug. 15, 1953*, ORNL-1614, p 65.

⁸R. M. Herriott, *J. Bacteriol.* 61, 752-754 (1951).

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2. After dialysis, the ultraviolet absorption in the region 220 to 250 $m\mu$ remains considerably elevated over that of the purified T4r+ DNA.

3. Nitrogen and phosphorus analyses of this fraction reveal a high N/P ratio.

4. Quantitative ninhydrin (amino acid) analyses on acid hydrolysates of the intact phage, the "ghosts," and the soluble fraction indicate that this last fraction contains about 25% of the phage protein.

5. Semiquantitative analysis of individual amino acids by paper chromatography reveals a considerable difference in constitution of the soluble protein compared with the ghost protein, the latter being relatively richer in glutamic and aspartic acids, tyrosine, arginine, and methionine, the former having a higher content of glycine.

6. The soluble protein is readily hydrolyzed by purified papain, whereas the ghost protein and intact phage are resistant.

7. In the analytical ultracentrifuge, the soluble fraction is seen as a single hypersharp peak, typical of DNA. After hydrolysis by a minute quantity of pancreatic DNAase, followed by dialysis, a new peak is observed, symmetrical in appearance, and having a much lower sedimentation rate.

That the soluble protein may actually enter the bacterial cell along with the DNA on infection is indicated by the following experiments with labeled phage, prepared by growing T4r+ in a medium containing a small amount of glycine-C¹⁴, so that the resultant phage incorporates some of the label in both DNA (purines) and protein (soluble and membrane):

1. Soluble DNA-protein, prepared by osmotic bursting of the phage, had a DNA:protein total radioactivity ratio of 2.1:2.2.

2. The soluble DNA-protein, recovered after the labeled phage had been adsorbed on bacterial membranes (prepared according to Weidel⁹), had a similar ratio of 2.1:2.2.

3. The soluble material liberated from bacteria where the ratio of phage particles to cells was 50:1 (lysis by excess phage) also had a ratio of 2.1:2.2.

A more highly labeled phage has since been prepared, again with glycine-C¹⁴, in order to determine if this soluble protein enters the bacteria under more normal conditions, that is, when the infection ratio is close to 1 phage per 1 bacterium. Attempts to repeat Hershey's experiments,¹⁰ in which the ghosts were removed from infected bacteria by blending, have thus far been unsuccessful owing to considerable damage of the infected cells. Therefore the cells after infection were immediately centrifuged in the cold, broken by alumina grinding, and the bacterial and phage membranes centrifuged off at high speed. By use of variable quantities of bacteria, suspended in buffer and infected with a constant amount of labeled phage to obtain ratios of 0.3:1, 1:1, and 3:1, the supernatant fractions thus prepared were found to contain somewhat varying amounts of labeled (phage) DNA and a fairly constant amount of labeled soluble (phage) protein. The ratios of these, although varying somewhat among themselves because of the variation in the DNA figure, were not too different from the ratio observed between the DNA and soluble protein of the infecting phage. This experiment, although imperfect from the quantitative standpoint, is also consistent with the other indicating the penetration of the soluble (nonmembrane) phage protein into the bacterial cell in "normal" infection.

Experiments are now in progress to characterize the soluble DNA-protein fraction in terms of its physical properties and to attempt to establish the function of the two moieties after entrance in the bacterial host cell.

⁹V. W. Weidel, *Z. Naturforsch.* B6, 251-259 (1951).

¹⁰A. D. Hershey and M. Chase, *J. Gen. Physiol.* 36, 39-56 (1952).

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TABLE 30. PERCENTAGE OF TOTAL C¹⁴ IN THE PRODUCTS FROM SEDOHEPTULOSE-C¹⁴ METABOLISM BY BARLEY LEAVES* IN THE LIGHT

COMPOUNDS	EXPERIMENTAL CONDITIONS					
	Air			Nitrogen		
	11 min	16 min	82 min	9 min	20 min	60 min
Sedoheptulose	78	48	35	75.0	66.4	42.7
Sucrose	5	16	23	0	8.6	6.6
Ribose	0	0	0	0	0	2.6
Upper phosphate area	4	10	7	4	10.2	8.6
Lower phosphate area	Trace	Trace	Trace	2	5.5	6.6
Alanine	5	5	8	10	7.8	21.7
Glyceric acid	8	9	2	7	1.5	2.0
Tetrose sugar area	0	0	5	0	Trace	0
Glycolic acid	Trace	Trace	Trace	Trace	Trace	Trace
Unknown (R _f ~ 0.8)	0	8	17	0	Trace	7.8

*Wintex barley.

depend on the environmental conditions of the experiments. Leaves of all plants tested, when placed in the light and air, rapidly converted sedoheptulose-C¹⁴ to sucrose which was labeled in both the glucose and fructose portions. In controls run in the light, the sedoheptulose was metabolized to respiratory products such as glutamic, aspartic, and succinic acids.

The metabolism of sedoheptulose by leaves in the air was so rapid that intermediary compounds in the process did not accumulate sufficient radioactive carbon to allow elucidation of the individual steps of this process. Consequently, experiments were run in a nitrogen atmosphere in which the conversion of sedoheptulose to sucrose was inhibited and the intermediary phosphate esters were formed in sufficient amounts to permit chromatographic identification. In such short-time experiments the major products from sedoheptulose were the two three-carbon compounds, glyceric acid and alanine, and a four-carbon phosphate ester which has been identified as a tetrose sugar phosphate. The results suggest the initial breakdown of sedoheptulose during metabolism *in vivo* into a triose and a tetrose sugar phosphate. The triose apparently was converted to alanine and glyceric acid, but the four-carbon sugar phosphate accumulated as such. The reverse of this reaction

would account for sedoheptulose formation in the path of carbon in photosynthesis.

Sedoheptulosan-C¹⁴, the anhydride of this seven-carbon sugar, was also used in metabolism studies identical with those for the free sugar. There was no metabolism of the anhydride in leaves kept in the dark and only trace metabolism of it in those kept in the light. Thus the use of the free sugar in experimentation is necessary.

Action of Acid on Sedoheptulosan

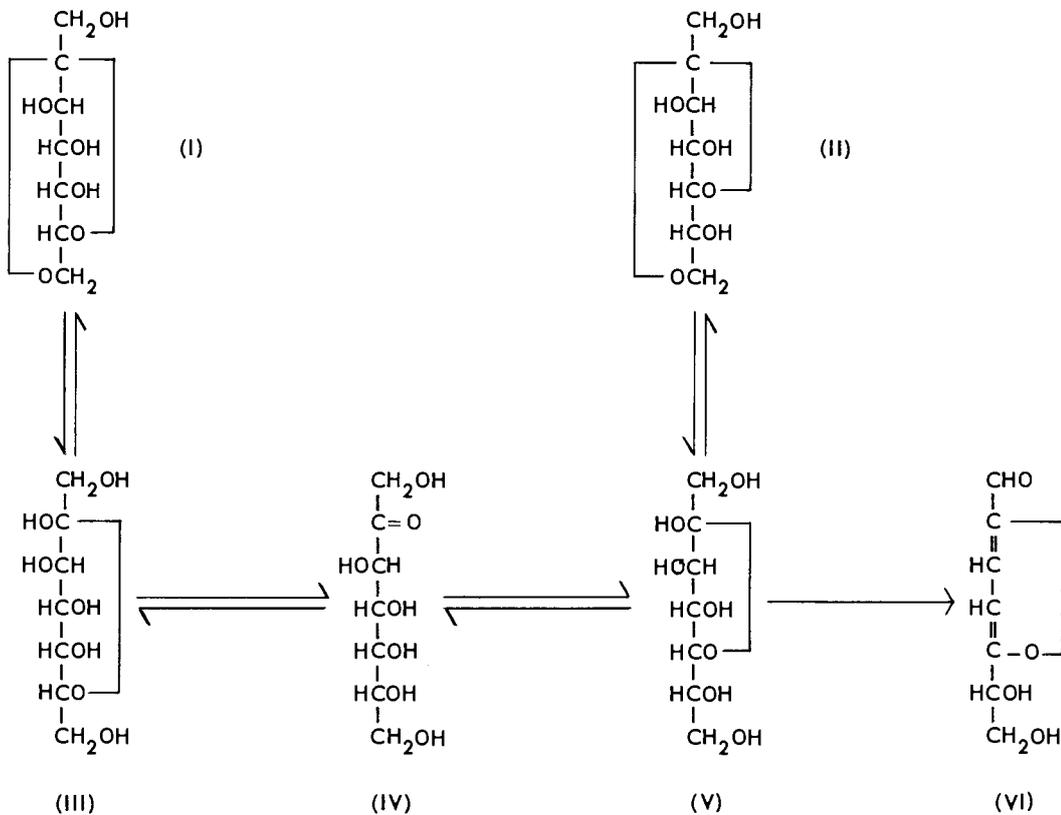
L. P. Zill

N. E. Tolbert

Sedoheptulosan occupies a position of major importance with respect to the chemistry of sedoheptulose from which it is derived. In addition, the formation of this anhydride is a requisite step in the isolation of sedoheptulose from natural sources. The formation of two compounds, other than sedoheptulose, by the action of acid on sedoheptulosan has been reported. In view of these considerations a study was made of the formation, isolation, and identification of these compounds. It was possible, after acid treatment of sedoheptulosan, to isolate these two unknown compounds by thick-paper chromatography. They have been tentatively identified as 2,7-anhydro- β -D-*altro*-heptulofuranose (II) and 5-(1,2-dihydroxyethyl)-2-furfuraldehyde (VI). These identi-

ties have been based on the behavior of their borate complexes on anion exchangers, ease of acid hydrolysis, ultraviolet absorption spectra, R_f values from paper chromatography in various solvents, periodate oxidation, reversibility of formation in acid, orcinol test, and chromatropic acid test. On the basis of this work the following scheme of interconversions among seven-carbon compounds stemming from sedoheptulose (IV) is proposed:

These compounds are identified as follows: (I) 2,7-anhydro-D-*altro*-heptulopyranose; (II) 2,7-anhydro-D-*altro*-heptulofuranose; (III) D-*altro*-heptulopyranose; (IV) D-*altro*-heptulose; (V) D-*altro*-heptulofuranose; (VI) 5-(1,2-dihydroxyethyl)-2-furfuraldehyde. This proposed scheme of conversions is completely analogous to similar reactions known to occur in the pentose and hexose sugars.





PHYSIOLOGY

PHYSIOLOGY

C. W. Sheppard J. N. Dent¹
N. G. Anderson P. J. Mathias
M. L. Anderson J. Ginsburg²

Circulatory Mixing of Injected Isotopes

C. W. Sheppard

Quantitative investigations of the rate of movement of substances in the body by the usual tracer methods requires the assumption that movement occurs between a collection of uniformly mixed compartments. This often imposes a serious limitation on the value of the data which can be obtained by kinetic analysis of the results of tracer experiments in mammals and in man. One particular situation arises when the disappearance from the circulation of injected labeled substances is being studied. Here, occasions arise where the injected material leaves the circulation at a rate comparable with the rate of circulatory mixing. An analysis of the corrections which must then be made to the results of experiments on the mixing and disappearance of K^{42} from the circulation of dogs has been presented,³ but the analysis requires an understanding of the kinetics of circulatory mixing. This process is studied experimentally by injecting substances such as labeled red cells which do not disappear from the circulation and observing their mixing kinetics. Physiologists have conducted many experiments of this sort on man and lower animals, particularly the dog. The curves of concentration of label vs time are obtained by injecting the label into the right heart and taking samples of blood from the aorta or a nearby tributary. The curves thus obtained show a delayed appearance following injection while the blood makes the first trip through the lung. There is then a sharp upsweep to a high peak, which is followed by one or more observable oscillations of concentration as one or more waves of recirculation appear, and finally a residual plateau of concentration when the label has been uniformly mixed with the circulating blood. The curves resemble those for certain types of transient phenomena in electric circuits.

¹Temporary employee.

²Research Participant.

By applying the mathematical methods of electric circuit transient analysis to these data, an increased understanding of the factors underlying the curves has been obtained, and a promising approach to the study by mathematical methods of circulatory mixing and transport has been achieved. An interesting electrical analogy which gives a good first approximation to circulatory mixing phenomena has been discovered. This analogy was tested on the electric analog computer in the laboratory of Dr. Gordon Brownell at Massachusetts General Hospital. The results indicated that the practical construction of such an electrical analog is feasible.

Distribution of Lithium in Amphibian Embryos

J. N. Dent

Considerable attention has been given to the action of lithium ions in bringing about developmental distortions producing microcephaly and hypomorphosis in amphibian embryos as shown by Gustafson.⁴ The question arises whether the lithium is generally distributed throughout the embryo or whether it is localized in those regions in which its effects are observed. The technique of Dent and Sheppard⁵ is being refined and used in an attempt to answer this question. The current procedure is as follows: Embryos of *Rana pipiens* or *Ambystoma maculatum* are immersed in solutions containing either LiCl, or LiCl with 75% enrichment of Li^6 , freeze-dried, embedded in paraffin and sectioned. The dry sections are mounted on 1 x 3-in. plates covered with Eastman 25- μ m NTA emulsion. These preparations are then exposed to thermal neutrons in the ORNL Graphite Reactor, developed photographically, and stained. Preliminary observations indicate that lithium becomes uniformly distributed throughout the embryo.

Movement of Lithium in Amphibian Embryos

J. Ginsburg J. N. Dent

Many workers have described the effects of lithium on the development of the amphibian

³C. W. Sheppard, R. R. Overman, W. S. Wilde, and W. C. Sangren, *Circulation Research*, 1, 284-297 (1953).

⁴T. Gustafson, *Rev. Suisse Zool.* 57, Suppl. 1, 77-92 (1950).

⁵J. N. Dent and C. W. Sheppard, *Biol. Semiann. Prog. Rep.* Aug. 15, 1953, ORNL-1614, p 72.

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embryo, and a program has been initiated to ascertain the effects of lithium on the distribution of naturally occurring electrolytes in the embryo. Lithium, potassium, and sodium contents of embryos have been analyzed by means of a Perkin-Elmer flame spectrophotometer.

Observations have been made concerning the movement of lithium into and out of amphibian embryos in early stages of development. Frog embryos in the neural-fold stage show a rapid accumulation of lithium during the first hour of immersion in a 0.15 M LiCl solution. The concentration of lithium in the embryos then holds constant, or increases only slightly, for as long as 8 hr and fails to show equilibration with the external medium. The release curve shows a rapid decline of lithium during the first hour after transferring the lithium-treated embryos to a lithium-free medium followed by a gradual loss of lithium for 38 hr, at which time the lithium concentration in the embryo approaches closely that of the external medium. The total amount of lithium which enters the embryo during a given period of time is directly proportional to the concentration of lithium in the external medium. The potassium content and nitrogen content of the embryos remain constant throughout the period observed, but there have been some indications that sodium may be lost. The problem is somewhat complicated by the large shifts in water content which accompany the immersion of the embryos in concentrated lithium solutions.

Iodine Metabolism of Terrestrial Salamander Embryos

J. N. Dent

The development of numerous specimens of the relatively rare bronzed salamander, *Aneides anaeus*, was followed from early limb-bud stages throughout hatching and gill resorption. Its developmental morphology was found to differ from that previously described for another entirely terrestrial plethodontid salamander, *Plethodon cinereus*,⁶ only in minor detail. The role of the thyroid gland in the development of typical amphibians has been studied extensively, and it is well known that removal or inhibition of the thyroid in the typical amphibian larva prevents metamorphosis. In the terrestrial amphibian, the larval and metamorphic stages are

greatly telescoped and altered, presumably, either because the thyroid functions precociously or because differentiation proceeds without the stimulus of the thyroid secretion. On the basis of histological observations, Dent concluded that the thyroid gland of *P. cinereus* functions only during the final stages of development. Autoradiographic preparations made from embryos of *A. anaeus*, which had been immersed in solutions containing I¹³¹, showed that their thyroids began to fix iodine shortly before their gills reached maximal growth, confirming the observations made on *P. cinereus*. The amount of iodine fixed in the thyroid then increased greatly as the gills regressed and hatching ensued. In a study of iodine distribution in the frog tadpole, Dent and Hunt⁷ found strong localizations of iodine in several extra-thyroidal structures (eye, gut, thymus, horny teeth). In *A. anaeus*, however, no very marked localizations of iodine were found outside the thyroid, although diffuse distributions of fixed iodine were observed in the developing skin and in the yolk of all stages studied and in the eye after the beginning of thyroid function. Destruction of the thyroid by immersion in solutions containing I¹³¹ at the stage of maximum gill extension did not prevent these animals from passing through the final stages of development. This would seem to indicate that, in the course of its evolution, *A. anaeus* had reached a state in which, unlike typical amphibians, it no longer required the stimulus of the thyroid for the attainment of adult form.

High Resolution Differential Centrifugation

N. G. Anderson

M. L. Anderson

Several workers have recently emphasized the fact that similar techniques for isolating cell components have not yielded reproducible results by various investigators. Techniques for differential centrifugation, on which the separation is based, have been empirically devised for the most part, and appear to have been based on the assumption that the various particulates would sediment according to accepted physical principles. However, simple calculations show that whole liver cells, nuclei, and mitochondria, assuming diameters of 200, 11, and 1.5 μ , and densities of 1.09, 1.10, and 1.11, should have relative sedimentation

⁶J. N. Dent, *J. Morphol.* 71, 577-601 (1942).

⁷J. N. Dent and E. L. Hunt, *J. Exptl. Zool.* 121, 79-98 (1952).

rates, in 0.25 M sucrose containing small amounts of salts, of around 130, 47, and 1, respectively. While these differences should allow clean separation by use of the layering system introduced by Wilbur and Anderson,⁸ clean separation of these components is difficult. In spite of the approximately 130:1 expected differences in the sedimentation rates of whole cells and mitochondria, the separation of these two components is not complete in one centrifugation.

The techniques of preparation and fractionation of liver homogenate have therefore been extensively reexamined in this laboratory. Data on the density and viscosity of various media, the effect of perfusion and pH on cell breakage, and data on the size of various cell constituents have been obtained. Factors affecting the aggregation of various components have been examined in detail. By the use of a stroboscopic-illumination system, it has

been found possible to observe the behavior of a number of layered systems in the centrifuge. It has been found that several anomalies occur which prevent ideal sedimentation during centrifugation. These include hydrodynamic effects which cause local areas containing a number of large particles to move as a unit dragging along associated small particles; convection currents which can mix rather completely the contents of a centrifuge tube during an ordinary run; and agglutination of homogenate constituents. A separation approximating the ideal is approached by a combination of suitable dilution of the homogenate, certain alterations in the suspending medium, careful layering over a gradient system sufficiently steep to prevent convection, and rigid adherence to a suitable acceleration and deceleration schedule. A speed-vs-time recording system allows integration of the total centrifugation done. Speeds are accurately measured by a stroboscopic system. The application of these principles to several different tissues is currently being examined.

⁸Karl Wilbur and N. G. Anderson, *Exptl. Cell Research* 2, 47-57 (1951).



BIOPHYSICS

BIOPHYSICS

J. S. Kirby-Smith E. B. Darden, Jr.
D. L. Craig M. L. Randolph

Effects of Ultraviolet Radiation in *Tradescantia*

J. S. Kirby-Smith D. L. Craig

The first phase of a comprehensive investigation of chromosome breakage in *Tradescantia* pollen by ultraviolet radiation has been completed. These experiments have been carried out to complement existing information on chromosome damage by ionizing radiation and, wherever possible, to make a comparative study of the mode of action of the two agents. Before pursuing the major points of this investigation, which is concerned with quantitative dose curves and wave length relations for ultraviolet-induced breakage, a number of basic preliminary factors have had to be examined and their importance evaluated. These include an understanding of the time-intensity (reciprocity) relations, a careful elimination of any possibility that the chromosome breakage figures have been modified by effects due to ozone, and studies designed to determine whether any photoreactivation processes or an oxygen effect might influence the observations. The results of these prerequisite researches and preliminary findings in the main program may be summed up as follows:

1. For equal doses, intensity of incident radiation may be varied by at least a factor of 10 with no change in aberration frequencies for one-hit breaks. Two-hit breaks, i.e., chromatid/chromatid exchanges, are too infrequent to allow any conclusions to be drawn for this situation at present.

2. Any possibility that ozone plays a role has been eliminated by the use of filters and by irradiation of pollen in some cases in a nitrogen atmosphere.

3. Irradiation of pollen in nitrogen leads to chromosome-aberration frequencies identical to those obtained for treatment in air. This lack of an oxygen effect for ultraviolet-induced breakage is contrary to the situation for ionizing radiation and verifies findings in the other materials.

4. Repeated exposures at various wave lengths and at widely different doses have shown that no photorecovery of ultraviolet-induced chromosomal damage by means of subsequent treatment with

visible or near visible radiation takes place in *Tradescantia* pollen.

5. A preliminary action spectrum for chromosome breakage covering the range 3650 to 2480 Å has been obtained, and is in substantial agreement with the well-known curves for the bactericidal action of ultraviolet as well as the nuclei and absorption spectrum.

Further experiments to extend these measurements and to obtain quantitative dose curves are in progress. Present results indicate a linear relation governing the variation of aberration frequency with dose at the lower ranges, and a trend toward saturation at the higher doses.

Effects of Ionizing Radiation in *Tradescantia*

J. S. Kirby-Smith D. L. Craig

X and Gamma Rays. Irradiation of *Tradescantia* pollen at different dose rates with X and gamma rays is in progress. These experiments are designed to determine the restitution half life for chromosome breakage in dry pollen. Since much of the work of this group involves this material, a need for information on this point has been felt for some time.

Fast Neutrons. The results for *Tradescantia* in the recent nuclear-detonation tests have been written up and published.¹ The most pertinent conclusion to be drawn from this work at the present time is that, for equal doses of fast neutrons of roughly the same energy distribution from the ORNL 86-in. cyclotron and under burst conditions from a nuclear detonation, chromosome breakage in *Tradescantia* is not widely different.

Magnetic Resonance

J. S. Kirby-Smith

Work along these lines continues to be largely in instrument development. The 3-cm (X-band) paramagnetic resonance apparatus has been pushed close to its maximum sensitivity and, although it is sufficiently sensitive to detect an annoying amount of stray paramagnetic contaminants, no radiation-induced free radicals have been observed.

¹J. S. Kirby-Smith and C. P. Swanson, with Appendix by C. W. Sheppard and E. B. Darden, Jr., *Science* 119, 42-45 (1954).

Efforts to find resonances in irradiated plastics and in germanium are under way.

Radiological Physics

M. L. Randolph E. B. Darden, Jr.
C. W. Sheppard

The Cockcroft-Walton accelerator, intended for generation of neutrons, has been in constant use for neutron-flux calibration measurements at low levels. Independent calibrations of a fast-neutron detector (long counter) by a standard neutron source and by the associated-particle technique, using either a proportional or scintillation counter for the protons from the $d(d,p)$ reaction which accompanies the $d(d,n)$ reaction, all agree within 15%. Neutron scattering and angular asymmetry factors are being evaluated. Since yields of the order of 3×10^7 neutrons/sec have been obtained from a drive-in target, it seems that this simple and very stable kind of target will be acceptable for low-level biological exposures.

Dosimetry is being increasingly emphasized along several lines of approach through use of the essentially monoenergetic neutrons produced in the $d(d,n)$ reaction. Two similar thimble-type ionization chambers, one with walls of polyethylene, with ethylene as the gas, the other polystyrene with acetylene, have been constructed according to the basic design of the gamma-ray thimble chamber.² Preliminary measurements made with the former are in fair agreement with the calculated dose due to fast-neutron energy absorption in idealized tissue for a given flux of fast neutrons of known energy.³ A special electrically conducting plastic of specified atomic composition with tissue-equivalent properties has just been procured for the construction of a third chamber.

²C. W. Sheppard and E. B. Darden, Jr., in J. S. Kirby-Smith and C. P. Swanson, *Science* 119, 42-45 (1954).

³L. D. Marinelli, *Nucleonics*, 8, No. 6, 5-20 - 5-32 (1951).

Work is also going forward on the count-rate method of fast-neutron dosimetry which employs a Hurst type polyethylene proportional counter.⁴ Plans are being made to move the accelerator to its permanent well-shielded installation during March. The first preliminary irradiations of *Tradescantia* inflorescences with fast neutrons are scheduled for the near future.

Major aspects of the fast-neutron dose studies made in the ORNL 86-in. cyclotron in preparation for the 1953 nuclear-weapons tests and nonclassified aspects of their application in the tests have been summarized in the open literature.¹

A 72-curie Co^{60} gamma-ray irradiation facility has been designed and built in the Y-12 and ORNL shops for the use of the Bureau of Entomology of the U. S. Department of Agriculture in their screw-worm fly control project. Previous experiments of the USDA, in which a smaller Co^{60} irradiation facility loaned to them by this laboratory was used, have shown the desirability of this method for the sterilization of masses of screwworm pupae used in the control work. The new facility makes use of the hollow, distributed type of source to provide a uniformly irradiated cavity into which the sample is inserted.⁵ Radiation hazards connected with operation have been reduced to a minimum by placing the source in the bottom of a vertical well in a lead shield and employing a light overhead chain-and-pulley arrangement operated remotely to handle the samples. The radiation chamber was calibrated by determining the dose rate at points inside by means of a standard polystyrene gamma-ray thimble chamber inserted in a phantom of the same material.⁶

⁴G. S. Hurst, R. H. Ritchie, and H. N. Wilson, *Rev. Sci. Instr.*, 22, 981-986 (1951).

⁵C. W. Sheppard, AECD-2566, 1949.

⁶E. B. Darden, Jr. and C. W. Sheppard, ORNL-1002, 1951.