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SEMIANNUAL PROGRESS REPORT

FOR PERIOD ENDING AUGUST 15, 1954

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BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

for Period Ending August 15, 1954

Alexander Hollaender, Director
Edited by E. J. Slaughter

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BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications. Since February 15 of this year 80 papers from the Biology Division have been published in open literature. A breakdown on this number shows 35 articles, 44 abstracts and book reviews, and one symposium. The "Symposium on Effects of Radiation and Other Deleterious Agents on Embryonic Development" was published as a 337-page Supplement to the Journal of Cellular and Comparative Physiology and comprises the material presented at the Sixth Annual Biology Research Conference in Oak Ridge. The proceedings of the Seventh Conference on "Genetic Recombination" are in an advanced phase of preparation for press and will be submitted for publication within a month.

In addition to the listed publications, there are 62 papers and 43 abstracts in press.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Alexander, M. L.	Mutation rates at specific autosomal loci in the mature and immature germ cells of *Drosophila melanogaster*	*Genetics* **39**, 409-428 (1954)
Arnold, W. A., and J. B. Davidson	The identity of the fluorescent and delayed light emission spectra in *Chlorella*	*J. Gen. Physiol.* **37**, 677-684 (1954)
Baker, W. K.	On the physical distance between breakage point and affected locus in a V-type position effect	*J. Heredity* **45**, 65-68 (1954)
Billen, D., and E. Volkin	The effect of X rays on the macromolecular organization of *Escherichia coli*	*J. Bacteriol.* **67**, 191-197 (1954)
Carlson, J. G.	Immediate effects on division, morphology, and viability of the cell	*Radiation Biology*, ed. A. Hollaender, Vol. I, McGraw-Hill, New York, pp 763-824 (1954)
Dent, J. N.	A study of regenerates emanating from limb transplants with reversed proximodistal polarity in the adult newt	*Anat. Record* **118**, 841-856 (1954)
Dolin, M. I.	The oxidation and peroxidation of $DPNH_2$ in extracts of *Streptococcus faecalis*, 10Cl (Comm. to ed.)	*Arch. Biochem. and Biophys.* **46**, 483-485 (1953)
Furth, J.	Morphologic changes associated with thyrotrophine-secreting pituitary tumors	*Am. J. Pathol.* **30**, 421-463 (1954)
Furth, J., and E. Lorenz	Carcinogenesis by ionizing radiations	*Radiation Biology*, ed. A. Hollaender, Vol. I, McGraw-Hill, New York, pp 1145-1201 (1954)
Giles, N. H.	Radiation-induced chromosame aberrations in *Tradescantia*	*Radiation Biology*, ed. A. Hollaender, Vol. I, McGraw-Hill, New York, pp 713-761 (1954)
Halmi, N. W., and W. D. Gude	The morphogenesis of pituitary tumors induced by radiothyroidectomy in the mouse and the effects of their transplantation on the pituitary of the host	*Am. J. Pathol.* **30**, 403-419 (1954)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Hollaender, A., ed.	Symposium on Effects of Radiation and Other Deleterious Agents on Embryonic Development	*J. Cellular Comp. Physiol.* **43**, Suppl. 1 (1954)
———	Biology Division Semiannual Progress Report for period ending February 15, 1954	Project Report ORNL-1693
Hollaender, A., and G. E. Stapleton	Modification of radiation damage after exposure to X rays	*Brit. J. Radiol.* **27**, 117-121 (1954)
Khym, J. X., and W. E. Cohn	Identification of the purine nucleotides *a* and *b* as the 2′ and 3′ phosphoribosides, respectively	*J. Am. Chem. Soc.* **76**, 1818-1823 (1954)
Mathias, P. J., and C. W. Sheppard	An upper limit for acetylcholine content and synthesis in human erythrocytes	*Proc. Soc. Exptl. Biol. Med.* **86**, 69-74 (1954)
Pomper, S., K. M. Daniels, and D. W. McKee	Genetic analysis of polyploid yeast	*Genetics* **39**, 343-355 (1954)
Russell, L. B.	The effects of radiation on mammalian prenatal development	*Radiation Biology*, ed. A. Hollaender, Vol. I, McGraw-Hill, New York, pp 861-918 (1954)
Russell, L. B., and W. L. Russell	An analysis of the changing radiation response of the developing mouse embryo	*J. Cellular Comp. Physiol.* **43**, Suppl. 1, 103-149 (1954)
Russell, W. L.	Genetic effects of radiation in mammols	*Radiation Biology*, ed. A. Hollaender, Vol. I, McGraw-Hill, New York, pp 825-859 (1954)
Sarvella, P. A.	Pearl, a new spontaneous coat and eye color mutation in the house mouse	*J. Heredity* **45**, 19-20 (1954)
Sheppard, C. W.	Mathematical considerations of indicator dilution techniques	*Minnesota Med.* **37**, (No. 2) 93-104 (1954)
Strehler, B. L., and F. H. Johnson	The temperature-pressure-inhibitor relations of bacterial luminescence *in vitro*	*Proc. Natl. Acad. Sci. U.S.* **40**, 606-617 (1954)
Strehler, B. L., E. N. Harvey, J. J. Chang, and M. J. Cormier	The luminescent oxidation of reduced riboflavin or reduced riboflavin phosphate in the bacterial luciferin-luciferase reaction	*Proc. Natl. Acad. Sci. U.S.* **40**, 10-12 (1954)
Strehler, B. L., and J. R. Totter	The determination of ATP and related compounds: Firefly luminescence and other methods	*Methods of Biochemical Analysis*, ed. David Glick, Vol. I, Interscience, New York, pp 341-356 (1954)
Tolbert, N. E., and L. P. Zill	Isolation of C^{14}-labeled sedoheptulose and other products from *Sedum spectabile*	*Plant Physiol.* **29**, 288-296 (1954)
———	Photosynthesis by protoplasm extruded from *Chara* and *Nitella*	*J. Gen. Physiol.* **37**, 575-588 (1954)
———	Metabolism of sedoheptulose-C^{14} in plant leaves	*Arch. Biochem. and Biophys.* **50**, 392-398 (1954)
Totter, J. R.	Incorporation of isotopic formate into the thymine of bone marrow deoxyribonucleic acid *in vitro*	*J. Am. Chem. Soc.* **76**, 2196-2197 (1954)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Upton, A. C., and J. Furth	The effects of cortisone on the development of spontaneous leukemia in mice and on its induction by irradiation	*Blood* 9, 686-695 (1954)
Volkin, E., and W. E. Cohn	The estimation of nucleic acids	*Methods of Biochemical Analysis*, ed. David Glick, Vol. I, Interscience, New York, pp 283-306 (1954)
Wheeler, H. E.	Genetics and evolution of heterothallism in *Glomerella*	*Phytopathology* 44, 342-345 (1954)
Wolff, S.	On a radiation-induced delay of chromosome rejoining in *Vicia faba* (Comm. to ed.)	*Nature* 173, 501-502 (1954)
Wolff, S., and K. C. Atwood	Independent X-ray effects on chromosome breakage and reunion	*Proc. Natl. Acad. Sci. U.S.* 40, 187-192 (1954)
Zill, L. P., and N. E. Tolbert	The action of acid on 2,7-anhydro-β-D-*altro*-heptulopyranose	*J. Am. Chem. Soc.* 76, 2929-2933 (1954)

Scientific Society Lectures and Traveling Seminars. Oral presentations before professional groups during the period of this report show an increase of more than 50% over the number presented in the last semiannual report. Listed are the presentations before scientific societies and on the ORINS-ORNL Traveling Lecture Program.

Anderson, N. G. (Tech. Asst., M. L. Anderson)	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City, N. J.	The sensitivity of deoxyribonucleic acid in crude fresh preparations to gamma and X rays [Abstr., Federation Proc. 13, 3 (1954)]
Arnold, W. A.	Medical School, University of Tennessee, Memphis	Problems connected with photosynthesis
_____	Oklahoma A & M College, Stillwater	As above
_____	University of Oklahoma, Norman	As above
_____	University of Arkansas, Fayetteville	Problems connected with photosynthesis
_____	Medical School, University of Arkansas, Little Rock	Population and energy
_____	Symposium on Fluorescence and Chemiluminescence in Photosynthetic Systems, National Science Foundation, Pacific Grove, Calif.	On the light saturation of delayed light production in green plants
_____	Department of Biology, University of Utah, Salt Lake City	Photosynthesis
_____	Central Arizona Regional Science Fair, Tempe	Photosynthesis and delayed light
Atwood, K. C.	Oceanographic Institute, Florida State University, Tallahassee	Indispensable gene functions
_____	Botany Department, College of Agriculture, University of Florida, Gainesville	Exploring the genome in *Neurospora*

_____	Marine Biological Laboratory, Woods Hole, Mass.	Some applications of heterokaryotic systems in *Neurospora*
_____	Biology Department, Brookhaven National Laboratory, New York	Radiation studies on *Neurospora* heterokaryons
_____	Radiation Research Soc., Cleveland, Ohio	X-ray inactivation of heterokaryotic cells
Baker, W. K.	Division of Biological Science, North Carolina State College, Raleigh	The relation between heterochromatin and variegation
Billen, D.	Medical and Psychological Section of the Georgia Academy of Science, Dahlonega	Radiation protection
_____	Department of Botany, University of Georgia, Athens	The effects of ionizing radiation on the physiology and cytology of bacterial cells
Billen, D., and E. Volkin	Soc. Am. Bacteriol., Pittsburgh, Penn.	The release of cellular constituents by X-irradiated *Escherichia coli* [Abstr., Bacteriol. Proc., pp 117-118 (1954)]
von Borstel, R. C. and S. Wolff	Radiation Research Soc., Cleveland, Ohio	Photoreactivation of the *Habrobracon* egg
Burnett, W. T., Jr.	Radiation Research Soc., Cleveland, Ohio	Cirrhosis and tumors of the liver produced in mice by colloidal gold^{198} (Upton, A. C., J. Furth, and W. T. Burnett Jr.)
Burnett, W. T. Jr., B. Anderson, E. L. Gadsden, and J. Furth	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City, N. J.	Quantitative TSH assays of pituitary tumors and of normal pituitaries of mice [Abstr., Federation Proc. 13, 21 (1954)]
Carlson, J. G., and N. Harrington	Radiation Research Soc., Cleveland, Ohio	X-ray-induced "stickiness" of the chromosomes of the *Chortophaga* neuroblast in relation to dose and mitotic stage at treatment
Carson, S. F.	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N. J.	Succinate decarboxylation systems in propionibacteria and *Veillonella* [Delwiche, E. A., E. F. Phares, and S. F. Carson. Abstr., Federation Proc. 13, 198 (1954)]
_____	Soc. Am. Bacteriol., Pittsburgh, Penn.	Biosynthesis of kojic acid [Denison, F. W., S. F. Carson, and J. W. Foster. Abstr., Bacteriol. Proc. p 99 (1954)]
Cohn, W. E.	Institute for Enzyme Research, University of Wisconsin, Madison	Nucleic acids and ion-exchange chromatography
_____	Department of Chemistry, Western Reserve University, Cleveland, Ohio	Frontiers in chemistry series
Dent, J. N.	Am. Assoc. Anatomists, Galveston, Texas	Observations on iodine metabolism in embryos of the terrestrial salamander *Aneides anaeus* [Abstr., Anat. Record 118, 294 (1954)]

Doherty, D. G.	Oklahoma A & M College, Stillwater	The kinetics of proteolytic enzymes
_____	Louisiana State University, Baton Rouge	1. As above. 2. Informal seminar 3. Equilibrium binding studies of enzyme-substrate complexes
_____	Biochemistry Club, Tulane University, New Orleans	As 3 above
_____	Southern Regional Research Laboratory, New Orleans, La.	As 3 above
_____	Southern Research Institute, Birmingham, Ala.	As 3 above
_____	Medical Center, University of Alabama, Birmingham	As 3 above
_____	Alton Ochsner Medical Foundation, New Orleans, La.	The synthesis of glyconyl peptide derivatives and the kinetics of their chymotrypsin-catalyzed hydrolysis
_____	Department of Chemistry, Georgia Institute of Technology, Atlanta	As above
Doherty, D. G., and L. Thomas	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N. J.	Hydrolysis of carbon-carbon bonds by α-chymotrypsin [Abstr., Federation Proc. **13**, 200 (1954)]
Dolin, M. I.	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N. J.	The enzymatic peroxidation of $DPNH_2$ [Abstr., Federation Proc. **13**, 200 (1954)]
_____	Biochemistry Department, Vanderbilt University, Nashville, Tenn.	$DPNH_2$ enzyme systems
Furth, J., J. N. Dent, W. T. Burnett, Jr., and E. Gadsden	Am. Goiter Assoc., Boston, Mass.	The induction mechanism and characteristics of thyrotropic pituitary tumors
Gaulden, M. E.	Winthrop College, Rock Hill, S. C.	Effects of radiation and chemicals on cell structure and mitosis as revealed in the living cell
_____	Medical College of the State of South Carolina, Charleston	As above
_____	University of Alabama School of Medicine, Birmingham	As above
_____	Southern Research Institute, Birmingham, Ala.	As above
_____	Spring Hill College, Spring Hill, Ala.	As above
Hollaender, A.	Sigma Xi, University of Florida, Gainesville	Basic studies on biological effects of radiation
_____	Biology Department, University of Florida, Gainesville	The reversal of radiation effects
_____	Oceanographic Institute, Florida State University, Tallahassee	Delayed effects of radiation on biological materials
_____	New York Academy of Sciences, New York City	A review of experiences in modifying radiation response

Khym, J. X., and W. E. Cohn	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N. J.	The separation of compounds of deoxyribose and ribose by ion exchange in the presence of borate [Abstr., Federation Proc. 13, 241 (1954)]
Kile, J. C.	Ohio State University, Veterinary Conference, Columbus	Possibilities for the use of radioactive materials in veterinary medicine
Kimball, R. F.	Louisiana State University, Baton Rouge	Some aspects of the induction of genetic changes by radiation
———	Plant Science Department, University of Oklahoma, Norman	The role of oxygen and peroxide in radiation damage, especially to *Paramecium*
———	Oklahoma A & M College, Stillwater	Some aspects of the induction of genetic changes by radiation
———	New York Academy of Sciences, New York City	The role of peroxide and oxygen in the production of radiation damage in *Paramecium*
———	Radiation Research Soc., Cleveland, Ohio	The influence of H_2O_2 on mutation production by X rays in *Paramecium aurelia*
Kirby-Smith, J. S.	School of Medicine, University of Oklahoma, Oklahoma City	The relative biological effectiveness of various ionizing radiations
———	University Hospital, Oklahoma City, Okla.	As above
———	Oklahoma A & M College, Stillwater	As above
———	Medical School, Department of Biological Sciences, University of Tennessee, Memphis	As above
Oakberg, E. F.	Radiation Research Soc., Cleveland, Ohio	Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of maturation in the mouse
Odell, T. T.	Tuskegee Institute, Tuskegee, Ala.	Platelets and the hemorrhagic syndrome of ionizing irradiation
Odell, T. T., F. G. Tausche, and J. Furth	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Atlantic City, N. J.	Life span of *in vivo* labeled transfused platelets [Abstr., Federation Proc. 13, 440 (1954)]
Passonneau, J. V., and J. R. Totter	Radiation Research Soc., Cleveland, Ohio	The effect of radiation on synthesis of DNA by chick embryos
Pittenger, T. H., and K. C. Atwood	Radiation Research Soc., Cleveland, Ohio	Diminution of thermal neutron effect by incorporated nitrogen-15
Russell, L. B., and W. L. Russell	Cold Spring Harbor Symposia Quant. Biol., Cold Spring Harbor, N. Y.	Pathways of radiation effects in the mother and the embryo
St. Amand, G. S., M. E. Gaulden, and J. R. Totter	Assoc. SE Biologists, Baton Rouge, La.	Effect of X rays on nucleic acid synthesis in embryos of the grasshopper *Chortophaga viridifasciata*
Schwartz, D.	North Carolina State College, Durham	Cytogenetic studies on X-ray-induced ring chromosomes in maize
———	University of Maryland, College Park	The effect of oxygen concentration on X-ray-induced chromosome breakage

_____	University of Pennsylvania, Philadelphia	Studies on the mechanism of crossing over
Sheppard, C. W., and P. J. Mathias	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City, N. J.	Do red cells produce acetylcholine? [Abstr., Federation Proc. **13**, 136 (1954)]
Stapleton, G. E.	Department of Biological Sciences, University of Delaware, Newark	Modification of the bactericidal effects of ionizing radiation by postirradiation treatments
_____	New York Academy of Sciences, New York City	The influence of pre- and postirradiation treatment on bacterial inactivation by ionizing radiation
Stapleton, G. E., A. J. Sbarra, and A. Hollaender	Radiation Research Soc., Cleveland, Ohio	Recovery of irradiated bacteria stimulated by extracts of biological materials
Tolbert, N. E.	Tuskegee Institute, Tuskegee, Ala.	Photosynthesis by protoplasm extruded from *Chara* and *Nitella*
_____	Duke University, Durham, N. C.	As above
_____	Georgia Institute of Technology, Atlanta	Path of carbon in photosynthesis
Totter, J. R.	University of Miami, Miami, Fla.	Formate as an intermediate in metabolism
_____	Tuskegee Institute, Tuskegee, Ala.	As above
_____	Southern Research Institute, Birmingham, Ala.	As above
_____	Medical Center, University of Alabama, Birmingham	As above
_____	Medical School, University of Tennessee, Memphis	As above
_____	Mound Laboratory, Miamisburg, Ohio	The metabolism of formate in bone marrow
_____	Radiation Research Soc., Cleveland, Ohio	The effect of radiation on synthesis of DNA by chick embryos
Upton, A. C.	Morristown, Tennessee (Sponsored by Tenn. State Medical Assoc.)	Radiation sickness and internal radiation problems
_____	Knoxville, Tennessee (Sponsored by Tenn. State Medical Assoc.)	As above
_____	Johnson City, Tennessee (Sponsored by Tenn. State Medical Assoc.)	As above
Upton, A. C., K. W. Christenberry, W. H. Benedict, and J. Furth	National Research Council, Washington, D. C.	Ocular changes in small animals induced by whole-body ionizing irradiation
Upton, A. C., and J. Furth	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Atlantic City, N. J.	Nephrosclerosis induced in mice by total-body irradiation [Abstr., Federation Proc. **13**, 445 (1954)]
Upton, A. C., J. Furth, and W. H. Benedict	Am. Assoc. Cancer Research, Atlantic City, N. J.	Radiation-induced carcinoma of the Harderian gland of the mouse
Volkin, E.	Medical Center, University of Alabama, Birmingham	Degradation products of nucleic acids

———	Southern Research Institute, Birmingham, Ala.	As above
———	Tuskegee Institute, Tuskegee, Ala.	Degradation products of ribo- and deoxyribonucleic acids: A tool in the study of their structure and function
———	Chemistry Department, Florida State University, Tallahassee	As above
———	Carnegie Institution of Washington, Cold Spring Harbor, Long Island, N. Y.	A nonmembrane protein from bacteriophage T4
———	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N. J.	A deoxynucleoptrotein in bacteriophage T4r+ [Abstr., Federation Proc. 13, 315 (1954)]
Wolff, S.	Radiation Research Soc., Cleveland, Ohio	On the rejoining of radiation-induced chromosome breaks in *Vicia faba*
Zill, L. P., and N. E. Tolbert	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N. J.	The action of acid on sedoheptulosan [Abstr., Federation Proc. 13, 327 (1954)]

Visiting Lecturers. Twenty-two outstanding scientists from the United States and two foreign countries have given lectures in the Biology Seminar Program since the middle of February. Speakers' names and their topics are listed.

Dr. Gosta C. H. Ehrensvärd	Department of Physiological Chemistry, Wenner-Gren Inst., University of Stockholm, Sweden	Problems in the biosynthesis of aromatic compounds
Dr. Otto Schales	Alton Ochsner Medical Foundation, New Orleans, La.	Decarboxylations and transaminations in higher plants
Dr. Leon A. Heppel	National Institutes of Health, Bethesda, Md.	Enzymatic studies on ribonucleic acid and nucleotides
Dr. John B. Buck	Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.	Bioluminescence in *Renilla* in relation to nerve net physiology
Dr. Cyrus P. Barnum	Department of Physiological Chemistry, School of Medicine, The University of Minnesota, Minneapolis	Phosphorus turnover in the nucleic acids of various tissues
Dr. George W. Schwert	Department of Biochemistry, Duke University, Durham, N. C.	Kinetics of enzyme action
Dr. R. W. Kaplan	Columbia University, New York City	Investigations on the color-sector-mutation process in *Serratia* (*Bacterium prodigiosum*)
Dr. Howard K. Schachman	Virus Laboratory, University of California, Berkeley	Recent applications of the ultracentrifuge with specific reference to the mechanism of enzyme action and to the structure of deoxyribonucleic acid
Dr. S. Spiegelman	University of Illinois, Chicago	On the nature of the precursor in the synthesis of enzymes

Dr. Andrew A. Benson	Radiation Laboratory, University of California, Berkeley	Path of carbon in photosynthesis
Dr. Robert MacVicar	School of Agriculture, Oklahoma A & M College, Stillwater	Nitrate reduction
Dr. William J. Blair	University of Rochester, New York	The role of electrolytes and starvation on the effects of radiation on yeast metabolism
Dr. Maurice H. Bernstein	Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, N. Y.	Some studies on deoxyribonucleoprotein
Dr. Corroll Williams	Biology Department, Harvard University, Cambridge, Mass.	Growth and metamorphosis: An experimental study on insects
Dr. D. J. D. Nicholas	British Agricultural Research Council's Unit of Plant Nutrition, Long Ashton Research Station, University of Bristol	Molybdenum and nitrate reductase in microorganisms
Dr. Paul F. Hahn	Cancer Research Laboratory, Meharry Medical College, Nashville, Tenn.	Radioisotopes in cancer therapy
Dr. R. A. Brink	Department of Genetics, University of Wisconsin College of Agriculture, Madison	The compound nature of the variegated pericarp allele in maize
Dr. Emily Bell	School of Medicine, Vanderbilt University, Nashville, Tenn.	Altered glucose metabolism of *Streptococcus pyrogenes*
Dr. Jackson W. Foster	Department of Bacteriology, University of Texas, Houston	Introspections on microbial metabolism
Dr. A. R. Gopal-Ayengar	Atomic Energy Commission of Bombay, India	Cytology and genetics of ascites tumors in mice, rats, and hamsters
Dr. Aubrey Gorbman	Department of Zoology, Columbia University, New York City	
Dr. H. B. Steinbach	Biological and Medical Sciences, National Science Foundation, Washington, D. C.	Sodium-potassium relation in muscle

Participation of Division Members in University Summer Courses. Members of the Biology Division have served on the instructoral staff of two university experiment stations during the summer term. Courses in Radiation Biology were conducted by Drs. R. F. Kimball, M. E. Gaulden, C. W. Sheppard, and G. E. Stapleton at the University of Virginia Biological Station at Mountain Lake, Virginia. At the Duke University Marine Biological Laboratory, at Beaufort, North Carolina, courses were offered by Drs. N. G. Anderson, M. E. Gaulden, C. W. Sheppard, and J. R. Totter.

Biology Division Speakers at Fall 1954 Meetings of Professional Societies. A number of papers decribing work performed in the Division will be presented this fall at scientific society meetings.

Drs. Alexander Hollaender, J. S. Kirby-Smith, J. Gordon Carlson, and C. P. Swanson will present papers at the *International Congress of Photobiology* in Amsterdam, The Netherlands, and at the *International Symposium of Radiobiology* in Liège, Belgium. Dr. Hollaender has been instrumental in the organization of programs for these two congresses and is serving as the official U.S. representative at each. He is also Secretary of the American Branch of the Comité International de Photobiologie.

Representing the Division at the *American Institute of Biological Sciences* in Gainesville, Florida, will be Drs. K. C. Atwood, W. K. Baker, R. F. Kimball, A. R. Krall, D. R. Parker, T. H. Pittenger, L. B. Russell, N. E. Tolbert, S. Wolff, L. P. Zill, and C. O. Doudney. The following Division members will also attend the meeting: D. Billen, K. C. Atwood, W. L. Russell, E. F. Oakberg, H. E. Luippold, A. D. Conger, A. H. Johnston, R. C. von Borstel, and M. E. Gaulden. Dr. Gaulden is Secretary of the Association of Southeastern Biologists, which meets with the AIBS.

Dr. A. C. Upton will present a paper at the *International Congress of Clinical Pathology* in Washington, D. C.

Dr. J. R. Totter will give a talk at the *Gordon Research Conference* (on Nucleic Acids) in New Hampton, N. H.

At the Southeastern Regional Meeting of the *American Chemical Society* in Birmingham, Alabama, two papers from the Biochemistry Section will be presented by J. X. Khym, one coauthored by L. P. Zill and W. E. Cohn, and the other coauthored by A. B. Ottinger and W. E. Cohn.

Dr. D. G. Doherty will present a report on work from his group at the *American Chemical Society* in New York City in September.

Dr. W. H. Benedict will present a paper on work done in this Division at the *Association for Research in Ophthalmology*, meeting with the Southern Medical Association, in St. Louis in November.

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
K. C. Atwood
R. T. Brumfield[1]
A. D. Conger
T. H. Pittenger
W. E. St. Amand[2]
D. Schwartz
S. Wolff
N. Gaither
A. H. Johnston
M. K. King
H. E. Luippold
A. D. Phelps

Lack of Prolonged Effects of High Doses of X Rays on Vegetatively Multiplying *Paramecium aurelia*

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

In the ciliate protozoan, *Tillina magna*, lethal effects of high doses of X rays are found[3,4] which may not express themselves for as long as 10 days after irradiation. Previous experience with the ciliate, *Paramecium aurelia*, had failed to show such very delayed effects after X irradiation, provided the paramecia did not undergo the sexual processes of autogamy or conjugation, although a really systematic search for them had not been made. Also, no study of the possible effects of X rays on the aging process in *Paramecium* had been undertaken. In view of the known effects of X rays on aging in higher organisms, it seemed worth while to check this matter.

A number of paramecia were X-irradiated with doses ranging from 66 to 115 kr under conditions in which peroxide could accumulate in the medium. Thus there was considerable death within the first 24 hours. A total of 30 survivors were obtained scattered over all dosage groups. These, together with 30 controls, were continued as daily isolation lines for approximately 50 days. Each day the number of progeny produced by division was recorded for each of the 60 lines, a single animal from each was transferred to fresh culture fluid to maintain the line, and a sample of the remaining progeny was stained to check for autogamy. Any line which underwent autogamy was discarded. The number of lines decreased during the experiment because of loss by autogamy and by the small number of deaths. Thus, at 49 days after the start, the number of lines was too small to provide a reliable sample for division rate determinations. The amount of death and autogamy in the controls and experimentals was not significantly different after the first day; it can therefore be concluded that delayed death similar to that found in *Tillina* does not occur in *P. aurelia*.

Paramecium aurelia, kept for some time without autogamy, shows a progressive decline in division rate and, as the division rate becomes quite low, death occurs with an increasing frequency.[5] The division rate records for the irradiated and control groups were averaged together over 2-day intervals and plotted in Fig. 1. It is apparent that there is no difference in the rate of decline of the two. The apparent periodicity in the decline, analyzed by A. W. Kimball of the Mathematics Panel, does not appear to be statistically significant.

The failure to find an effect of high doses of X rays on aging excludes one hypothesis which might have been suggested for this phenomenon. A priori, the decline could be attributed to the accumulation of spontaneous mutations in the

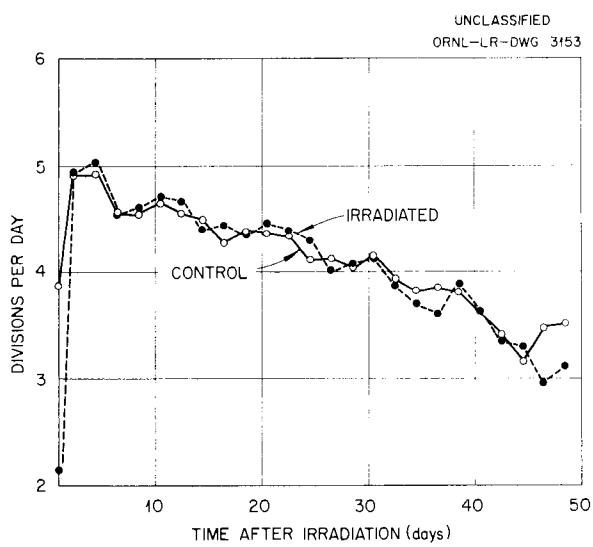


Fig. 1. Division Rate, Expressed as Divisions per Day and Based on 2-Day Averages Plotted Against Days After Irradiation for *Paramecium aurelia*. ●, Irradiated; O, control.

[1] Consultant.

[2] Research Associate.

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

[4] A. J. Bridgman and R. F. Kimball, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, p 13.

[5] T. M. Sonneborn, *J. Protozool.* 1, 38-53 (1954).

macronucleus. Owing to the amitotic division of the macronucleus, such mutations would have some probability of segregating out over the course of many divisions and so of producing a deleterious effect. However, this explanation is not correct, for if it were such high doses of X rays would have had a distinct effect on aging.

Stage in the Division Cycle and X-Ray Mutagenesis in *Paramecium aurelia*

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

Some years ago, it was reported[6] that paramecia were considerably less sensitive to the mutagenic action of ionizing radiation for a period of 1 hour or more before visible cytoplasmic division commenced than during the earlier part of the interdivision interval. The precise insensitive period was not ascertained, but it was suggested that it might coincide with the division of the micronucleus. Recently it has been possible to define the period more exactly and to show that the degree of insensitivity is much greater than was previously realized.

Dividing paramecia were picked out at various times before irradiation, allowed to develop for what was judged an appropriate time, and then all groups were irradiated together. Shortly after irradiation, a sample from each group was fixed and stained to ascertain the condition of the micronuclei. Another sample from each group was isolated and carried through autogamy in the usual way to ascertain the percentage of normal clones. The data in Table 1 make it obvious that the whole prophase and perhaps late interphase are highly insensitive. It is not yet clear whether metaphase and anaphase are insensitive, though it is possible. The presence of some interphases in all groups may have been due to errors in classifying micronuclei, to inclusion of nondividing animals in the sample of dividing ones with which each group was started, or to irregularities in the division interval. It may be noted that the visible differences between early prophase and interphase are not equally clear in all nuclei.

Table 1 also shows data on the dose relations for animals in the middle of the insensitive period.

[6] R. F. Kimball, *Genetics* 34, 210-222 (1949).

TABLE 1. EFFECT OF STAGE IN THE DIVISION CYCLE ON MUTATION INDUCTION

Dose (r)	Time after division when irradiated (hr)	Stained sample[a]						Mutation production	
		Number in:						Number of paramecia	Percentage of normal exautogamous clones
		I	EP	MP	LP	MA	?		
3,000	4.50	3	4	14	7	3	1	24	98
3,000	4.25	5	22	9	4	0	2	24	99
3,000	4.00	4	9	11	0	0	2	24	97
3,000	3.75	5	7	1	0	0	0	24	99
3,000	3.50	8	12	0	0	0	0	24	97
3,000	1.00	12	0	0	0	0	0	24	49
None	Combined	Not checked						18	99
20,400	4.0	Not checked						60	74
11,700	4.0	Not checked						60	93
5,800	4.0	Not checked						60	92
2,900	2.6	Not checked						60	47
None	Combined	Not checked						20	94

[a] I, interphase; EP, early prophase; MP, midprophase; LP, late prophase; MA, metaphase or anaphase; ?, state uncertain.

The dose curve is not completely defined but it appears to have a long shoulder at lower doses and so is not of the same form as the curve for the sensitive period, which is nearly linear on a semi-log plot. Thus a single number cannot adequately represent the difference between the periods. However, the magnitude of the difference is shown by the fact that 20,000 r at prophase produces about the same effect as 1500 r at interphase.

It is interesting to note that the prophase of the first meiotic division is also found to be extremely insensitive in the eggs of the wasp, *Habrobracon*,[7] and prophase in most organisms seems to be relatively insensitive in relation to other stages, especially metaphase and anaphase.[8] However, the interphase in most organisms is also relatively insensitive, unlike the situation in *Paramecium*. The mitotic cycle in *P. aurelia* differs in several important respects from that in other organisms, notably in that the chromatin is relatively condensed in the interphase. Thus it is tempting to try to relate the sensitivity of interphase in *Paramecium* and of metaphase in other organisms to the condensed condition of the chromatin. The difficulty is that the early prophase stages, which are clearly insensitive, are nearly as condensed as the interphase and there is even reason to believe that late interphases are also insensitive. It will therefore be necessary to look elsewhere if the changes in sensitivity during the division cycle are to receive an explanation common to all organisms.

Estimations of the Concentration of Peroxide Inside the Micronucleus of *Paramecium aurelia*

R. F. Kimball J. Z. Hearon[9]

Evidence that H_2O_2 does not play an important role in X-ray mutagenesis has already been presented.[10] The present report summarizes the procedures for estimating the concentration inside the micronucleus upon which this negative conclusion rests.

The concentration inside the micronucleus can be shown to be given approximately by

$$\frac{C}{(1 + k\Lambda)(1 + k\Lambda_m)} \qquad (1)$$

when a constant concentration, C, of H_2O_2 is present in well-stirred medium surrounding the cells. This is the condition which obtains when washed paramecia are exposed to peroxide added to the medium. In Eq. 1, k is the unimolecular rate constant for the catalase activity within a paramecium and is assumed to be the same throughout the cell, and Λ and Λ_m are constants which depend on the dimensions of the whole animal and the micronucleus respectively and also on the diffusion and permeability coefficients for H_2O_2. The dimensions of the paramecia and micronuclei are known and the diffusion coefficient can be estimated with some certainty from the diffusion constant for water, by use of a mass correction. The values of k and h, the permeability coefficient, must be obtained experimentally.

The rate constant k was estimated from the rate of destruction of peroxide by dried, disintegrated paramecia. The measurements were made at ice-bath temperature and corrected to 25°C. Peroxide was estimated colorimetrically with titanium sulfate. Several methods of preparing the paramecia were tried and the one giving the largest k was used. This method allowed the paramecia to remain alive almost to the moment of drying, thereby providing some reason for thinking that little of the activity was lost. However, the value of k of 6 sec^{-1} which was obtained must be considered a minimum estimate and may be much too low.

The value of Λ, and so of h, can be estimated directly from the rate of destruction of H_2O_2 by living paramecia. The paramecia were washed nearly free of bacteria, and concentrated. Peroxide was added to aliquots of this suspension, and the amount of peroxide remaining was measured at appropriate times. A control consisting of fluid from which the paramecia had been removed by gentle centrifugation was also run. The fraction of peroxide remaining after time t can be shown[11] to approximate $\exp(-nvt/V\Lambda)$ when $k\Lambda$ is, as in this instance, very much larger than unity. The values for n, the number of paramecia per aliquot; v, the

[7] A. R. Whiting, *Am. Naturalist* 79, 193-227 (1945).

[8] B. P. Kaufmann, in *Radiation Biology*, Vol. 1, pp 688-691, ed. by A. Hollaender, McGraw-Hill, New York, 1954.

[9] Mathematics Panel.

[10] R. F. Kimball, N. Gaither, and M. K. King, *Biol. Semiann. Prog. Reps. Aug. 15, 1953*, ORNL-1614, pp 12-13; *Feb. 15, 1954*, ORNL-1693, pp 12-13.

[11] J. Z. Hearon, *Math. Semiann. Rep. June 30, 1954*, ORNL-1751, pp 11-12.

volume of a paramecium; and V, the volume of the aliquot are known so that Λ can be estimated from the data after correction for the control activity. The value is 3.7 seconds. The permeability, b, can be estimated[11] from this value to be 17.8×10^{-5} cm sec^{-1} since the diffusion coefficient and dimensions of a paramecium are known.

The constant Λ_m for the micronucleus is given approximately by $r/3b$ since this body can be treated as a sphere with a radius of 2 microns. The value of b for the micronucleus cannot be determined experimentally but is probably no smaller than that for the whole animal, and quite possibly considerably larger. Thus the maximum value of Λ_m is 0.37 second.

The value of k cannot be computed directly from the destruction of peroxide by the living paramecia since the rate of destruction is nearly independent of k when $k\Lambda$ is as large as it is in this instance. However, when k is made small by cyanide poisoning, a direct estimate from the living animal is possible provided Λ is the same as in unpoisoned cells. The fraction of H_2O_2 remaining is given for this case by $\exp[-k_c nvt/V(1 + k_c\Lambda)]$ where the symbols have their previous meaning and k_c is the residual catalase activity of the poisoned animals. The determination was made with paramecia in 0.003 M KCN and k_c calculated to be 0.18 sec^{-1}. Thus, in those experiments in which tests for mutagenic activity of H_2O_2 were made with 0.003 M KCN present, the value for k can be considered better known than when cyanide was not present.

On the basis of these values of the parameters, it can be calculated from Eq. 1 that paramecia exposed to H_2O_2 in the absence of cyanide have approximately 1% of the outside concentration in their micronuclei whereas those exposed with cyanide present have about 50%.

The concentration of peroxide inside the micronucleus during irradiation can be estimated[12] to be

$$\frac{R\Lambda_m}{1 + k\Lambda_m} + \left(\frac{R\Lambda}{1 + k\Lambda} + \frac{C}{1 + k\Lambda}\right)\frac{1}{(1 + k\Lambda_m)} . \quad (2)$$

The external concentration, C, is given by

$$\frac{R}{\beta}(1 - e^{-\beta t}) ,$$

where R is the rate of formation (moles/liter/sec) of H_2O_2 by X rays and

$$\beta = \frac{nvk}{V(1 + k\Lambda)} + b .$$

For those X-ray exposures made in the presence of many bacteria, it is necessary to know b, the rate of use of H_2O_2 by the bacteria. This was determined experimentally, in much the same way as the use by living paramecia, to be 3.45×10^{-3} sec^{-1} for the standard 24-hour culture of *Aerobacter aerogenes* in baked lettuce infusion.

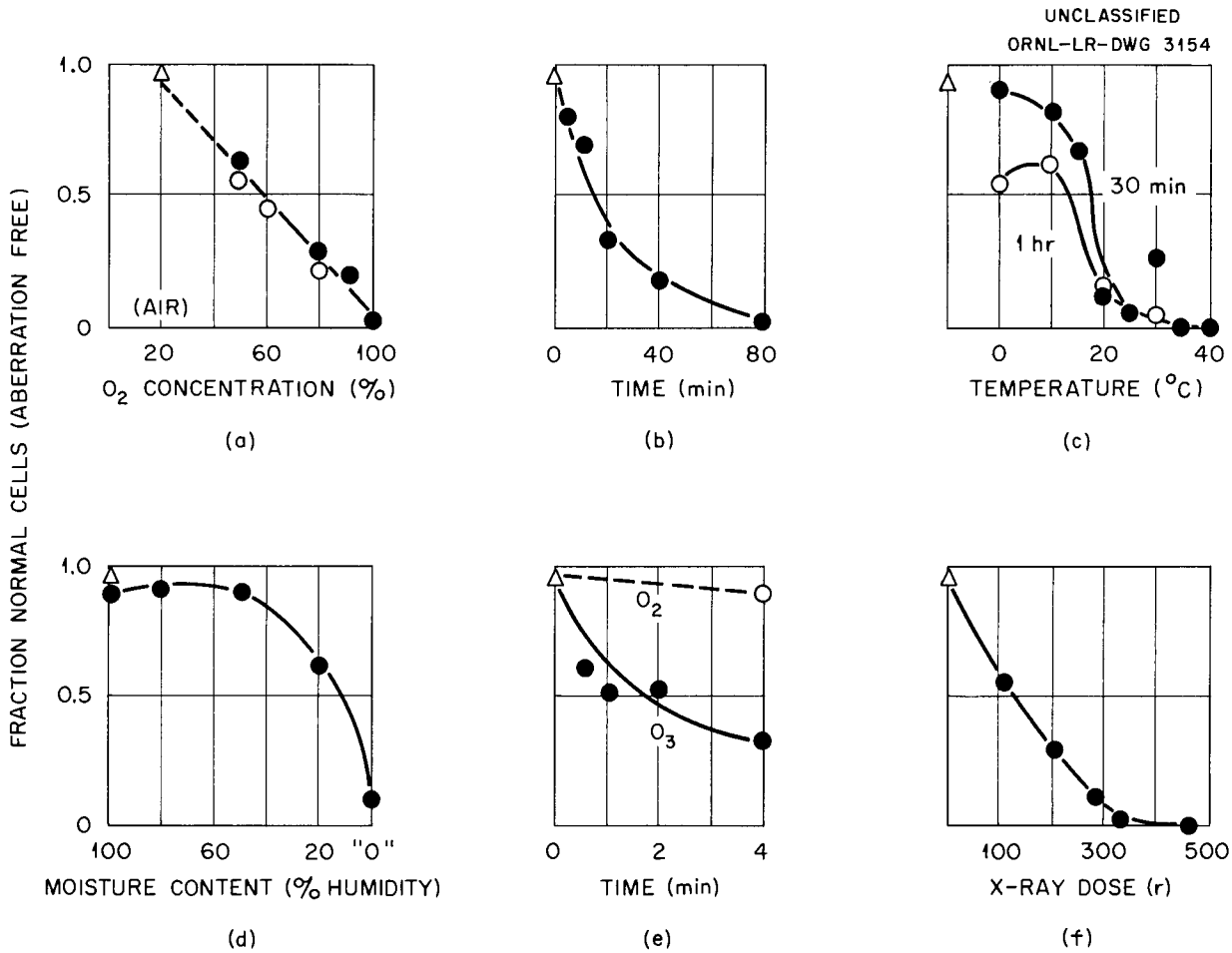
For the R values and irradiation times employed, the terms $R\Lambda_m/(1 + k\Lambda_m)$ and $R\Lambda/(1 + k\Lambda)$ do not contribute in a major way to Eq. 2. If these terms are neglected, comparison of Eqs. 1 and 2 shows that the micronuclear concentration is the same fraction of the external concentration in radiation experiments and peroxide exposures. Thus errors in the values of the constants would affect the two estimations in approximately the same way and so would not seriously disturb arguments based on quantitative comparisons between the effects of radiation and peroxide. However, the radiation and peroxide exposures differ in that the concentration in the former continues to increase during irradiation periods of the sort used in this work, whereas the concentration in the latter remains nearly constant throughout exposure. This difference would, if anything, make peroxide exposures more effective than radiation exposures when they are compared on the basis of equal maximum concentration. Thus, even if there are gross errors in the determination of the constants, the conclusion stands that H_2O_2 does not contribute appreciably to the mutagenic action of X rays.

Chromosome Breakage by Oxygen

A. D. Conger A. H. Johnston

Work has been resumed on the induction of chromosome aberrations in *Tradescantia* dry pollen grains by exposure to oxygen. After treatment, the grains are grown on a culture medium and chromosomal aberrations observed at the pollen tube division. Figure 2 shows a compilation of most of the quantitative data assembled prior to this year. Aberration frequency may be so high in some of the treatments as to prevent accurate determinations of it, so, instead, a plot was made of the accurately observable frequency of normal (i.e., aberration-free) cells as a function of treatment. From other experimental data, not shown on the graphs, it is

[12] J. Z. Hearon, *Math. Semiann. Rep. June 30, 1954*, ORNL-1751, pp 12-13.



VARIABLES OF DOSE

(a) NORMAL PRESSURE–1 HOUR

(b) 100% O_2, NORMAL PRESSURE

VARIABLES OF ENVIRONMENT

(c) 100% O_2, NORMAL PRESSURE 30 min–1 hr

(d) 100% O_2, NORMAL PRESSURE, 30 min

(e) IN 100% O_2 OR OZONIZED O_2

Fig. 2. Breakage of Chromosomes by Oxygen in *Tradescantia* Pollen Grains.

also shown that: (1) the chromosome breakage was being caused by the oxygen, not by any contaminants or by the methods of handling; (2) light was not necessary for the effect; and (3) oxygen could cause breakage in the flower buds or in the dry pollen grains, at mitosis or meiosis, and at resting stage or at prophase.

The previous results led to the conclusion that the oxygen was not causing the breakage directly, but acted indirectly by stimulating the cells to some physiological response which, secondarily, caused the breakage. The reasons for this conclusion are too numerous to recapitulate here; but an obvious one is the effect that temperature has on oxygen breakage (Fig. 2, C), which is different from and in the opposite direction to the effect of temperature on oxygen solubility but is very similar to an enzyme activity-temperature curve.

To test this idea, measurements have been made of the effect of a number of known enzyme inhibitors – cold temperatures, cyanide, and carbon monoxide – for their effect on oxygen-induced breakage. Data showing the results are shown in Figs. 3, 4, and 5. A minimum of 150 cells were observed for each point.

The temperature curves are an extension of the earlier one, and test down to the important below-zero range, where enzyme activity should be completely inhibited, or almost so. The curve shows that at enzyme-inactive temperatures, oxygen has almost no effect, even when treatment is prolonged for as much as 4 hours. Cyanide has a profound inhibitory effect on oxygen-induced breakage. The effect is specific, for addition of equal percentages of an inert gas, such as nitrogen, would not cause any detectable inhibition. Carbon monoxide by itself, in one of the two experiments, seemed to have some breakage effect; but there is no question that it, too, is a powerful inhibitor of oxygen. Here again, the effect is specific, for monoxide inhibition is reversible by treating in the light. Incomplete reversal of monoxide inhibition by simultaneous exposure to light is to be expected. The usual monoxide experiments, in which complete light reversal is found, are run at about 5% oxygen; these oxygen-breakage pollen experiments are, by necessity, run at higher oxygen concentrations, since no breakage occurs until oxygen concentration exceeds that of air (Fig. 2,A).

It now seems clear that our earlier notion that oxygen caused chromosome breakage indirectly, by an effect on cellular physiology, was correct. Inhibition of oxidative enzyme systems clearly inhibits the oxygen effect. It is significant that such a very specific and well-known cellular effect of ionizing radiations, namely, chromosome breakage, can be caused by cellular enzymatic activity in oxygen-treated material.

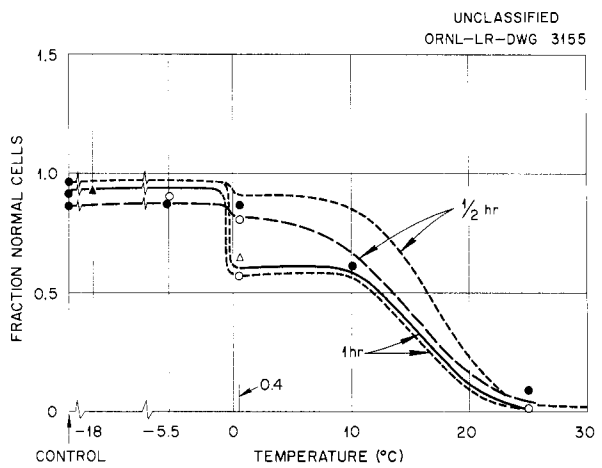


Fig. 3. Effect of Temperature on Chromosome Breakage by Oxygen. ----, Feb. 1952; ——, Dec. 1953; – – – –, May 1954; ●, ½ hr; O, 1 hr; Δ, 2 hr; ▲, 4 hr.

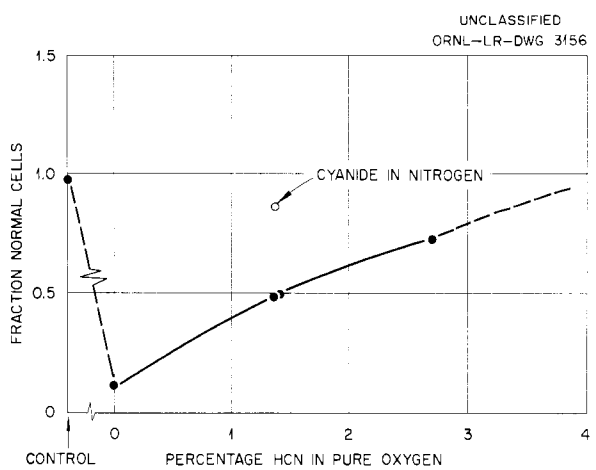


Fig. 4. Cyanide Inhibition of Oxygen-induced Chromosome Breakage.

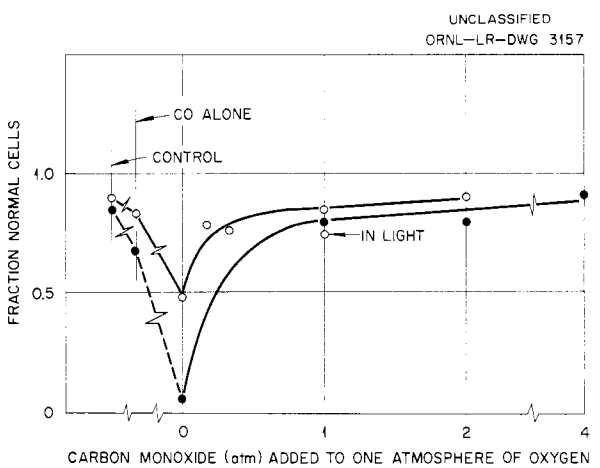


Fig. 5. Carbon Monoxide Inhibition (in the Dark) of Oxygen-induced Chromosome Breakage. ●, 19 May, 1 hr; O, 1 July, ½ hr.

Spontaneous Chromosomal Aberrations in Mouse Ascites Tumors

A. D. Conger A. H. Johnston

Accurate measurement can be made of chromosomal aberration frequency in ascites tumors by observation of the fraction of cells in anaphase of division which show abnormalities – chromosome bridges or fragments, or both – resulting from chromosome breakage and fusion. The observation serves as a useful measurement of radiation damage to the cells.

Remarkable variations in the spontaneous abnormality frequency have been found in four different strains of the Ehrlich mouse ascites tumor, all of which were derived from a single original source (the laboratory of Dr. George Klein, Stockholm) and have subsequently been grown in separate laboratories (Table 2).

Tumor 1 (England), throughout 1953, showed a constant fraction of spontaneously abnormal anaphases of 8.5 to 9%, which was unaffected by handling, age of inoculum, or a large number of other variables. Number 2 (Philadelphia) has displayed an extremely high and extremely variable frequency of spontaneously abnormal cells, from 12% minimum to 45% maximum. The frequency can vary within this range from day to day within one animal, and between brother animals inoculated at the same time. Tumors 3 and 4 are less abnormal than tumor 2, but more so than tumor 1. They clearly show that from one original tumor four distinct sublines have evolved with different spontaneous abnormality rates.

A large number of observations have shown there is a correlation between spontaneous abnormality rate and: (1) specific strain differences, mentioned in the preceding paragraph; (2) age of the tumor after inoculum, increasing with age; (3) size of initial inoculum, higher with larger inoculum; (4) growth rate, inversely related to abnormality frequency; or (5) inoculating the tumor into a new strain of mice. However, none of the factors affected the abnormality frequency of tumor 1, which is nothing more than a subline supposedly identical with the other three tumors.

Ring Chromosome Behavior

D. Schwartz A. G. Phelps

An extended experiment has been conducted to determine if ring chromosomes in maize are ever converted into rod fragments by breakage and healing of broken ends. The experiment consisted of backcrossing plants trisomic for a small chromosome 9 ring to plants homozygous recessive for the aleurone color factor *c*. The ring-bearing plants carry the dominant *C* marker on the ring chromosome and the recessive *c* marker on the other two normal #9 rod chromosomes.

Ring chromosomes are unstable and show a high degree of loss of chromatin from the ring. As a result, the dominant marker is frequently lost from the ring. In the absence of ring-to-rod conversions two types of kernels should result from the backcross described here: (1) colorless kernels – these lack the ring or contain a modified ring which has lost the dominant marker in a prezygotic division, and (2) color mosaic kernels which show frequent losses of the dominant marker in all stages of endosperm development. Unlike the rings, rod fragments are stable and a kernel carrying the dominant marker on such a fragment would be self-colored and thus easily distinguished.

Of approximately 300 backcrosses one self-colored kernel was found. However, further study revealed that although the dominant marker was now stabilized it did not involve a ring-to-rod conversion. The exceptional kernel contained a

TABLE 2. SPONTANEOUS ABNORMALITY FREQUENCY IN FOUR SUBLINES OF EHRLICH MOUSE ASCITES

Tumor no.	Source laboratory	Derived from Klein in	Spontaneous abnormality frequency (%)
1	L. H. Gray, Hammersmith Hosp., London	1953	8.5 to 9 (constant)
2	Dr. T. Hauschka, Lankenau Hosp., Philadelphia	1950	12 min. to 45 max. (extremely variable)
3	Dr. N. L. R. Bucher, Mass. Gen. Hosp., Boston	1950	12 to 20
4	Dr. E. C. Horn, Duke University, Durham, N. C.	?	12 to 25

reverse duplication of the short arm of chromosome 9 as was revealed in pachytene configurations. The chromosome folds back on itself frequently showing pairing of the duplicated sections. This is shown in Fig. 6e. The duplicated chromosome most probably arose as a result of crossing over between the ring and rod (a rare event) followed by the breakage-fusion-bridge cycle as is diagrammed in Fig. 6. As a consequence of crossing over between the ring and the rod, a dicentric chromosome is formed. The bridge produced at anaphase 1 was broken close to the ring centromere. Fusion of sister strands forms another dicentric which includes the short arm in duplicate between the centromeres. Breakage of this bridge close to one of the centromeres at anaphase of the microspore division and subsequent healing of the broken ends would result in a chromosome with a reverse duplication of the short arm.

Studies to date have failed to reveal any instances of healing of broken ends of ring chromosomes even though in maize broken ends of rods heal regularly.

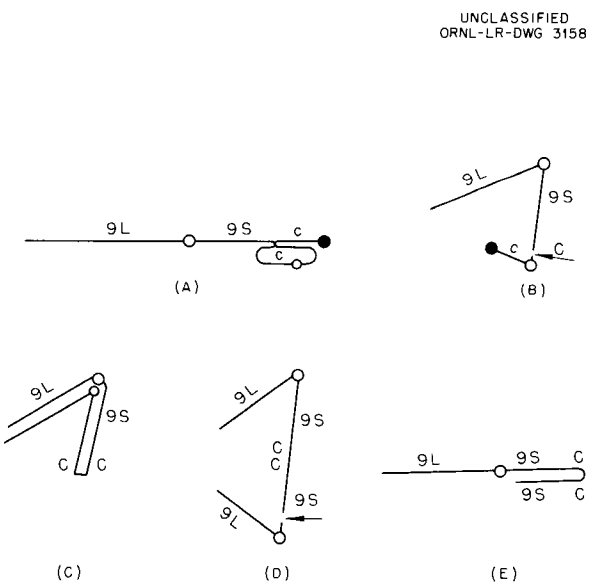


Fig. 6. Diagrammatic Representation of Events Leading to the Formation of a Chromosome 9 with a Reverse Duplication of the Short Arm (See Text). The arrows indicate points of breakage.

The Relation of Growth Rate to Nuclear Ratio in *Neurospora* Heterokaryons

T. H. Pittenger K. C. Atwood

In *Neurospora crassa*, the hyphal fusion of two different mutant strains results in a heterokaryotic mycelium. Where the two mutant strains are unable to grow on minimal medium because they lack the ability to synthesize certain growth factors, the heterokaryon can grow on such medium since each of the different mutant nuclei carry the normal allele of the other and consequently the heterokaryon can synthesize the nutrient requirement of each of the component strains. The heterokaryotic mycelium is in essence a nuclear pool with no mechanical fixation of a definite nuclear ratio since the cross walls of the hyphae are perforated, which allows the nuclei to pass freely between the "cells." Consequently, in the absence of selection, the nuclear ratio is free to shift continually. However, the rate of growth of the heterokaryon will presumably depend on the proportion of the two nuclear types, and certain ratios would be expected to give maximum rate of growth and other ratios less favorable would give submaximal growth rates.

Practically all reported heterokaryons have a growth rate equal to that of wild-type strains or about 4 mm per hour. The normal growth rate of heterokaryons has been explained as follows. During the growth of the heterokaryon on unsupplemented medium some hyphae will come to have a more favorable ratio of nuclear types than others and, consequently, will have a faster growth rate. Since such rapidly growing hyphae will eventually be expected to form the frontier of the growing mycelia, they will continue by natural selection to contribute that favorable proportion of nuclear types which will give the maximum rate of growth.

It is possible to test the validity of the assumption that there is a continual selection for nuclear ratios which will give the maximum rate of growth by synthesizing heterokaryons with such a high proportion of one type of nucleus that the growth rate would be expected to be submaximal and then determining whether selection for a more favorable nuclear ratio gives a corresponding increase in growth rate. Such heterokaryons can be experimentally produced by mixing conidial suspension of two nuclear types in various proportions.

Nuclear ratios as great as 350:1 have been produced with various modifications of this method. Conidial mixtures of the desired proportions (usually in the range of 10:1 to 100:1) are grown on minimal medium, after which samples of conidia are removed and the nuclear ratios are estimated by plating on minimal and supplemented media. Growth tubes 300 mm long were used in most of the experiments, and conidia could be sampled at both ends. In some experiments 1000-mm tubes were used and could be sampled at 100-mm intervals. Strains tested in various heterokaryotic combinations included those with requirement for pantothenic acid (*pan*), nicotinic acid (*nic*), lysine (*lys*), arginine (*arg*), and one strain had a double requirement for pantothenic acid and *p*-aminobenzoic acid (*paba*). The *pan* and *nic* strains each carried a different nonallelic albino mutant, *al-1* and *al-2*, respectively.

In an analysis of over 50 heterokaryons from several experiments involving these five mutant strains in different nuclear ratio, it was found that the relation between growth rate and nuclear ratio was variable, depending on the heterokaryon involved and the initial nuclear ratio.

In some heterokaryons there was a definite selection for one of the mutant components (Table 3). Heterokaryons of *lys+pan, paba* strains consistently showed a very definite selection of *lys* nuclei regardless of the initial nuclear ratio. However, there was no apparent selection for nuclear ratios giving maximum rate of growth.

On the other hand, heterokaryons involving *pan,al-1* and *arg* grown on minimal and on supplemented media showed little or no evidence of any selection even though growth was submaximal (Table 4). In these cases, the proportion of *pan,al-1* nuclei averaged about 97%. The growth

TABLE 3. COMPARISON OF PERCENTAGE *lys* NUCLEI AND GROWTH RATE FROM THREE *lys+pan,paba* HETEROKARYONS WITH DIFFERENT INITIAL PROPORTIONS OF *lys* NUCLEI

Heterokaryon number	Percentage *lys* nuclei		Growth rate (mm/hr)
	Beginning of growth tube	End of growth tube	
1	0.8699	0.9256	2.29
2	0.3167	0.5742	2.57
3	0.0161	0.0588	2.40

TABLE 4. NUCLEAR RATIOS AND AVERAGE GROWTH RATES OF HETEROKARYONS BETWEEN *pan, al-1* AND *arg* GROWTH ON MINIMAL, ARGININE, AND PANTOTHENIC ACID-SUPPLEMENTED MEDIA

Heterokaryon number	Media	Percentage nuclear types		Growth rate (mm/hr)
		Beginning of growth tube	End of growth tube	
VI-1	Min.	0.9444 *pan* 0.0556 *arg*	0.9408 *pan* 0.0592 *arg*	2.31
VI-2	Min.	0.9696 *pan* 0.0304 *arg*	0.977 *pan* 0.023 *arg*	2.31
VI-3	*pan*	0.9761 *pan* 0.0239 *arg*	0.9768 *pan* 0.0232 *arg*	3.31
VI-4	*pan*	0.9708 *pan* 0.0292 *arg*	0.9901 *pan* 0.0099 *arg*	3.22
VI-5	*arg*	0.9688 *pan* 0.0312 *arg*	0.9791 *pan* 0.0209 *arg*	2.11
VI-6	*arg*	0.9655 *pan* 0.0342 *arg*	0.9794 *pan* 0.0206 *arg*	2.26

rate on both minimal and arginine-supplemented media were submaximal but on pantothenic-supplemented medium the growth rate was significantly higher. This suggests that the low proportion of the normal allele of the *pan* mutant was responsible for the slow growth, but there was no apparent selection for nuclei containing the normal allele of *pan*.

The most striking case of lack of selection for a nuclear ratio giving maximum rate of growth involved a heterokaryon of *pan,al-1* and *nic,al-2* (Table 5, Fig. 7). In a typical case, the initial nuclear ratio was about 350 *pan*:1 *nic*. Selection quickly brought the ratio to about 99:1 but even after 30 days growth there was no additional change in the nuclear ratio of any significance although changes in growth rate occurred. Growth rate was submaximal at all times and averaged less than 1.5 mm/hour. However, heterokaryons involving the same two mutant strains but in different nuclear ratios had growth rates averaging about 3.5 mm/hour.

In many of the cases studied the initial proportion cf one type of nucleus was 90% or more, but there were no significant differences between the nuclear ratio at opposite ends of the growth tubes even though growth was submaximal. In a few cases heterokaryons were grown in 1000-mm growth tubes where conidia could be sampled at 100-mm intervals and in such cases changes in nuclear ratio could be followed more closely. Table 5 and Fig. 7 show the results of such tests with *lys+nic* and *lys+pan*. In these cases, when the growth rate was compared to the nuclear ratio of each conidial sample, it was found that almost identical nuclear ratios often gave different growth rates which were sometimes submaximal; also, in some cases, the same growth rate was associated with different nuclear ratios. Increases in growth rate were not always associated with corresponding changes in the nuclear ratio.

From these data it is apparent that in many heterokaryons there is no evidence for selection of nuclear ratios which will give maximum growth rates.

Nuclear Mixing During Heterokaryon Formation in *Neurospora*

K. C. Atwood T. H. Pittenger

Experimental control of the nuclear ratios in *Neurospora* heterokaryons has not been previously reported, although it has a wide variety of applications. For instance, the dominance relations of biochemical mutants could be studied quantitatively if it were possible to vary the proportions of the mutant nuclei at will. One means by which genetically different nuclei are brought into a common cytoplasm is simply by the mixing together of conidia from the homokaryotic stocks. Upon germination, many cytoplasmic connections are formed in such mixtures, allowing the nuclei to

TABLE 5. COMPARISON OF PERCENTAGES OF NUCLEAR TYPES AND GROWTH RATES IN DIFFERENT HETEROKARYONS SAMPLED AT 100-mm INTERVALS FROM THE BEGINNING TO THE END OF 1000-mm GROWTH TUBES[a]

Heterokaryon	Percentages of nuclear types (*) and growth rates (**) (mm/hr) in indicated sample number											Av. growth rate (mm/hr)
	1	2	3	4	5	6	7	8	9	10	11	
pan,al-1+lys	* 0.9702	0.9692	0.9449	0.9409	0.9450		0.9360		0.9363		0.9443	3.00
	**3.38	3.08	1.82	2.96	2.67		3.41		3.33		3.60	
lys+pan,al-1	* 0.9680	0.9360	0.9190		0.858			0.8610	0.9240	0.937	0.903	3.17
	**3.54	2.03	3.03		3.17			3.43	3.35	3.53	2.58	
nic,al-2+lys	* 0.988	0.939			0.963			0.913	0.914	0.914	0.813	3.35
	**3.96	2.23			3.44			3.48	3.31	3.67	3.75	
lys+nic,al-2	* 0.953	0.926			0.958	0.934		0.928	0.962	0.961	0.890	3.15
	**3.54	2.29			3.08	3.08		3.25	3.08	3.88	3.63	
pan,al-1+nic,al-2	* 0.997	0.989	0.990	0.994	0.994	0.990	0.986	0.971	0.994	0.992	0.985	1.43
	**3.33	3.28	2.00	2.33	1.61	2.22	3.60	1.40	1.41	1.45	1.44	

[a]Beginning of growth tubes supplemented with the growth requirement of mutonts just prior to inoculation.

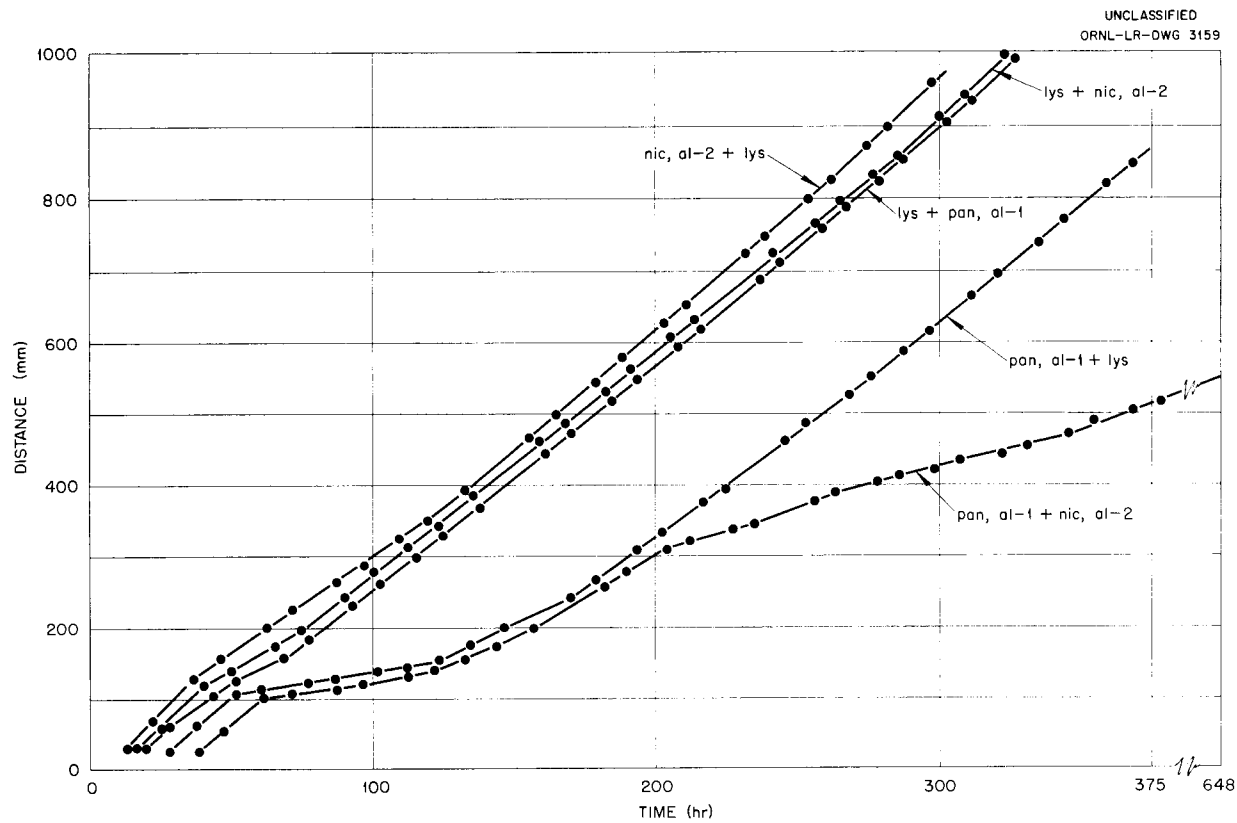


Fig. 7. Growth Curves for *Neurospora crassa* Heterokaryons in Which the Proportion of Nuclei of the First Component Is 95% or Greater. The beginning end of growth tubes supplemented with the growth requirements of the mutants prior to inoculation.

intermingle. Experiments have been done to determine whether there is, under these conditions, any significant barrier to the transference of nuclei which enter the mixture sequestered within homokaryotic cells.

The experimental design is the mixing of multinucleate macroconidia of a heterokaryotic stock with those of a homokaryotic stock, and the analysis of the nuclear constitution of heterokaryotic conidia produced by the resultant culture. The heterokaryotic stock contributes to the mixture a certain proportion of cells which are themselves heterokaryotic; that is, with the first and second genetically distinguishable nuclear types already within the same cell. The conidia contributed by the homokaryotic stock contain only the third type of nucleus. Therefore, among conidia formed by the resultant culture, the presence of heterokaryons of the first and second nuclear types is, to a great extent, independent of fusions occurring in the mixture. On the other hand, the presence of heterokaryons involving the third type, which entered the mixture entirely within separate cells, is ultimately dependent on such fusions. The relative frequencies with which heterokaryotic conidia involve the first and third nuclear types, separate prior to mixing, and the first and second types, which were associated in heterokaryotic cells before mixing, is a measure of the ratio of the second and third types in the complex heterokaryon, and hence should reveal the efficiency of nuclear mixing.

Conclusions from such experiments will be valid only if natural selection affecting the nuclear types, or affecting their local combinations following cytoplasmic fusion, has not obscured the quantitative relation of nuclear mixing to the end result. Similarly, this relation would be difficult to evaluate unless the relative numbers of nuclei of the three types present at the time of mixing are approximately known.

In the first experiment conidia from the heterokaryon, (*pan,al-1*)+(*nic,al-2*), involving requirements for nicotinic and pantothenic acids, and two nonallelic albino factors, were mixed with conidia from a *pan* stock. After the culture resulting from this mixture had conidiated, certain new combinations of nuclei were sought among the conidia. The new combinations to be looked for were those in which *nic,al-2* and *pan* nuclei were both present in the same conidium. These may be distinguished by the colors of the colonies on plates. Colonies homokaryotic for *pan,al-1* are white. Pigment is produced in heterokaryons of *pan,al-1* and *nic,al-2* but under the present experimental conditions it is usually dilute relative to that of *pan* homokaryons. Thus the plates in which the medium is supplemented with pantothenate can be sorted into three classes. In the first, only pink colonies appear, a mixture of heterokaryons and *pan* homokaryons. Each such plate represents a conidium containing *nic,al-2* and *pan* nuclei, a new combination. In the second, both white and dilute pink colonies are present, but no intense pink colonies. On these plates are *pan,al-1* homokaryons and (*nic,al-1*)+(*pan,al-1*) heterokaryons, representing conidia bearing the original nuclear combination. Finally, the class of plates having white, dilute pink, and intense pink colonies represents conidia with all three nuclear types, a new and old combination simultaneously.

The second experiment is the same as the first, except that *pan* is substituted for *pan,al-1*, and vice versa. Thus the *pan* nuclei enter the mixture in conidia of the heterokaryon (*pan*)+(*nic,al-1*), whereas the *pan,al-1* nuclei enter the mixture entirely within separate cells. Thus the old and new combinations are reversed in the final result. The purpose of the reciprocal experiment is to rule out the possibility that a selective differential between *pan* and *pan,al-1* nuclei plays any important part in determining the result.

Actually, in both of the experiments which were performed the initial concentrations of the conidial suspensions were so adjusted as to contribute approximately equal numbers of *pan* and *pan,al-1* nuclei to the mixture. In order to determine whether this adjustment had been properly accomplished, in each case the viable count of conidia from the homokaryotic stock was obtained by plating on pantothenic medium, the nuclear ratio of the heterokaryotic stock was estimated by plating on differential media, and the average number of nuclei per cell was obtained for both stocks by counts on stained preparations. The relative frequencies of *pan* and *pan,al-1* nuclei computed from these data were about equal.

The frequencies of old and new combinations are also essentially equal. It may be concluded that the rapidity and frequency of anastomosis was such as to allow preexisting combinations no advantage over those newly formed. This suggests that variation of the input ratios of conidia in simple mixtures can have a decisive influence on the nuclear ratios in heterokaryons, since the fusion process itself is not a serious limiting factor. In experiments now in progress heterokaryons with widely differing nuclear ratios have produced at will by this simple expedient.

Mediation of Chromosome Rejoining by Cytochrome Oxidase

S. Wolff

Radiation-induced chromosome breaks in the seed of *Vicia faba* usually rejoin within 30 min, at room temperature, if irradiation is performed *in vacuo*.

Therefore, irradiation with 600 r of X rays *in vacuo* followed by a waiting period of 75 min before giving a second dose of 400 r in air, provides a method of determining from the numbers of two-hit aberrations whether or not the chromosomes broken by the first dose of X rays have rejoined or healed. The delay period of 75 min between doses allows time for various treatments designed to modify the rejoining time of the chromosomes. In this way information regarding radiation damage and its biochemistry may be obtained.

The experiments described in Table 6 indicate that cytochrome oxidase is necessary for the healing of X-ray-induced breaks in chromosomes, and also that this function of cytochrome oxidase is distinct from its ordinary function in oxidative metabolism.

Experiments 1 and 2 show that rejoining is temperature sensitive. This is consistent with the idea that rejoining is dependent on enzymatic processes. Furthermore, experiment 3 indicates that rejoining is cyanide sensitive and therefore probably dependent on a heavy metal enzyme. Experiments 4 and 5 show that rejoining is inhibited by 95% carbon monoxide in the dark and that this inhibition is light reversible. This is the

TABLE 6. EFFECT OF CONJUNCTIVE TREATMENTS ON CHROMOSOME REJOINING

Expt. no.	Dose I at time, *t in vacuo*	Conjunctive treatment immed. following dose I	Dose II at $t + 75$ min in air (r)	No. of two-hit aberrations per cell			Occurrence of rejoining within 75 min
				Observed	Theoretical value		
					With rejoining	Without rejoining	
1	600	Room temperature	400	0.220	0.195	0.380	+
2	600	0°C	400	0.367	0.195	0.380	–
3	600	2×10^{-3} *M* KCN	400	0.367	0.236	0.440	–
4	600	95% CO + 5% O_2 dark	400	0.340	0.195	0.380	–
5	600	95% CO + 5% O_2 light	400	0.210	0.195	0.380	+
6	600	Vacuum	600[a]	0.200	0.232	0.465	+
7	600	2×10^{-3} *M* BAL	400	0.133	0.134	0.258	+

[a]*In vacuo.*

specific test for cytochrome oxidase which, of course, is a heavy metal enzyme. This enzyme plays a central role in oxidative metabolism. However, if oxidative metabolism is inhibited at any position other than cytochrome oxidase as in experiment 6 in which oxygen was removed and experiment 7 in which BAL [British antilewisite (2,3-dimercaptopropanol)] was used to inhibit Slater's factor, then rejoining still occurs. This indicates that cytochrome oxidase is exerting its effect on chromosome rejoining in some fashion other than merely stopping oxidative metabolism. Two possibilities immediately come to mind: (1) that cytochrome oxidase acts by itself to catalyze the rejoining of broken chromosome ends and (2) that cytochrome oxidase acts on a series of unknown intermediates which in turn can effect chromosome rejoining.

The Effect of Pre- and Postirradiation Centrifugation on the Chromosomes of *Tradescantia* and *Vicia*

S. Wolff R. C. von Borstel

The most widely accepted theory of the action of X rays on chromosomes holds that the radiation breaks the chromosomes, that the broken ends stay open for varied lengths of time, and that the ends then either rejoin or give rise to aberrations. The aberrations are consequently formed either by a broken end remaining open or by its uniting with ends from other breaks. Part of the corroborative evidence for this theory is the work of Sax wherein mechanical disturbance of the chromosome breaks, by centrifugation, increased the numbers of aberrations formed. However, in these past experiments, the centrifugation had to be employed simultaneously with the radiation to get the effect.

The experiments reported in Table 7 show that, in addition to simultaneous centrifugation and irradiation, postirradiation centrifugation can also increase the numbers of two-hit chromosome aberrations formed provided that the centrifugation is begun before 4 min have elapsed. The time of 4 min represents the average time that chromosome breaks stay open in *Tradescantia*. In *Vicia* seed this average time is about 90 min under these conditions of irradiation. All experiments were performed on *Tradescantia* inflorescences or *Vicia faba* seed. The first microspore division 5 days after irradiation was scored in *Tradescantia*, whereas the first root tip division of the germinating seed was used in *Vicia*.

It may be noted from experiments 3 and 5 that if the inflorescences are kept cold, the posttreatment centrifugation may be begun later than 4 min after the cessation of radiation. This is consistent with experiments performed on *Vicia* which indicate that cold inhibits rejoining of broken ends.

Experiments 3 and 6, 7 and 8, and 10 and 11 show that by pretreatment with centrifugation, a decrease appears in the numbers of aberrations produced by a given dose of X rays.

We interpret these experiments to indicate that indeed breaks in chromosomes are formed by

TABLE 7. EFFECT OF CENTRIFUGATION ON X-RAY-INDUCED CHROMOSOMAL ABERRATIONS[a]

Expt. no.	Dose (r)	Dose rate (r/min)	Centrifugation	Percentage of two-hit aberrations
			Tradescantia	
1	200	10	None	9.4
2	200	10	Simultaneous	17.0
3	200	200	None	18.3
4	200	200	Immediately following irradiation for 20 min	22.2
5	200	200	7°C for 10 min after raying, then centrifugation	23.0
6	200	200	Preirradiated centrifugation for 20 min	6.4
7	200	100	None	16.7
8	200	100	Preirradiation centrifugation for 15 min	8.3
9	200	100	Immediately following raying for 15 min	22.7
			Vicia	
10	800	200	None	32.3
11	800	200	Preirradiation centrifugation for 20 min	16.0
12	800	200	Postirradiation centrifugation for 20 min	Too numerous to be scored

[a]Centrifugotion was performed to give ± 1700 *g*.

radiation, that these breaks do remain open for varied lengths of time, and that if the material is centrifuged while these breaks remain open, the ends move apart and are less likely to rejoin. These ends then increase the number of aberrations formed. If, however, centrifugation is performed prior to irradiation, the nuclear material is compressed centrifugally and movement of breaks formed in the compressed area is inhibited. This in turn enhances rejoining and decreases aberration yield.

By the same technique an attempt has been made to gain some information regarding the present controversy about the "oxygen effect," whereby a given dose of X rays produces fewer aberrations if the material is irradiated in an oxygen-free atmosphere. One group holds that fewer breaks are formed in the absence of oxygen; another group believes that the same number of breaks are formed whether or not oxygen is present and that the decrease in anaerobic radiation damage is due to increased "healing" processes. We believed that if the same number of breaks were formed under anaerobic conditions as under aerobic ones, then posttreatment centrifugation would raise the number of anaerobically formed aberrations to the number formed in air. As seen from the data in Table 8, such was not the case.

The Effect of Pretreatment with Indoleacetic Acid and 2,4,6-Trichlorophenoxyacetic Acid on Curvatures Induced by Ultraviolet Radiation in Timothy Roots

R. T. Brumfield

Curvatures in plants result from the unequal growth of cells on opposite sides of the organ and the development of curvatures indicates that some change in the factors controlling cell growth has occurred. This affords a means of studying the factors which control cell growth in plant organs. At least two types of curvatures can be readily induced in the timothy root. Positive geotropic curvatures develop when the growing root is put in a horizontal position. Curvatures can also be

TABLE 8. EFFECT OF POSTIRRADIATION CENTRIFUGATION ON ANAEROBICALLY INDUCED ABERRATIONS

Dose (r)	Dose rate (r/min)	Centrifugation	Percentage two-hit aberrations
175	200	None	6.0
175	200	20 min	7.7
300	200	None	13.4
300	200	20 min	13.7

induced by unilateral exposure to ultraviolet radiation. Since the root is an absorptive structure, its cells will take up chemical agents applied to it. The effect of several compounds on growth and geotropic curvature in the timothy root was the subject of a previous report[13] in which it was shown that 2,4,6-trichlorophenoxyacetic acid (2,4,6-TCPA) inhibited the geotropic curvature whereas indoleacetic acid (IAA) did not appreciably influence the amount of curvature per unit of root growth. This report concerns the effect of 2,4,6-TCPA and IAA on curvatures induced by ultraviolet radiation.

The type of curvature induced by ultraviolet varies with dosage. Low dosages (96,000 ergs/cm^2) induce a curvature away from the source, the center of curvature being in the region of cell elongation of the root. Higher dosages (192,000 ergs/cm^2) induce two curvatures, one of which occurs soon after irradiation and is toward the source (a "positive" curvature), its center of curvature lying in the region of elongation. A second curvature, away from the source (a "negative" curvature) later develops and its center is nearer the root apex. Thus the two curvatures differ in direction, space, and time. Since it was desired to test the effect of chemical pretreatment on both positive and negative curvatures, a dosage of 192,000 ergs/cm^2 was used in all experiments described here. The source of radiation was a G.E. germicidal lamp (15-watt) which delivers about 80% of its energy in the 2537 A region. The intensity was measured with a G.E. germicidal lamp meter.

[13]R. T. Brumfield, *Biol. Semiann. Prog. Rep. Aug. 15, 1953*, ORNL-1614, pp 19-21.

The type of moist chamber used for the growth and observation of the timothy seedlings is shown in Fig. 8. It consists of a rectangular piece of lucite 75 × 55 mm and 3 mm thick. A rectangular opening 17 × 18 mm is cut in the center and coverslips, affixed to opposite sides of the opening with a heavy silicone stopcock grease, form a thin moist chamber. Strips of filter paper inside the chamber support the growing seedling and serve as a wick to supply it with water from the bottom of the chamber. A hole 1.5 mm in diameter extends from the edge of the lucite to the chamber and through it solutions may be added or removed with a blunt syringe. A groove 12.5 mm wide and 1.5 mm deep extends from one edge of the lucite rectangle to the chamber and the growing root is exposed to the ultraviolet tube by directing the groove toward it.

A photographic method is used to study root growth and to observe curvatures. The chamber is placed on the stage of a microscope in the horizontal position (stage vertical) and the image of the root projected onto sensitized paper, the microscope and its light source being used essentially as photoenlarger. In the experiments described here a 10× objective and a 5× ocular were used and the distance from the ocular to the paper holder was adjusted to give a magnification of 100×. Illumination was supplied by a Bausch and Lomb ribbon-filament lamp with an absorption cell containing $CuSO_4$ solution.

Seedlings of timothy (*Phleum pratense*) were germinated on moist filter paper in Coplin jars until the roots were 4 to 5 mm in length. In all experiments, four seedlings were transplanted, one to a chamber, and at least 1 hr allowed for the roots to become adjusted to the new environment. Photographs were then taken at 20-min intervals for 1 hr to determine the rate of growth prior to treatment. The chamber was then flushed with the solution being tested by withdrawing the water and adding the solution to a point just below the root and withdrawing the solution. After flushing, fresh solution was added to a depth that covered the distal 3 to 4 mm of the root and was withdrawn after 2 minutes. The roots were not washed after being immersed in the solution, but were exposed to ultraviolet immediately after treatment. Control roots were treated in the same way, distilled water being used. Photographs were taken immediately after exposure and at 20-min intervals for 2 hr.

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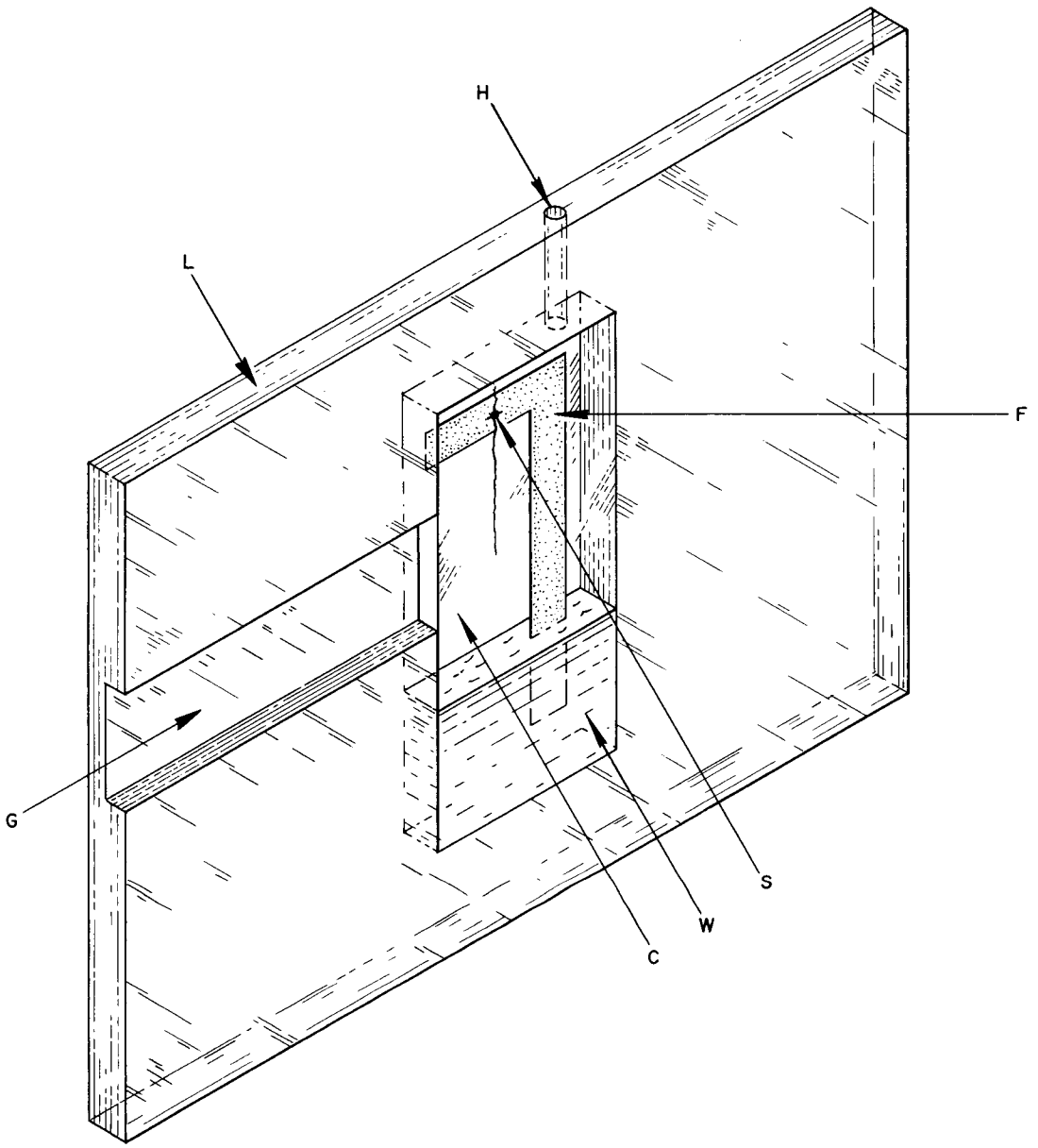

Fig. 8. Diagram of Moist Chamber Used for Observation of Living Roots and for Unilateral Exposure to Ultraviolet. L, lucite rectangle 75 × 55 mm and 3 mm thick with rectangular opening 17 × 38 mm in the center. C, coverslip; F, filter paper; S, seedling; W, water; H, hole through which solutions may be introduced or removed. A groove, G, extends from one edge to the moist chamber to admit ultraviolet radiation.

Curvatures were measured on the photographs with a protractor, the linear cell rows being used as guide lines. Root length was measured with a pair of dividers and with curved roots; the total length was taken as the sum of short distances measured along the photograph.

All experiments were carried out at room temperatures, 27° to 29°C.

Data showing the results are presented in Table 9 and are shown graphically in Fig. 9. Table 1 shows the average growth made by each group of four roots prior to treatment, the treatment given, growth at 20-min intervals after treatment, and the amount of curvature at each 20-min interval. Since curvatures in plants represent differential cell growth on opposite sides of the organ, root curvatures can be clearly interpreted only when related to root growth. This relation is made clear in Fig. 9 which shows the angle of curvature as ordinates plotted against root length as abscissas. It is apparent that pretreatment with 2,4,6-TCPA markedly influences both the positive and negative curvatures induced by ultraviolet radiation, whereas pretreatment with auxin has little effect.

There is evidence that 2,4,6-TCPA acts as an antiauxin and it may be through an effect on auxin or its distribution that the inhibition of geotropic curvatures and ultraviolet-induced curvatures are mediated. Evidence bearing on this possibility can be obtained by comparing the effects of auxin and 2,4,6-TCPA on cells at different distances from the root tip. Methods for observing the effects of applied substances on the growth of individual cells have been developed and research in this direction is now in progress.

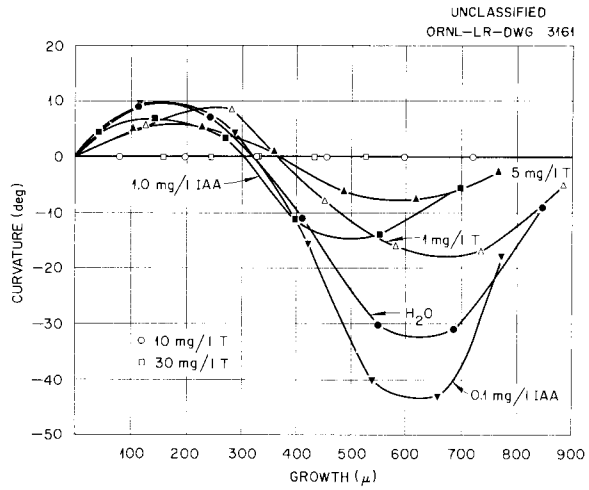

Fig. 9. Curves Showing Amount of Curvature in Timothy Roots Induced by Ultraviolet Following Treatment with IAA and 2,4,6-TCPA. Abscissas are growth in μ 20, 40, 60, 80, 100, and 120 min after treatment and ordinates are curvatures in degrees at the same time intervals. Curvatures toward the source are positive and those away from the source are negative. Each point is an average of four roots.

TABLE 9. GROWTH AND CURVATURE IN TIMOTHY ROOTS INDUCED BY ULTRAVIOLET AFTER VARIOUS CHEMICAL PRETREATMENTS[a]

Growth in 60 min prior to treatment (μ)	Solution used for pretreatment (mg/liter)	Growth (μ) after indicated time (min)						Curvature (°) at time after treatment (min)					
		20	40	60	80	100	120	20	40	60	80	100	120
497.5	H_2O	113	245	411	549	688	851	+9.0	+7.0	−11.1	−30.3	−31.4	−9.0
474.0	1 – TCPA	128	287	451	584	740	898	+5.5	+8.5	−8.0	−16.2	−17.2	−5.0
374.2	5 – TCPA	103	229	359	483	620	772	+5.0	+5.5	+1.0	−6.1	−7.2	−2.7
435.0	10 – TCPA	79	197	327	456	591	726	0	0	0	0	0	0
450.0	30 – TCPA	75	153	245	331	432	527	0	0	0	0	0	0
491.0	0.1 – IAA	119	291	425	540	669	783	+9.2	+4.7	−15.7	−40.1	−43.0	−17.5
453.0	1.0 – IAA	41	140	270	404	563	715	+4.7	+6.7	+3.6	−11.9	−14.0	−5.7

[a]In each experiment the root was treated with the solution indicated for 2 minutes then exposed unilaterally to 192,000 ergs/cm^2 of 2537 A ultraviolet. The figures are averages of four roots in each experiment. Curvatures toward the source are positive and those away from the source are negative.

INSECT CYTOLOGY AND GENETICS

M. E. Gaulden
R. C. von Borstel
J. G. Carlson[14]
H. S. Roberts[15]
S. R. Tipton[14,16]
A. R. Whiting[14]
G. S. St. Amand
N. A. Sloan[17]

A Paradoxical Inequality in Recessive Lethal Frequency as Determined by Different Methods

R. C. von Borstel K. C. Atwood
A. R. Whiting

Approximately two-thirds of the eggs are fertilized in mated females of the parasitic wasp *Habrobracon*. The unfertilized eggs develop normally to become haploid males; the fertilized eggs develop into diploid females.

This phenomenon allows the determination of induced recessive lethals by two distinct methods. The first method is a test, in the F_1 generation, which discloses the frequency of recessive lethals induced in the haploid eggs. The second method is a second generation (F_2) analysis which reveals the frequency of recessive lethals induced in eggs which were fertilized subsequent to the irradiation. As will be shown here, at a given dose of X rays, the frequency of recessive lethals appearing by F_1 analysis far exceeds the frequency appearing by F_2 analysis.

After X irradiation of females subsequently fertilized, the viable proportion of fertilized eggs, V_f, may be expressed as

$$V_f = \frac{m - V_u(1 - f)}{f}$$

where m is the viable proportion of eggs from mated females, V_u is the viable proportion of unfertilized eggs from mated or unmated females, and f is the proportion of fertilized eggs. Mated controls for this experiment gave a value for f of 0.61.

The viable proportion of unfertilized eggs, V_u, following X irradiation of virgin females is composed of the surviving embryos which have neither a dominant lethal nor a recessive lethal mutation. In the haploid egg the results of an induced recessive lethal would be expressed in the F_1 generation. Therefore

$$V_u = (1 - r)(1 - D) \quad ,$$

where r is the frequency of genomes having at least one recessive lethal mutation, and D is the frequency of genomes having at least one dominant lethal mutation.

Since the recessive lethals are masked in the diploid genome, the frequency of induced dominant lethality is

$$D = 1 - V_f \quad ;$$

therefore, the frequency of induced recessive lethal mutations is

$$r = 1 - \left(\frac{V_u}{V_f}\right) \quad .$$

The data for the F_1 embryos following X irradiation of females with 875 r are given in Table 10. Only the eggs which were in the first meiotic metaphase during the time of irradiation are included. From these data the value of r is determined to be 0.362.

The frequency of recessive lethal mutations can be directly determined by F_2 analysis. Any recessive lethal mutation which has been masked by the presence of another genome will appear in half the offspring of such individuals. All females from the F_1 experiment were set unmated. In this experiment 99 virgins were set and 19 of them possessed at least one embryo recessive lethal mutation, a frequency of 0.192. If the criteria for measuring the frequency of induced recessive lethal mutations are correct, it appears that a reduction of approximately half the mutations has occurred between generations or else there is a profound difference between recessive lethal mutation induction in haploid nuclei and haploid nuclei subsequently made diploid.

Three separate hypotheses can reasonably explain the large discrepancy between the frequency of recessive lethals expressed by death of the embryo in the F_1 and F_2 analyses.

Hypothesis I. Postembryo dominant lethals. In this hypothesis it is assumed that X rays induce a certain class of dominant lethals which are expressed by death of the haploid embryo and by death of the diploid larva or pupa. In order for this hypothesis to be correct, the frequency of postembryonic deaths in the offspring of mated females would have to be much higher than that

[14]Consultant.
[15]Research Participant.
[16]To June 1, 1954.
[17]Visiting Assistant.

TABLE 10. EGG HATCHABILITY AND ADULT SURVIVAL DATA FROM X-IRRADIATED *HABROBRACON* FEMALES

	Mated	Unmated
Hatchability of F_1		
Treated	220/577 = 0.381	158/559 = 0.283
Control	102/104 = 0.981	118/118 = 1.000
Adult survival of F_1		
Adults (treated)/larvae	155/220 = 0.682	123/158 = 0.788
Males (treated)/adults	54/155 = 0.348	123/123 = 1.000
Males (treated)/dead pupae	7/13 = 0.538	9/9 = 1.000
Adults (control)/larvae	95/102 = 0.931	106/118 = 0.898
Males (control)/adults	37/95 = 0.389	106/106 = 1.000

of the offspring of virgin females. Since they are not significantly different, this hypothesis appears to be excluded.

Hypothesis II. Multifactor lethals. This hypothesis is based on the assumption that a class of recessive mutations is induced of which two or more must be present in one genome in order to cause death of the embryo. These multifactor lethals would be together in the genome of the F_1 generation causing death of the haploid embryo and would be masked in the diploid. The factors would segregate independently in the F_2 and, therefore, would not cause embryonic death. This hypothesis appears to be excluded since multifactor lethals apparently do indeed occur and the frequency can be determined. In Fig. 10 the 19 embryos containing recessive lethals by the F_2 test are plotted according to the hatchabilities of eggs from the heterozygous mothers. Females having one recessive lethal yield hatchabilities of 0.50, those having two recessive lethals would give hatchabilities of 0.25, three would give 0.125, etc. Those females yielding hatchabilities of 0.75 could be considered as having multifactor lethals whereby two recessive mutations must be in the same genome in order to cause death. These females are few in number, can be detected, and do not make up for the wide discrepancy as shown by F_1 and F_2 analyses.

Hypothesis III. Genome regeneration. This hypothesis states that the processes leading to gene mutation can be reversed or a mutated gene can be repaired if a normal nucleus is combined with the irradiated nucleus; otherwise the mutation would become "fixed" in the haploid nucleus. This combination and "regeneration" would probably have to take place before the first round of gene synthesis in the embryo. The cited discrepancy between F_1 and F_2 recessive lethal data is found when the irradiated chromosomes are in the nonsynthesizing first meiotic metaphase. However, preliminary data indicate that there is no discrepancy between the frequency of induced recessive lethals in the F_1 and F_2 generations if the chromosomes are in the synthesizing first meiotic prophase when treated. The "damage" can be visualized as being repaired during the synthetic process in this case.

Of course, hypothesis III must be regarded with unusual caution since it is arrived at by elimination of other hypotheses rather than by positive evidence to prove its validity. It is not yet certain whether all reasonable alternative possibilities have been considered.

Cytokinesis of Isolated Neuroblasts of *Chortophaga viridifasciata*

H. S. Roberts

Neuroblasts of *Chortophaga viridifasciata* divide unequally, giving rise to large daughter neuroblasts and smaller cells which become ganglion cells. The current investigation was undertaken in the expectation that an analysis of the factors which result in this unequal division might contribute to knowledge of the fundamental mechanisms of cytokinesis.

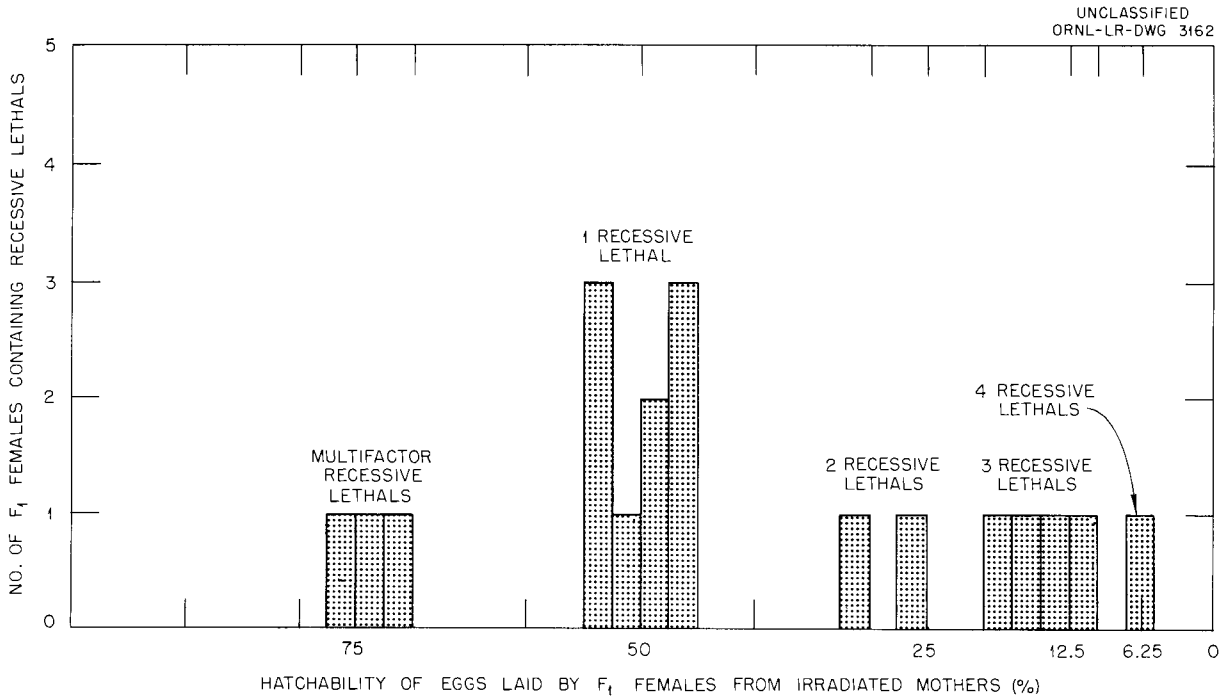

Fig. 10. Frequencies of Recessive Lethals in *Habrobracon* Females from Irradiated Mothers.

Neuroblasts were isolated from embryos of *Chortophaga* by the method of St. Amand and Tipton.[18] These were suspended in a modified Tyrode's solution and studied in hanging-drop preparations. Divisions were recorded on 16-mm film by phase contrast time-lapse photomicrography.

In such isolated neuroblasts the first evidence of the initiation of cytokinesis is a clearly visible expansion of the cell membrane at that pole which will give rise to the daughter neuroblast. This expansion increases in size and progresses toward the opposite pole. As a result, the cell membrane expands away from the spindle which is, in effect, shifted toward that pole of the cell which will give rise to the ganglion cell. Later an expansion of the cell membrane begins at the ganglion cell pole and progresses toward the neuroblast pole. The approach of these polar expansions toward each other establishes the constriction furrow between them. The subsequent advance of the furrow constricts the cell, giving rise to the large daughter neuroblast and smaller ganglion cell. Examination of dividing neuroblasts in intact embryos makes it clear that this sequence of events occurs in all dividing neuroblasts, although in many cases the polar expansions are too gradual for their first appearance to be accurately timed.

The events described appear to agree with those postulated by Swann[19] and Mitchison[20] in their "Expanding Membrane Theory of Cytokinesis" except that in this case the division is an unequal one in which the polar expansions do not occur simultaneously. It thus provides a means of testing this theory by applying it to a quite different type of division from that considered by the authors.

On the basis of the theory it would be predicted that the relative size of the daughter neuroblast and ganglion cell would be related to the time interval between the polar expansions. The greater the time interval, the farther the first expansion would advance toward the ganglion cell pole before the establishment of the constriction furrow. To test this prediction, the time interval between

[18] G. St. Amand and S. R. Tipton, *Science* 119, 93-94 (1954).

[19] M. M. Swann, *Symposia Soc. Exptl. Biol.* 6, 89-106 (1952).

[20] J. M. Mitchison, *Symposia Soc. Exptl. Biol.* 6, 106-127 (1952).

expansions was plotted against the ratio of daughter neuroblast volume to ganglion cell volume (Fig. 11). The prediction is borne out by the results. With a 2-min interval between expansions the daughter neuroblast is approximately twice as large as the ganglion cell. When the time interval is 8 min the ratio of volumes is approximately 10:1.

This is rather dramatic support for the theory of Swann and Mitchison. However, it is a common observation that in dividing cells the constriction furrow cuts through the equator of the spindle. This also appears to be the case in dividing neuroblasts. The question then arises as to whether the critical factor in determining the inequality of division is the interval between polar expansions or the shift in position of the spindle resulting from the first expansion. An effort has been made to determine from measurement of projected film images just how precisely the constriction furrow cuts through the equator of the spindle. In 11 of 12 neuroblasts at roughly comparable stages of cytokinesis, the constriction furrow was displaced toward the ganglion cell spindle pole a distance varying from 1 to 9.8% of the spindle length. In nine of these cells the displacement was 2 to 5% of the spindle length. In one of 12 cases the constriction furrow coincided precisely with the equator of the spindle. The relation of the constriction furrow to the equator of the spindle in five dividing neuroblasts was determined at 1-min intervals from the establishment of the constriction furrow to late constriction. In each of the five cases the constriction furrow, when first established, was displaced toward the ganglion cell pole by a distance of 7 to 15% of the spindle length. As cytokinesis progressed, the displacement decreased and the constriction furrow approached, but in only two cases reached, the equatorial position.

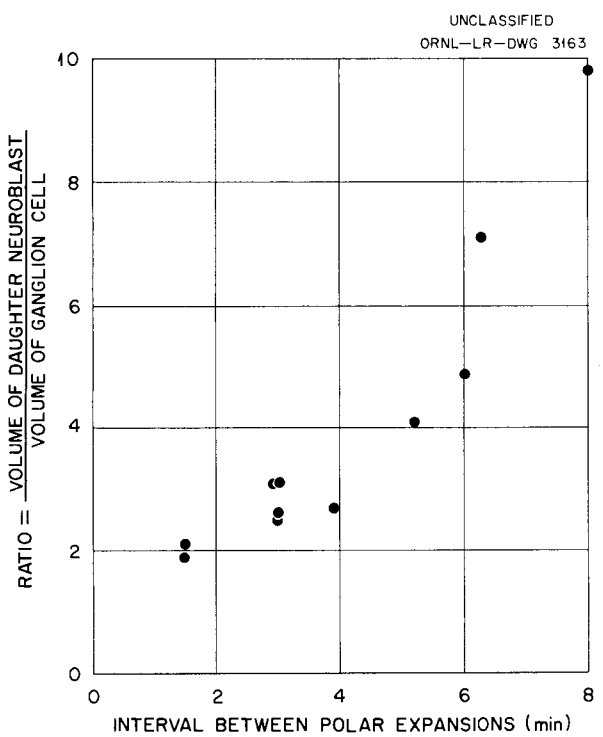

Fig. 11. Relation of Interval Between Polar Expansions to Relative Volume of Daughter Neuroblasts and Ganglion Cells.

In an effort to evaluate further the relative importance of the position of the spindle in determining the inequality of division, cells are being irradiated with ultraviolet, which has been shown by Carlson and Hollaender[21] to result in decreased spindle size and equal division of neuroblasts. Although this work is incomplete, preliminary observations suggest that the position of the constriction furrow is determined by the time interval between expansions, but that the structure of the spindle impedes constriction near the poles and results in the furrow being shifted toward the equator of the spindle.

DROSOPHILA GENETICS

W. K. Baker
D. R. Parker[22]
D. L. Lindsley[23]
C. W. Hinton[24]
C. W. Edington[25]
P. Ballard

Evidence on the Mechanism of the Oxygen Effect by Use of a Ring Chromosome

W. K. Baker

Fewer chromosomal aberrations are recovered from chromosomes irradiated in low oxygen concentrations. Whether this modification of the X-ray-induced damage is caused by a reduced number of initial breaks being produced or whether it is due to a greater reunion of broken ends, is a question which is still not entirely resolved. In the ring-X

[21] J. G. Carlson and A. Hollaender, *J. Cellular Comp. Physiol.* 31, 149-173 (1948).

[22] Research Participant.

[23] Arrived Aug. 11, 1954.

[24] Research Associate.

[25] ORINS Fellow.

chromosome of *Drosophila*, it is known that all chromosome breaks rejoin. Therefore, if oxygen were acting on the reunion process no effect on intrachromosomal rearrangements of this chromosome would be observed; whereas, conversely, a large oxygen effect would be seen if the breakage mechanism were valid.

Intrachromosomal rearrangements of the ring chromosome (X^c) can involve loss of this chromosome, an event which can be detected genetically if the Y chromosome is marked (the bw^+ insertion was used in the experiments reported here). This loss of the X^c chromosome in the first cleavage division of a female zygote also converts the female into an X-O male and thus the sex ratio is altered. Therefore, the loss of the ring chromosome can be followed by two criteria – the appearance of X-O males, and the shift in sex ratio.

Drosophila melanogaster males bearing the ring chromosome were irradiated in either air or nitrogen with 250-kvp X rays and mated to females bearing appropriate marker genes. Mating was allowed to proceed for 48 hours. The offspring resulting from this mating were observed to determine the frequency of X-O males and the sex ratio.

The relation between these two effects, the oxygen concentration and the dosage of X rays, is depicted in Fig. 12. It will be noted that at 1000 r there is no difference in the frequency of X-O males between the air and nitrogen series. Even at the higher dosages the difference is not statistically significant, although a slight oxygen effect would be expected even on the reunion hypothesis of oxygen action because of induced loss of the marker on the Y chromosome. Likewise, oxygen has no effect on the sex ratio at the low dose; but at the higher doses, significantly more males are produced when irradiation is carried out in air as contrasted to nitrogen. The latter result is not unexpected on the reunion hypothesis, since the ring would be lost and the female zygote would die by the formation of a dicentric chromosome when the ring was involved in a chromosomal exchange with another chromosome. This would alter only the sex ratio and not the frequency of X-O males.

On the basis of these findings, it is concluded that, at least in *Drosophila*, the oxygen effect is observed because irradiation in low oxygen concentrations makes chromosome ends more likely to rejoin rather than making fewer initial chromosome breaks.

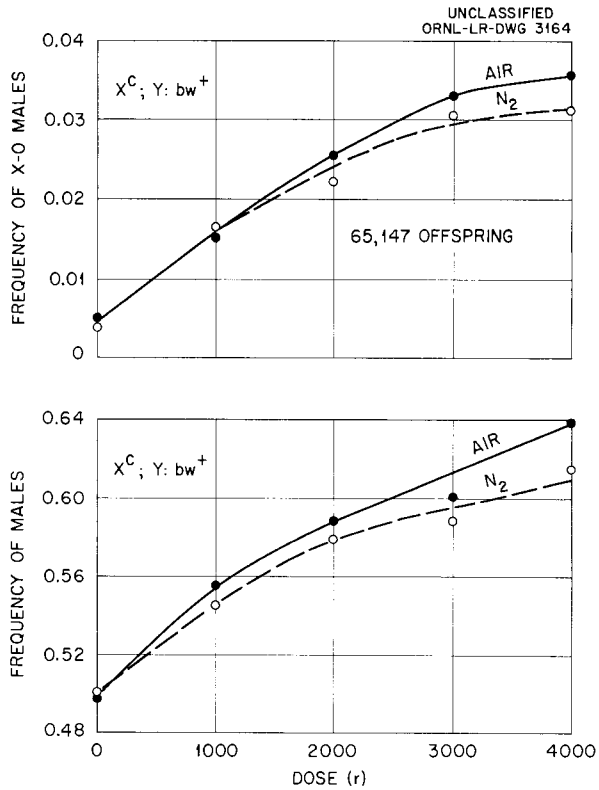

Fig. 12. Dose Effect Relation of Loss of the X^c Chromosome.

Radiation-Induced Exchanges in *Drosophila* Females

D. R. Parker

It has been found that radiation-induced detachment of attached-X chromosomes in the absence of a Y chromosome most often involves exchange with chromosome 4, whereas with a complete Y chromosome present, about four-fifths of all detachments result from induced exchanges of attached-X with Y.

Various incomplete Y chromosomes have been tested to determine their effects on frequency and type of radiation-induced detachment of attached X. The Y chromosomes that have been used in these experiments are:

(1) $sc^8 \cdot Y$ – a complete Y, structurally similar to a normal Y, marked with y^+ac^+, near the tip of the long arm.

(2) $sc^{V1} \cdot Y^S$ – a rod-shaped short arm marked at the centromere with y^+ac^+.

(3) Y^S – an unmarked rod-shaped short arm.

(4) $Y^S \cdot Y^S \#2$ – a V-shaped chromosome, made of two short arms.

(5) $sc \cdot Y^L$ – a J-shaped chromosome, morphologically similar to a normal Y but with short arm marked distad to bb^+ with y^+ac^+ and fertility factors, short arm wanting.

(6) $sc^{S1} \cdot Y^L \#2$ – similar to $sc \cdot Y^L$, but with bb^+ locus absent; a nearly rod-shaped chromosome.

(7) Y^{Lc} – a ring-shaped long arm of the Y.

Table 11 gives the results obtained by treatment with 2000 r (80 kv, 30 ma, 3-mm Al filter, 100 r/min) of attached-X females marked with y^2 su w^a w^a bb and with the Y chromosome(s) indicated. The adjusted frequencies are obtained after multiplying the number of regular females by four.

The higher frequencies obtained with rod-shaped chromosomes show these shorter chromosomes to be more effective than J-shaped Y's in undergoing exchanges with attached-X. However, the V-shaped $Y^S \cdot Y^S \#2$ gives about the same frequency as a single rod-shaped short arm, but seems less effective than two separate rod-shaped short arms. With the possible exception of the latter, increase in frequency of detachment with rod-shaped as compared to J-shaped Y chromosomes does not involve a lowering of frequency of X-4 exchanges. With the V-shaped $Y^S \cdot Y^S \#2$, the frequency of X-4 translocating may be somewhat higher than with Y's other than Y^{Lc}. It is difficult to explain the results on the basis of random reunion of randomly produced breaks. It is suggested that some pairing between X and 4 occurs, with part of the Y chromosome in some way altering this "pairing" relation, thereby affording chromosome 4 some "protection" from exchanges with X.

The results with Y^{Lc} are difficult to explain; perhaps because of its shape, it "pairs" with itself and is seldom involved in any exchange with the X.

TABLE 11. FREQUENCY OF DETACHMENTS WHEN VARIOUS Y CHROMOSOMES ARE USED

A	B	C	D	E	F = D × E
Y chromosome used	No. of cases of detachment recovered	No. of regular F_1 females	Adjusted percentage of detachment	Percentage of detachment showing X-4 linkage	Adjusted percentage of detachments with X-4 linkage
None	66	3314	0.50	59.8 (61/102)[b]	0.30
$sc^8 \cdot Y$	30	870	0.85	13.2 (9/68)[b]	0.11
$sc \cdot Y^L$	97	2839	0.85	13.0 (10/77)	0.11
$sc^{S1} \cdot Y^L \#2$	67	879	1.91	4.8 (2/42)	0.09
Y^{Lc}	20	944	0.53	80.0 (12/15)	0.42
$sc^{V1} \cdot Y^S$	247	3927	1.55	8.9 (8/90)	0.14
$sc^{V1} \cdot Y^S, Y^S$	44	584	1.93	? (0/14)	?
$Y^S \cdot Y^S \#2$	95	1446	1.62	12.8 (6/47)	0.21

[a]Only one of the two products of X-4 exchange is detectable by the test used, hence frequency of this exchange is somewhat larger than these figures indicate.

[b]The stocks tested include some obtained in other experiments not included in this table.

RADIATION PROTECTION AND RECOVERY

PROTECTION AND RECOVERY IN BACTERIA

A. Hollaender
G. E. Stapleton
D. Billen
C. O. Doudney
A. J. Sbarra
I. W. Bacon
D. O. Galloway[1]
M. R. Zelle[2]
A. S. Angel
J. E. Ogg[3]

Postirradiation Recovery of *Escherichia coli* B/r

G. E. Stapleton
A. J. Sbarra
I. W. Bacon

Simultaneously with the attempts at purification of natural materials for the recovery factor, an attempt was made to duplicate a chemically defined medium which would substitute for yeast or spleen extract in promoting recovery of bacteria exposed to ionizing radiations (X and gamma rays). A modified lactobacillus assay medium[4] was found to give approximately the same activity as yeast extract. Deletion of the vitamins from this medium resulted in no loss of activity. Omission of the purines and pyrimidines or amino acids, however, brought about a drastic reduction in activity. The four previously mentioned amino acids[5] were found to substitute for the eighteen amino acids in this medium. Also, guanine and uracil sufficed for the purine and pyrimidine requirement. The lactobacillus assay medium was thus reduced to a single amino acid, purine and pyrimidine, and salts A and B, as shown in Table 12. The importance of the salts in medium II is indicated by the finding that glutamic acid, uracil, and guanine added to medium I as listed in Table 13, gives less than 20% of the activity obtained by addition of yeast extract. Removal of either salts A or B from medium II resulted in a marked decrease in the number of survivors on this medium. Preliminary data indicate salts A to be more essential than salts B. More work will be required to elucidate the role of inorganic salts in the recovery process. The requirement for a purine and a pyrimidine, normal constituents of ribonucleic acid (RNA), plus glutamic or aspartic acid, the two amino acids most widely implicated in bacterial transamination suggests the role of synthesis of RNA and protein in partial recovery of *Escherichia coli* from the damaging effect of ionizing radiations.

Preliminary data indicate that the chemically defined medium with added glutamic acid, uracil, and guanine will substitute for yeast extract when the cells are incubated at 18°C as well as at 37°C. However, the survival on extracts of natural materials is greater when the incubation temperature is reduced from 37° to 18°C. It is interesting to consider the possibility that irradiated cells might be supplied with some intermediates in nucleic acid or protein synthesis which would elevate the survival level at 37° to that obtained on complex materials at 18°C. No evidence in this regard has been obtained to date.

[1] ORINS Fellow.
[2] Consultant.
[3] Visiting Assistant.
[4] J. M. Ravel, L. Woods, B. Felsing, and W. Shive, *J. Biol. Chem.* 206, 391-400 (1954).
[5] G. E. Stapleton, A. J. Sbarra, and C. W. Edington, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, pp 35-36.

TABLE 12. COMPOSITION OF MEDIUM II

Component	Amount
L-Glutamic acid or glutamine or aspartic acid	150 μg
Guanine	30 μg
Uracil	30 μg
Salts A (K_2HPO_4, 25 g; KH_2PO_4, 25 g; H_2O to make 250 ml)	1 ml
Salts B ($MgSO_4 \cdot 7H_2O$, 10 g; NaCl, $FeSO_4 \cdot 7H_2O$, and $MnSO_4 \cdot 4H_2O$, 0.5 g; H_2O to make 250 ml)	1 ml
Glucose	2 g
Agar	3.4 g
Water	200 cc

TABLE 13. COMPOSITION OF MEDIUM I

Component	Amount (g)
KH_2PO_4	1
$(NH_4)_2HPO_4$	4
$MgSO_4$	0.7
Na citrate	0.5
Glucose	10
Distilled water	1000 ml

It is also interesting to consider the possibility that the factors required for bacterial recovery might be applicable to recovery of higher forms of life. On the basis of the evidence presented by Jacobson,[6] Cole,[7] and Barnes and Loutit,[8] it seems unlikely that the "factors" required for recovery of mammals from part of the damaging effect of X rays could be as simple as those shown here to be operative in bacterial recovery. It is tempting, however, to consider the possibility that a similar fundamental mechanism is involved in bacterial recovery and cells of certain tissues of the mammal.

Studies on the Effect of Cysteamine on Survival of X-Irradiated *Escherichia coli* B/r

C. O. Doudney A. Hollaender

A previous report[9] has described modifications of X-ray sensitivity of *E. coli* B/r by cysteamine (β-mercaptoethylamine) and β-mercaptoethanol. In view of the remarkable protection afforded *E. coli* by cysteamine [dose reduction factor (DRF) = 12] as compared to other protective compounds (BAL: DRF = 5; β-mercaptoethanol: DRF = 8), further studies have been initiated in an attempt to elucidate the mechanism involved in the protective action of this compound. It has been found that, at energy levels below 60 kr, cysteamine is not additive in protective ability to a number of other protective compounds (BAL, ethanol, cysteine, β-mercaptoethanol, and sodium hydrosulfite) which readily add to the protective ability of each other. This was taken as presumptive evidence that cysteamine may protect *E. coli* by another mechanism than that postulated for the compounds listed (reducing the formation of toxic radicals produced by radiation by lowering the oxygen tension of the cells and surrounding medium).[10]

A comparison of survival of *E. coli* B/r on nutrient plates (salts–glucose + yeast extract) and on minimal plates (salts–glucose) indicates that *E. coli* treated with cysteamine during irradiation possess a marked ability to recover from the killing effects of irradiation on nutrient plates as opposed to minimal plates (Fig. 13). For example, at 90 kr about 1 thousand times the number of cells survive to form colonies on nutrient plates as compared to minimal plates. This is in marked contrast to the protection of β-mercaptoethanol where the difference between survival on nutrient and minimal plates is only twofold at this energy level. A chemically defined medium developed in this laboratory and which promotes recovery of unprotected, irradiated *E. coli*[11] will also support recovery of cysteamine protected *E. coli*. This medium contains, in addition to salts and glucose, a purine (guanine), a pyrimidine (uracil), and an amino acid (glutamic acid). Thus recovery of cysteamine-protected

[6] L. O. Jacobson, E. K. Marks, M. J. Robson, E. Gaston, and R. E. Zirkle, *J. Lab. Clin. Med.* 34, 1538-1543 (1949).

[7] L. J. Cole, M. C. Fishler, and V. P. Bond, *Proc. Natl. Acad. Sci. U.S.* 39, 759-772 (1953).

[8] D. W. H. Barnes and J. F. Loutit, *Nucleonics* 12, 68-71 (May, 1954).

[9] A. Hollaender and C. O. Doudney, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, p 33.

[10] A. Hollaender, W. K. Baker, and E. H. Anderson, *Cold Spring Harbor Symposia Quant. Biol.* 16, 315-326 (1951).

[11] G. E. Stapleton, A. J. Sbarra, and I. W. Bacon, this report.

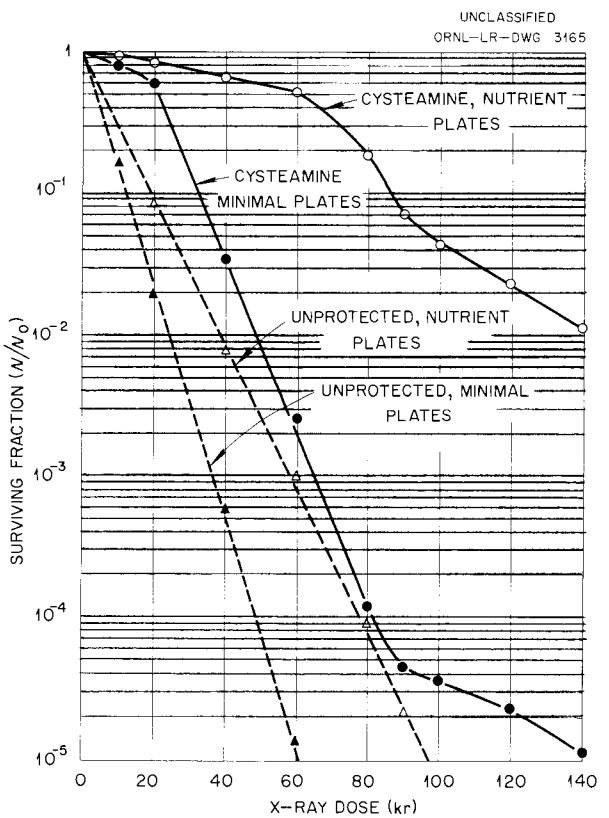

Fig. 13. The Ability of Nutrient Media (Salts-Glucose-Yeast Extract) and Minimal Media (Salts-Glucose) to Support Postirradiation Recovery of *E. coli* Pretreated with Cysteamine.

cells appears to involve the same synthetic processes as that of unprotected cells. It would seem that pretreatment with cysteamine enables a far greater proportion of the irradiated bacteria to recover. A study of the effect of holding cells for 24 hours following irradiation at various temperatures has shown that marked recovery takes place at 18°C (Fig. 14) a temperature low enough to slow cell division and yet high enough to allow continuance of metabolic processes which must be involved in the process of repair of the destructive effects of radiation.

The ability of cysteamine to protect *E. coli* is reduced significantly by phosphate salts. For example, at 60 kr, about one-tenth as many cells survive when irradiated in presence of both cysteamine and phosphate as survive in the absence of phosphate. Furthermore, a significantly lower concentration of phosphate as compared to cysteamine (<1:10) is required for this effect, suggesting that phosphate does not interfere with protection by direct interaction with cysteamine, but, indirectly, by some effect on the bacterial cell which interferes with the mechanism of cysteamine protection.

Figure 15 presents evidence that cysteamine does not protect *E. coli* simply by keeping oxygen away from the bacterial cell. Cells treated with cysteamine prior to irradiation, washed, and resuspended in a salt solution show a very high degree of radiation resistance as compared to control cells. It seems clear, therefore, that the major protective effect of cysteamine is intracellular.

As stated above, β-mercaptoethanol or other protective agents do not add to cysteamine protection at levels of radiation below 60 kr. However, at levels in excess of 60 kr where, as Fig. 16 shows, the effectiveness of cysteamine is reduced, β-mercaptoethanol as well as other protective

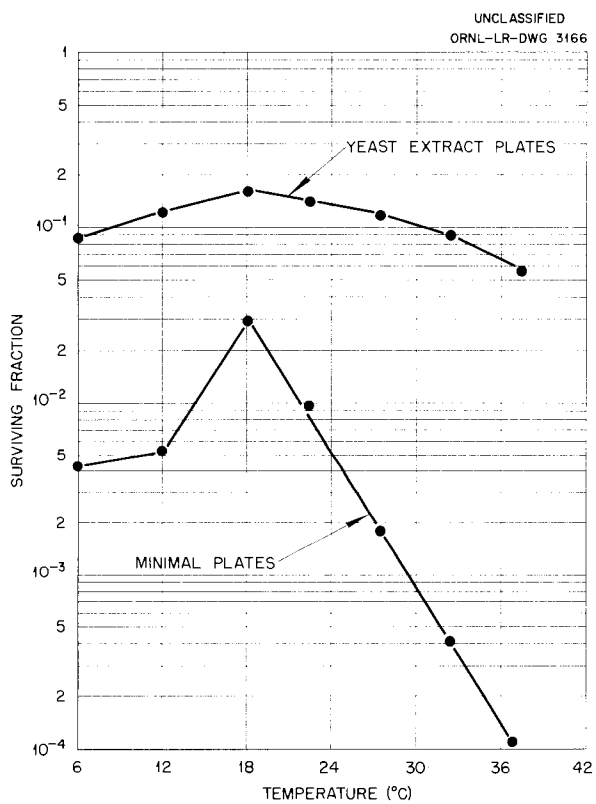

Fig. 14. The Effect of Temperature on Postirradiation Recovery of Cysteamine-Treated *E. coli* B/r Exposed to 90 kr of X Rays.

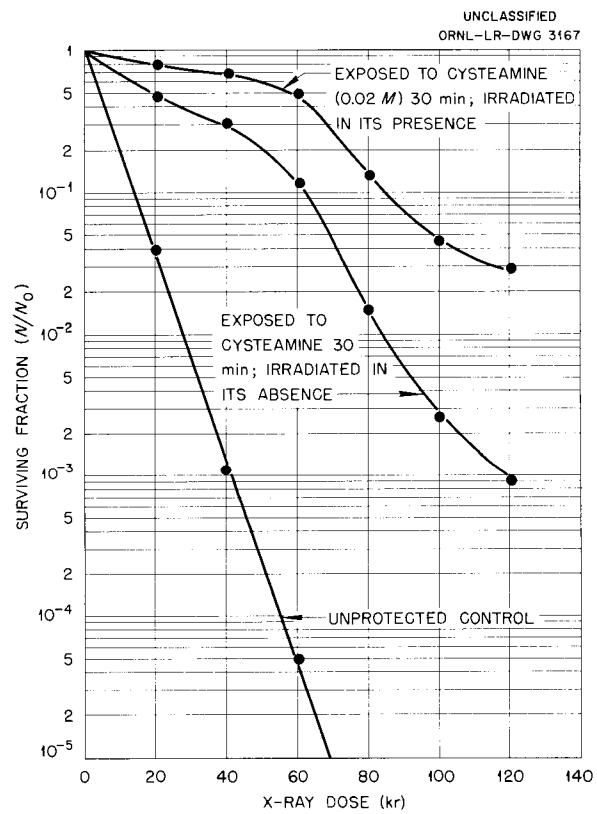

Fig. 15. The Effect of Preirradiation Removal of Cysteamine on Survival of Cysteamine-Treated Cells.

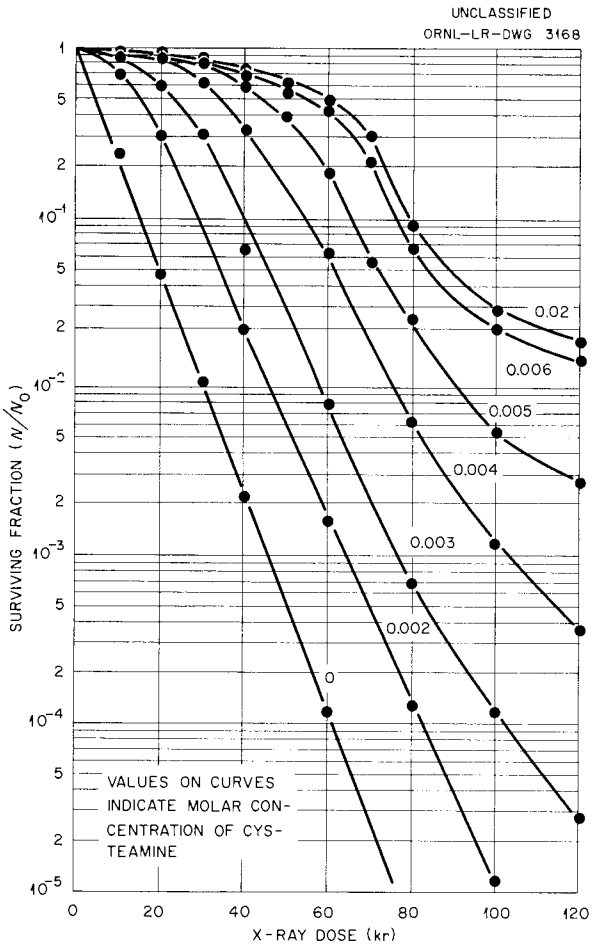

Fig. 16. The Effect of Cysteamine Concentration on Protection of *E. coli* B/r from the Killing Effects of X Irradiation.

agents will add readily to the protective ability of cysteamine. The decrease in protective ability of cysteamine at energy levels above 60 kr is not due to a direct effect of X radiation on cysteamine, since solutions of cysteamine preirradiated up to 120 kr give essentially the same survival curve as that of unirradiated cysteamine.

It is clear from these results that cysteamine not only protects *E. coli* from ionizing radiation at a very high level as compared to other protective compounds, but protects by a different mechanism. This mechanism appears to be some interaction of cysteamine with the bacterial cell which leaves a far greater proportion of the cells able to recover from the killing effects of radiation, when supplied with certain nutrients or held at favorable temperatures, than would otherwise recover.

The Protection of X-irradiated *Escherichia coli* B/r by Pretreatment with Acetylated β-mercaptoethylamine Derivatives

C. O. Doudney D. G. Doherty[12]
A. Hollaender

In view of the marked ability of cysteamine (β-mercaptoethylamine) to protect *E. coli* from the lethal effects of ionizing radiation,[13] further studies have been carried out which sought to determine the effect of "covering" of the end groups ($-NH_2$, $-SH$) of cysteamine by various other groups on the protection from X rays afforded to *E. coli*. These studies were initiated as a preliminary survey to determine the feasibility of the application of such an approach to the problem of increasing the protection afforded mammals by cysteamine.[14]

The toxicity, instability, and therapeutic index of this compound are not satisfactory for practical protection of mammals from radiation. It was felt that, by modifying the structure of cysteamine, a more stable molecule might be evolved which, following administration, could be degraded in the animal tissue to liberate cysteamine. By this means, it was hoped that cysteamine might penetrate in greater concentration to sites of protection of the animal against radiation. Furthermore, such a compound might liberate cysteamine slowly in the animal body, thus extending the protective state over longer periods of time. In addition, toxicity of the protective agent to mammals might be reduced below that of cysteamine by this method.

The protection afforded by compounds formed by acetylating the end groups of crysteamine have been determined (Table 14). Both N-acetyl-β-mercaptoethylamine and S-acetyl-β-mercaptoethylamine give a very high level of protection against X rays at 60 kr, though not surpassing that afforded by cysteamine. However, at 100 kr, where the protective ability of cysteamine falls off (see Fig. 16), the protective ability of these compounds remains high. Whereas the protective ability of the N-acetyl compound appears to be about the same

[12] Of Biochemistry.

[13] cf. C. O. Doudney and A. Hollaender, this report.

[14] cf. D. G. Doherty et al. (Biochemistry) and C. O. Doudney, this report.

TABLE 14. THE PROTECTION AFFORDED TO X-IRRADIATED *ESCHERICHIA COLI* B/r BY PRETREATMENT WITH ACETYLATED β-MERCAPTOETHYLAMINE DERIVATIVES

Protective compound (0.02 *M*)	Dose (kr) ⟶ 60		100	
	Temp. (°C) ⟶ 2	37	2	37
	Percentage of cells surviving irradiation			
Unprotected, control	3×10^{-3}	3×10^{-3}	5×10^{-4}	5×10^{-4}
β-Mercaptoethylamine (cysteamine)	55	48	9	6
N-Acetyl-β-mercaptoethylamine	40	32	17	15
S-Acetyl-β-mercaptoethylamine	20	48	9	29
N,S-Diacetyl-β-mercaptoethylamine	2×10^{-2}	24	2×10^{-3}	9

Cells were held for 30 min at the indicated temperature in solutions of the compounds, then irradiated.

TABLE 15. COMPOUNDS PRODUCING INCREASED KILLING OF *ESCHERICHIA COLI* BY X RAYS

Compound	Molar concn. ⟶ 0.02	0.08
	Percentage of cells surviving to form colonies	
2-Mercaptothiazoline	12	8
S,β-Aminoethylisothiouronium bromide HBr	3	1

when held at either 2° or 37°C prior to irradiation, the S-acetyl compound is more protective at 37°C. It is interesting to note that the 29% survival obtained at 100 kr with this compound represents the highest level of protection afforded *E. coli* by any compound yet tested at higher energy levels. N,S-diacetyl-β-mercaptoethylamine is only slightly protective when held at 2°C but highly protective at 37°C. Possibly some enzymatic process is required for this compound to protect. These results demonstrate that the end groups of cysteamine may be "covered" by acetylation, and the resulting compounds yet remain highly effective protective agents for *E. coli*.

Compounds Producing Increased Killing of *E. coli* by X rays. Two compounds studied which represent modifications of structure of cysteamine (2-mercaptothiazoline and S,β-aminoethylisothiouronium bromide HBr) increased the rate of killing by X rays of *E. coli* B/r significantly (Table 15). This effect with aminoethylisothiouronium is in marked contrast to that obtained in studies of the protection afforded mammals by this compound[15] which indicate that aminoethylisothiouronium is as effective as cysteamine in protecting mice from X rays. This marked difference between the results with mice and bacteria probably lies in the likelihood that the actively metabolizing mammalian body is able to degrade this compound to cysteamine, whereas *E. coli*, in the resting-cell state as studied here, is unable to do so. It is possible that these compounds may act in *E. coli* as competitive antagonists to a structurally related compound (cysteamine?) responsible for part of the innate radiation resistance of the bacteria. Further studies are being carried out to ascertain the mode of action of these compounds in increasing radiation killing.

The Effects of Cysteamine and β-mercaptoethanol on Mutation Induction by X rays

C. O. Doudney A. Hollaender

Experiments are under way to determine the effects of cysteamine and β-mercaptoethanol on the induction by X irradiation of reversions to the

[15] cf. D. G. Doherty et al. (Biochemistry) and C. O. Doudney, this report.

nonrequiring state of several mutant strains of *E. coli* which require specific growth factors (adenine, tyrosine, proline, threonine, and methionine). In addition, an effort is being made to determine the effect of recovery on X-ray-induced reversions of these strains since it has been shown that a large part of the increase in survival of cysteamine-treated *E. coli* is due to recovery following irradiation.[16] It is of considerable interest to determine whether or not this marked increase in survival due to recovery is related to a genetic "recovery" involving a return to the preirradiated state of genes which have been modified by irradiation. Owing to technical difficulties which are being overcome, only the reversion rate of cysteamine treated cells which do not have available the recovery factors following irradiation can be reported.

Data on one strain (adenineless) on which extensive work has been carried out will illustrate the results obtained. Previous studies in this laboratory have shown that the X-ray-induced reversion rate of this strain is profoundly increased in the presence of oxygen.[17] Figure 17 shows that β-mercaptoethanol markedly decreases the reversion rate of this strain as compared to that obtained in an air-equilibrated system. At low energy levels cysteamine is effective in decreasing the reversion rate, but at energy levels higher than 40 kr, where survival falls off rapidly due to increasing requirements of the irradiated cells for recovery factors, a corresponding rapid increase in the reversion rate occurs. Thus a plot of the reversion rate of either β-mercaptoethanol or cysteamine-treated cells as a function of survival gives curves which fall along approximately the same line as that of unprotected cells. This compares with the results obtained by Anderson who showed a definite relation of mutation rate to survival of cells of this strain irradiated in the presence and absence of oxygen.[16]

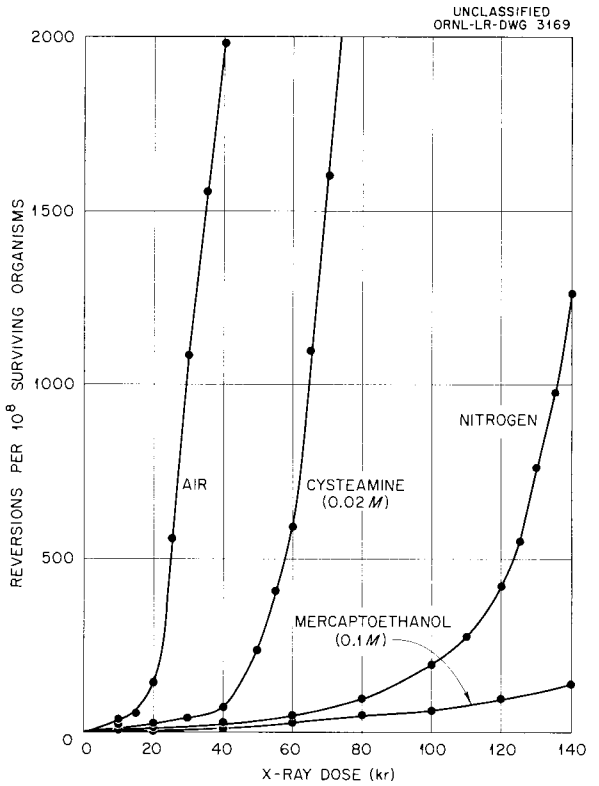

Fig. 17. X-Ray Induced Reversion of *E. coli* Strain 82-r (Purine-Requiring).

Studies on the Postirradiation Temperature Effect on Induced Mutations in *Escherichia coli*

D. Billen R. W. Whittle

It was previously reported that the X-ray-induced mutation rate of auxotrophic strains of *E. coli* is dependent on the temperature at which the plated cells are incubated for a 48-hr period following exposure.[18] Since those studies were carried out with cell suspensions flushed with oxygen prior to irradiation it was of interest to determine whether anoxic conditions during exposure would alter the postirradiation temperature treatment response. It was found that in two strains, T83-8 (tyrosine) and P82/r (purine), exposed to 40 kr, the postirradiation temperature treatment had a similar influence on the induced mutation rates for cells exposed either in oxygen or nitrogen, as shown in Table 16. In these two strains a rising induced reversion rate with increasing temperature of incubation was observed in both the oxygen- and nitrogen-treated cells. However, in strain P62, the gas present during exposure was found to influence the pattern of response to type of the temperature treatment. Whereas a maximum induced mutation rate for the cells exposed in an atmosphere of oxygen was obtained by incubation of the plated cells at 24°C,

[16] C. O. Doudney and A. Hollaender, this report.

[17] E. H. Anderson, *Proc. Natl. Acad. Sci. U.S.* 37, 340-349 (1951).

[18] D. Billen and R. W. Whittle, *Biol. Semiann. Prog. Rep. Aug. 15, 1953*, ORNL-1614, pp 38-40.

TABLE 16. EFFECT OF THE GAS DURING X-RAY (40 kr) EXPOSURE ON THE MUTATION RESPONSE OF *ESCHERICHIA COLI* AUXOTROPHS TO POSTIRRADIATION TEMPERATURE TREATMENT

Strain	Mutation rate per 10^8 survivors of postirradiation treatment for suspensions exposed in:					
	Oxygen			Nitrogen		
Temp. (°C) ⟶	12	24	37	12	24	37
P82/r	3,173	4,337	9,761	347	489	569
T83-8	8,875	11,202	13,496	188	212	378
P62	3,805	38,660	16,470	6	75	310

the cells exposed in nitrogen showed a maximum mutation rate at 37°C (Table 19). Thus, in this one strain, it appears that the gas present during exposure can influence the genetic response to a postirradiation treatment.

Studies on the effect of the gas phase on the induction of mutation at several doses of X rays have also been carried out. As reported by Anderson[17] for the purineless strain of *E. coli* (P82/r), the mutation rates for strains T83-8 and P62 increased with increasing dosage when exposed either in oxygen or nitrogen. It has also been found that exposure of these two strains in either oxygen or nitrogen to several doses of X rays produces a similar mutation rate for an equivalent surviving fraction under the two conditions of treatment (see Fig. 18; only the data for T83-8 are presented). This is in agreement with similar observations of Anderson (*ibid.*) for strain P82/r as contrasted to his findings with a streptomycin-dependent mutant in which the mutation rates were different at equal surviving fractions of cells exposed either in oxygen or nitrogen.

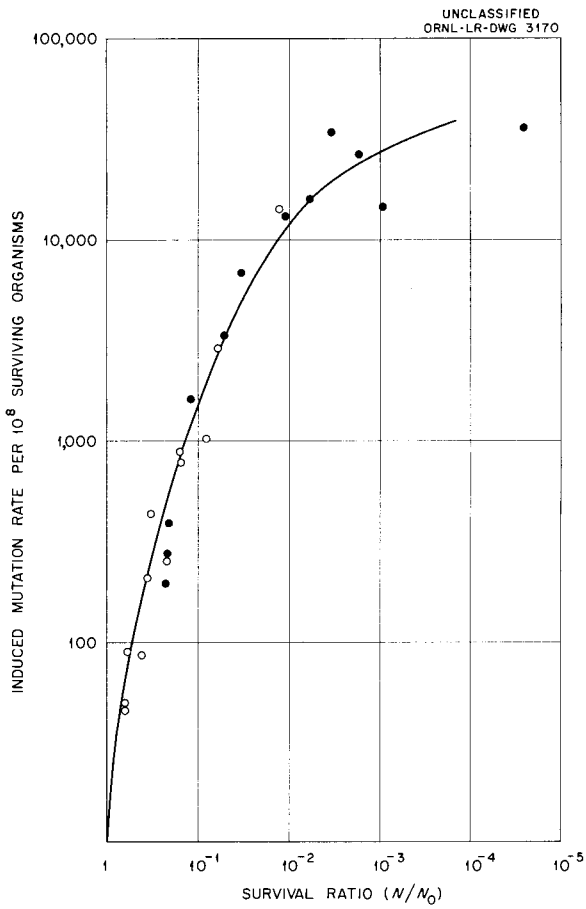

Fig. 18. Rate of X-Ray Induction of Back Mutations as a Function of Survival Ratio in *E. coli* T83-8. ●, Oxygen; O, nitrogen.

The Effects of Some Chemical Protective Agents on the Lethal and Mutagenic Action of Ionizing Radiations on *Aspergillus terreus*

D. O. Galloway A. Hollaender
G. E. Stapleton

Aspergillus terreus spores have been previously used to study killing and morphological variation induced by radiations.[19-23] Spores of *A. terreus*

[19] A. Holloender, K. B. Raper, and R. D. Coghill, *Am. J. Botany* 32, 160-165 (1945).

[20] K. B. Raper, R. D. Coghill, and A. Hollaender, *Am. J. Botany* 32, 165-176 (1945).

[21] L. B. Lockwood, K. B. Raper, A. J. Moyer, and R. D. Coghill, *Am. J. Botany* 32, 214-217 (1945).

[22] G. E. Stapleton, A. Hollaender, and F. L. Martin, *J. Cellular Comp. Physiol.* 39, Suppl. 1, 87-100 (1952).

[23] G. E. Stapleton and A. Hollaender, *J. Cellular Comp. Physiol.* 39, Suppl. 1, 101-113 (1952).

were suspended in 1:1,000,000 dilution of sodium lauryl sulfonate before chemical treatment and irradiation. All chemicals were used in concentrations which gave maximum protection without detectable toxic effects. The suspensions were plated upon potato dextrose agar (Difco) immediately following irradiation and incubated at 29°C for 4 days before counting. One hundred colonies were transferred to potato dextrose agar slants and incubated for 7 days before examining for morphological variants.

X rays are 20% more effective than Co^{60} gamma rays for killing of oxygen-saturated spore suspensions, as well as production of morphological variants. Bubbling nitrogen through the suspension gave the same degree of protection as a 0.02 *M* solution of British antilewisite (BAL) against gamma rays. The dosage required to give equal killing was 1.8 times that required in suspensions saturated with oxygen. Propylene glycol (3 *M*) and 0.00125 *M* sodium hydrosulfite were equally effective in protecting and required doses 1.4 times as great as the oxygen-saturated suspensions. No differences in protection were observed when the spores were incubated with chemical agents for various periods of time preceding irradiation. Incubation of the plated, irradiated spores for 24 hr at various temperatures has not revealed a temperature recovery element as observed with bacteria. The effect of cysteamine alone and in combination with various other agents is being studied at present. Likewise, the effect of holding in liquid suspension for various lengths of time after irradiation is being investigated.

Ultraviolet Quantum Yields for Inactivation of Bacteriophages

M. R. Zelle J. E. Ogg
A. Hollaender

Zelle and Hollaender[24] present action spectra and quantum yields for inactivation of bacteriophages T1 and T2 by monochromatic ultraviolet radiation. Table 17 presents the major results of the earlier studies.

The action spectra for both phages are of the nucleic acid type with maxima at about 2650 A, minima at about 2400 A, and with increasing efficiency at the shorter wave lengths. The quantum yields show no systematic variation with increasing wave length and the quantal efficiency for T1 is roughly twice that for T2. Thus, on the basis of absorbed energy, T1 appears more sensitive to ultraviolet whereas about 2½ times as much incident energy is required for inactivation of T1.

The quantum yield is computed as

$$QY = \frac{1}{(E_{.37})(A_p)} ,$$

where $E_{.37}$ is the inactivation dose (0.37 survival

[24] M. R. Zelle and A. Hollaender, *J. Bacteriol.*, in press.

TABLE 17. RELATIVE INACTIVATION EFFICIENCY AND QUANTUM YIELDS FOR INACTIVATION OF BACTERIOPHAGES T1 AND T2 FOR NINE MONOCHROMATIC ULTRAVIOLET WAVE LENGTHS

Wave length (A)	T1		T2	
	Inactivation efficiency relative to 2650 A	Quantum yield ($\times 10^{-4}$)	Inactivation efficiency relative to 2650 A	Quantum yield ($\times 10^{-4}$)
2250	1.25	4.95	0.90	2.65
2300	0.83	5.08	0.56	2.69
2378	0.51	5.47	0.53	3.75
2483	0.82	6.88	0.78	3.49
2537	0.93	6.41	0.81	2.99
2650	1.00	6.42	1.00	3.26
2804	0.60	5.70	0.77	3.55
2967	0.17	8.05	0.13	2.43
3022	0.04	7.26	0.03	1.76

ratio) in quanta incident per square centimeter and A_p is the absorption coefficient in units of square centimeters per particle.

The studies now in progress are designed to furnish more accurate estimates of the quantum yields for several different phages. Improved physical techniques, reduction of nonspecific absorption, and increased replication will yield more accurate values of $E_{.37}$.

More accurate values of A_p will be obtained by measuring the ultraviolet absorption by means of integrating spheres which permit measuring the absorption with no error due to light scattering. Secondly, the absorption coefficients will be related to the total number of phage particles as determined by electron micrographic particle counts instead of the number of plaque-forming particles. Values of A_p related to the number of plaque-forming particles can be twice as great as the true value due to the presence of inviable phage particles in the purified preparations.

It is planned to use four different phages: T1, T2, and T4 of the T series of *E. coli* phages, and the S13 dysentery phage. S13 is one of the smallest phages known, and, presumably, is a less differentiated and complex entity than the larger phages. This series of phages will furnish additional evidence on the apparent inverse relation between biological complexity and quantum yields observed with phages T1 and T2 (Table 17) and for a number of enzymes.[25]

At this writing, accurate survival curves for T1 and T2 phages at seven wave lengths have been determined. Ultraviolet absorption measurements have been made on a number of purified preparations of these phages, and electron micrographic particle counts are in progress but not complete. Hence no new values of quantum yields are yet available. Owing to the instability of purified preparations of the S13 phage, it now appears doubtful if accurate values of quantum yields can be obtained, although attempts to find conditions which increase the stability are under way.

[25] A. D. McLaren, *Acta Chem. Scandinav.* 4, 386-389 (1950).

ENZYMOLOGY AND PHOTOSYNTHESIS

ENZYMOLOGY

J. R. Totter
W. T. Burnett, Jr.
J. V. Passonneau[1]
A. N. Best
M. J. Cormier
J. E. Wilson[2]

Studies on Enzyme Systems in *Achromobacter fischeri*

M. J. Cormier J. R. Totter

Further investigations on the bioluminescence of *Achromobacter fischeri* have revealed that cytochrome c reduces light output by what appears to be an enzymatic reaction. The manner of interference by cytochrome c suggested a competition for the hydrogens of reduced diphosphopyridine nucleotide ($DPNH_2$) through the "Slater Factor." The results of a series of experiments designed to test this hypothesis may be visualized by reference to the chain of postulated hydrogen and electron transfers shown in Fig. 19.

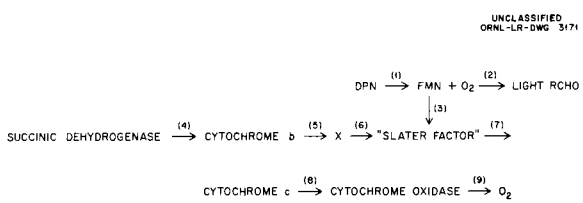

Fig. 19.

The bacterial extract appeared to contain the enzymes necessary for both the production of light [reactions (1) and (2)] and for transfer of electrons through the "Slater Factor" to oxygen without light production [reactions (3), (7), (8), and (9)]. In addition, menadione (2-methyl-1,4-naphthoquinone) reductase was present. Menadione can substitute for the "Slater Factor" in the reduction of cytochrome c by reduced flavin mononucleotide ($FMNH_2$) and, in fact, may be identical with or closely related to this factor. Succinic dehydrogenase was absent from the bacterial extracts. It is not yet known whether cytochrome b and substance "X" were present. Cytochrome c is limiting in the extracts; consequently, addition of this enzyme resulted in reduction of light output by increasing competition for the hydrogens of $FMNH_2$. The "Slater Factor" was originally defined as the site of action of 2,3-dimercaptopropanol (BAL) which abolishes oxygen uptake in the presence of succinate and the complete succinic oxidase system, but does not interfere with the transfer of hydrogen from succinate to cytochrome b. In agreement with the postulated series of reactions, BAL increased light output by the bacterial extracts and reversed the inhibition brought about by either cytochrome c alone or a combination of menadione and cytochrome c.

Other investigators have shown that antimycin A interferes with succinate oxidation in a manner somewhat similar to BAL. Tests conducted with antimycin showed that it did not enhance light output by the bacterial extracts either in the presence or absence of an added succinic oxidase system. It is necessary, therefore, to postulate an additional reaction between succinic dehydrogenase and the "Slater Factor." There is as yet insufficient information about the system to permit establishment of the precise position of the reaction with respect to cytochrome b. In Fig. 19 the possible intermediate is represented by "X" between cytochrome b and the "Slater Factor."

In summary, the following points have been established: Extracts of *Achromobacter fischeri* contain (1) the "Slater Factor," (2) a flavin-linked menadione reductase, and (3) a limited amount of the cytochrome oxidase system linked to the "Slater Factor." Application of this information has led to additional elucidation of the pathways of oxidation of $DPNH_2$. It was established that the inhibitory action of BAL differed from that of antimycin A, thus clarifying the relation of the "Slater Factor" to other components of the succinic oxidase system.

Influence of Inhibitors on Formate Incorporation in Bone Marrow

J. R. Totter A. N. Best

In continuance of studies on the incorporation of formate into rabbit bone-marrow nucleic acids *in vitro*, an improvement in the method has been made, and specific activities for the nucleic acid purines and pyrimidines may now be obtained. With this improvement it is possible to study the effects of various agents on the incorporation of formate

[1]Consultant.

[2]Research Participant.

into each purine or pyrimidine independently. As indicated previously,[3] the reactions leading to the incorporation of formate carbon into the nucleic acid purines and thymine are complex. A demonstration that the formation of purines and their incorporation into deoxyribonucleic acid (DNA) might be affected differently from the production and incorporation of thymine, for instance, would be of considerable interest since such a finding could be used to help elucidate the mechanism of purine and pyrimidine biosynthesis. Furthermore, present day theories of the structure of DNA have an implicit suggestion that the incorporation of 1 molecule of adenine into a newly synthesized fragment of DNA must be accompanied simultaneously by 1 molecule of thymine; and similar considerations would hold for guanine and cytosine. Thus a change in specific activity for a purine, relative to its conjugate pyrimidine, suggests that either an alternative synthetic route is brought into use by the application of the agent tested or that the picture of DNA synthesis implied by the Watson-Crick formulation of the structure of DNA needs modification.

[3]J. R. Totter and A. N. Best, *Biol. Semiann. Prog. Rep. Aug. 15, 1953*, ORNL-1614, p 40.

The data presented in Table 18 are the results of several experiments in which use was made of aminopterin, the inhibitor previously tested, and several purine analogues.[4] It may be seen that two of the compounds tested showed differential inhibition, namely, aminopterin and 2,6-diaminopurine. A third, 5,7-dimercaptotriazolopyrimidine showed differential enhancement of the activities. Further experiments on these compounds are under way.

PHOTOSYNTHESIS

W. A. Arnold E. S. Meek

Accessory Pigments and Delayed Light Production

W. A. Arnold E. S. Meek

The study of the action spectrum for the production of delayed light by blue-green algae and red algae has been completed and is here summarized.

In 1951, Strehler and Arnold[5] showed that green plants emit light for some time after they have

[4]The triazolopyrimidines listed in Table 19 were kindly furnished by Dr. Carl T. Bahner of Carson-Newman College, Jefferson City, Tennessee.

[5]B. L. Strehler and W. Arnold, *J. Gen. Physiol.* 37, 809-820 (1951).

TABLE 18. RELATIVE ACTIVITY OF DNA

Inhibitor tested	Concn. of inhibitor per ml	Adenine	Guanine	Thymine
5-Amino-7-mercaptotriazolopyrimidine	0	100	100	100
	10	100	125	103
	40	112	126	107
	100	95		86
5-7-Dimercaptotriazolopyrimidine	10	117	74	
	40		126	
	100	252	138	
5-Mercapto-7-hydroxytriazolopyrimidine	10	97	94	97
	40	91	114	86
	100	58	72	66
2,6-Diaminopurine	10	77	107	70
	40	65	92	51
	100	37	44	22
Aminopterin	2	88	65	18
	5	47	40	15
	10	58	38	10

been illuminated. That the delayed light comes from energy absorbed by chlorophyll is shown by the fact that the action spectrum for the delayed light production is the same as the absorption spectrum of the chlorophyll-protein in the green plants. Figure 20, the action spectrum for the green plant, *Chlorella pyroidenosa*, was taken from the paper by Strehler and Arnold[5] in which may also be found a diagram of the apparatus used and the method of calculating the curve.

With the same method the action spectrum has been determined for the blue-green alga, *Anacystis nidulans*. The results are given in Fig. 21, along with the transmission of the suspension as determined by the Beckman spectrophotometer. The action spectrum consists of a single band at 620 mμ, corresponding to the absorption of the pigment, phycocyanin, regardless of the fact that the chlorophyll band at 680 mμ is quite pronounced in the transmission curve.

The curves for the red alga, *Porphyridium cruentum*, are given in Fig. 22. Here the action spectrum consists of two bands. One band is in the position of phycocyanin and a much larger one is at 550 mμ, corresponding to the absorption of phycoerythrin. Again, although the absorption of chlorophyll at 680 mμ is quite pronounced in the transmission curve, there is no trace of it in the action spectrum.

The emission spectrum of the delayed light in green plants is identical with the fluorescent spectrum of chlorophyll in the living plant,[6] and thus it is chlorophyll that emits the delayed light. The emission spectrum has been determined for the delayed light from both blue-green and red algae and they have been shown to be the same as the

[6]W. Arnold and J. B. Davidson, *J. Gen. Physiol.* 37, 677-684 (1954).

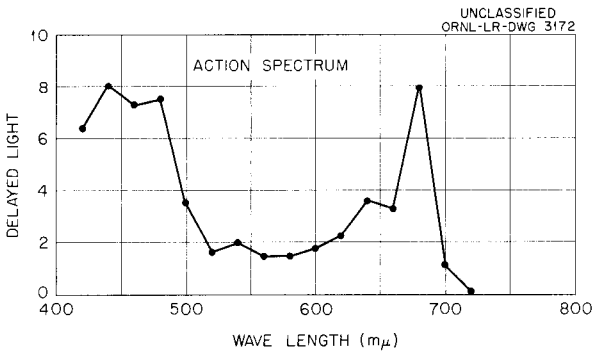

Fig. 20. The Relative Delayed Light Signal per Incident Quantum as a Function of Wave Length for the Green Plant, *Chlorella pyroidenosa*.

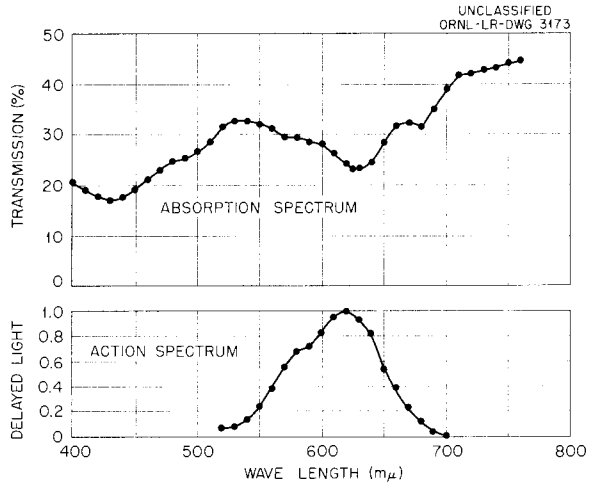

Fig. 21. The Relative Delayed Light Signal per Incident Quantum as a Function of Wave Length for the Blue-Green Alga, *Anacystis nidulans* and the Transmission of the Suspension as Determined by the Beckman Spectrophotometer.

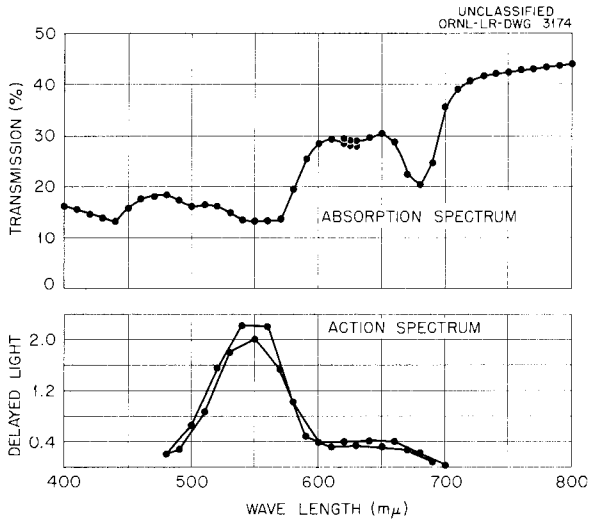

Fig. 22. The Relative Delayed Light Signal per Incident Quantum as Function of Wave Length for the Red Alga, *Porphyridium cruentum*, and the Transmission of the Suspension as Determined by the Beckman Spectrophotometer.

fluorescent spectrum of chlorophyll in the green plant. The action spectrum for photosynthesis in the red algae, as given by Haxo and Blinks,[7] and the action spectra for both chlorophyll fluorescence and photosynthesis in the blue-green and red algae, as given by Duysens,[8] agree with our results from these studies.

The results can be summarized with the following statements: In the green plant (1) chlorophyll does photosynthesis and makes the delayed light; (2) chlorophyll absorbs the energy used for both processes. In the blue-green and red algae, (1) chlorophyll does photosynthesis and makes the delayed light; (2) phycocyanin and phycoerythrin absorb the energy used for both processes; and (3) generally, the energy absorbed by chlorophyll is wasted.

[7] F. T. Haxo and L. R. Blinks, *J. Gen. Physiol.* 33, 389-422 (1950).

[8] L. M. N. Duysens. Transfer of Excitation Energy in Photosynthesis, *Doctoral Thesis, Univ. of Utrecht* (1952).

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell, Section Chief

E. F. Oakberg
L. B. Russell
J. S. Gower
J. C. Kile, Jr.
L. Wickham
R. Auerbach[1]
J. W. Bangham
M. K. Freeman
J. C. Furney
E. M. Kelly
M. H. Major
L. H. Millemann
R. J. Spear

Radiation-Induced Mutations in the Mouse

W. L. Russell
E. F. Oakberg
J. S. Gower
J. W. Bangham
E. M. Kelly
M. H. Major
L. H. Millemann
R. J. Spear

Some of the presumed mutations obtained from the 0-, 300-, 600-, and 1000-r X-ray experiments on specific loci are still being tested, and additional offspring are being obtained in the 300-r experiment. However, the following conclusions already appear justified.

It has been stated in earlier reports that the mutation rate at the 1000-r dose was lower than expected, on the hypothesis of linearity, from the rates obtained at 0 and 600 r. This finding has been substantiated by additional data, the departure from linearity now being clearly significant. Quantitative details will be presented when tests of the mutants are complete. A plausible explanation for the lowering in mutation rate at the higher dose is that there is differential sensitivity to radiation among spermatogonia and that the responses of cell death and mutation in these cells are correlated. Thus, at the higher dose, more of the sensitive spermatogonia would be killed and the survivors would be cells having a lower sensitivity to induction of mutations. Part of the basis on which this hypothesis rests is being tested by detailed quantitative studies on radiation damage to spermatogonia (see data of E. F. Oakberg in these reports).

It has been pointed out earlier that the lack of linearity in mutation rates for the 0-, 600-, and 1000-r doses indicated the importance of determining mutation rates at doses between 0 and 600 r. Data from the 300-r experiment continue to show a mutation rate slightly, but not significantly higher than would be expected, on the hypothesis of linearity, from the 0- and 600-r rates. As far as it goes, this information is reassuring. However, in view of the high mortality of spermatogonia found by Oakberg at doses much lower than 300 r, there is still considerable uncertainty as to whether mutation rates between 0 and 300 r may differ markedly from linear interpolation between these doses. Experiments with doses below 300 r should, therefore, be undertaken.

Sensitivity of Spermatogonia of the Mouse, and Killing of Spermatogonia by 20 r of Gamma Rays

E. F. Oakberg J. C. Furney

The effects of 600 r on spermatogonia reported previously were acute and near a maximum possible response.[2,3] Accordingly, dosage curves on sensitivity of spermatogonia are necessary for adequate planning of subsequent experiments on radiation response of these cells. Mice were exposed to 5, 10, 25, 50, 75, 100, 150, and 200 r of gamma rays from a Co^{60} source at a dose rate of 6 r/minute. Eighteen mice were used, two for each dose level and two for controls. All animals were killed 48 hr after exposure. This interval allows most necrosis of spermatogonia to occur, especially of intermediate and B types. Also, by 48 hr, cells irradiated as type B spermatogonia will have passed through at least one division, and degeneration of damaged cells will be reflected as a deficiency of resting primary spermatocytes. Scoring at this stage is advantageous, owing both to distinctive morphology of resting primary spermatocytes and to the large number of these cells per tubule. Some latent damage to type A spermatogonia probably has not been expressed at this time, but extreme sensitivity of a portion of the type A nevertheless can be demonstrated.

The reduction in number of resting primary spermatocytes (Table 19) observed 48 hr after irradiation is due to damage of cells which were type B spermatogonia at time of irradiation. Sensitivity of type B cells was unexpectedly high, with

[1]Research Associate (arrived July 9, 1954).

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

[3]E. F. Oakberg and J. C. Furney, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, p 46.

TABLE 19. NUMBER OF TYPE A SPERMATOGONIA AND RESTING YOUNG SPERMATOCYTES 48 HOURS AFTER DOSES OF 5 TO 200 r OF GAMMA RAYS

Dose (r)	Type A spermatogonia				Young spermatocytes[a]			
	No. of tubules scored	No. of cells counted	No. of controls	Ratio, exptl./control	No. of tubules scored	No. of cells counted	No. of controls	Ratio, exptl./control
0	342	1361			51	2805		
5	253	1056	1110	0.951	33	1546	1815	0.852
10	344	1232	1396	0.883	33	1543	1815	0.850
25	234	594	941	0.631	26	938	1430	0.656
50	307	597	1229	0.486	45	593	2475	0.240
75	198	379	786	0.482	22	57	1210	0.047
100	275	435	1153	0.377	37	33	2035	0.016
150	184	256	762	0.336	32	4	1760	0.002
200	282	347	1165	0.298	77	0	4235	0.000

[a]Irradiated as type B spermatogonia.

24% survival at 50 r and only 0.2% survival at 150 r.

Comparable survival of type A and type B cells was observed at doses of 5 to 25 r, with a divergence of response above 50 r. A portion of this discrepancy at higher doses could be due to latent damage of type A cells which is not expressed by 48 hr, or to a heterogeneity of sensitivities of the type A population. The existence of differences of sensitivities within type A spermatogonia is the more attractive hypothesis, for it would reconcile the large amount of killing observed at low doses with the survival of a few type A cells at high doses of radiation.

It may be questioned if loss of spermatogonia demonstrated with low doses of gamma rays is due to killing of cells as with 600 r of X rays, or if it may be due to inhibition of mitosis. Twenty roentgens was selected as a dose which should give sufficient reduction in number of spermatogonia to distinguish between these possibilities. Mice were irradiated with gamma rays from a Co^{60} source at a dose rate of 6 r/minute. Four experimental mice were killed at each time-interval, one control was killed with each of the 6-, 12-, 18-, and 24-hr groups. A frequency distribution of tubule stages based on a random sample of 1072 tubule cross sections from control mice was used in selecting the 60 tubule sections scored for each mouse. Thus expected numbers are the same for each group, and problems arising from sampling atypical distributions of qualitatively different tubules are avoided.

The number of normal and necrotic spermatogonia counted in controls and in 3- to 72-hr experimental groups is given in Table 20. Survival has been expressed as experimental/control ratios. The rapid decrease observed after 600 r also occurs with 20 r. Survival at comparable time is, of course, higher with the lower dose, with a leveling off at approximately 60% survival of both type A and type B cells at 24 to 72 hours.

The number of necrotic type A spermatogonia increased rapidly, with a high of 10% recorded at 12 hours. Peak incidence of degeneration for 600 r likewise occurred at 12 hours. The increase in proportion of necrotic cells at all intervals from 6 to 18 hr, inclusive, were significant at the 95% confidence level. Increases above controls at 21, 24, 30, and 36 hr were not significant. Type B spermatogonia show the same rapid increase in degeneration. Here all experimental groups are significantly above controls. The peak incidence of necrosis occurred at 15 and 18 hr, which is slightly later than observed for type A cells.

Following 20 r of gamma rays, spermatogonia of all types show a significant rise in frequency of necrosis which coincides with the time of most rapid decline in number of apparently normal cells. Therefore, as has been previously demonstrated

TABLE 20. NUMBER OF SPERMATOGONIA OBSERVED 3 TO 72 HOURS AFTER 20 r OF GAMMA RAYS

Time after irradiation (hr)	No. of tubules scored	Type A spermatogonia					Intermediate and type B spermatogonia				
		Total cells	"Normal" cells	Necrotic cells	Ratio, irrad./control[a]	Proportion necrotic	Total cells	"Normal" cells	Necrotic cells	Ratio, irrad./control[a]	Proportion necrotic
0 (Control)	240	1152	1118	34		0.0295	1577	1577	0		0
3	240	1140	1083	57	0.969	0.0500	1399	1367	32	0.867	0.0229
6	240	953	886	67	0.792	0.0703	1278	1236	42	0.784	0.0329
9	240	1090	1019	71	0.911	0.0651	1413	1371	42	0.869	0.0297
12	240	967	869	98	0.777	0.1013	1280	1214	66	0.770	0.0516
15	240	833	755	78	0.675	0.0936	1283	1173	110	0.744	0.0857
18	240	884	814	70	0.728	0.0792	1224	1138	86	0.722	0.0703
21	240	852	820	32	0.733	0.0376	1269	1202	67	0.762	0.0528
24	240	673	641	32	0.573	0.0475	1139	1051	88	0.666	0.0773
30	240	723	701	22	0.627	0.0304	958	942	16	0.597	0.0167
36	240	705	675	30	0.604	0.0426	1022	976	46	0.619	0.0450
48	240	743	728	15	0.651	0.0202	982	965	17	0.612	0.0173
72	240	675	662	13	0.592	0.0193	932	920	12	0.583	0.0129

[a]Limited to count of "normal" cells.

with 600 r, the decrease in number of spermatogonia after low doses of radiation is due to killing of cells rather than to inhibition of mitosis.

Dominant Lethals Induced in Female Mammalian Germ Cells by X rays

L. B. Russell W. L. Russell
R. J. Spear

The search for radiation-induced dominant changes induced in *female* mammalian germ cells is complicated by the virtual impossibility of irradiating germinal tissue without also irradiating certain somatic tissues of the mother, thus potentially affecting the prenatal environment of the F_1. It has been known for many years that female rodents become permanently sterile soon after relatively low doses of radiation and the reduced litter sizes observed[4,5] between irradiation and onset of complete sterility has been interpreted to be due, at least in part, to the rapid destruction of the follicular elements of the ovary.[6] However, from genetic experiments involving irradiation of males, it is known that dominant lethal changes may be induced in mature germ cells of mammals.[7] A series of experiments was, therefore, undertaken to determine whether dominant lethals may be induced in female mammalian germ cells and, if possible, to obtain quantitative comparisons of their induction in different stages of oogenesis.

Females of tested fertility were placed with males at various intervals following 0 or 400 r of X rays and the F_1 from the resulting matings was scored *in utero* at about 2 weeks' gestation. Corpora lutea were counted at the same time. Figure 23 gives data showing the results (irradiated/control ratios) for this series.

Possible causes for the reduced litter size (lower line in Fig. 23) may be listed as follows: (1) reduction in number of ovulated eggs, (2) reduction in the proportion of ovulated eggs that are fertilizable in the normal manner, or (3) increase in prenatal death. Each of these possible causes of litter size reduction may trace to one or both of two explanations: (1) damage to germ cells, induced at some stage in oogenesis, (2) radiation effects on maternal physiology. To decide between these causes, additional experimental series were undertaken which, to date, cover only the matings made ½ to 4½ days (i.e., in the first estrus) following irradiation. The account, from here on, will refer mostly to this interval.

Cause (1) (preceding paragraph) can be ruled out with relative ease. The number of corpora lutea is not only *not* decreased but actually increased (see also an earlier report[8]) for about 2 weeks following irradiation. The peak is at about one week postirradiation (Fig. 23, upper line) but the increase is already highly significant days ½ to 4½ postirradiation (Table 21, row 3). Since it might otherwise have been questioned whether other types of radiation-induced changes in the ovary were mimicking the appearance of corpora lutea, tubal eggs were counted within 24 hr after ovulation, both in the intact oviduct and after

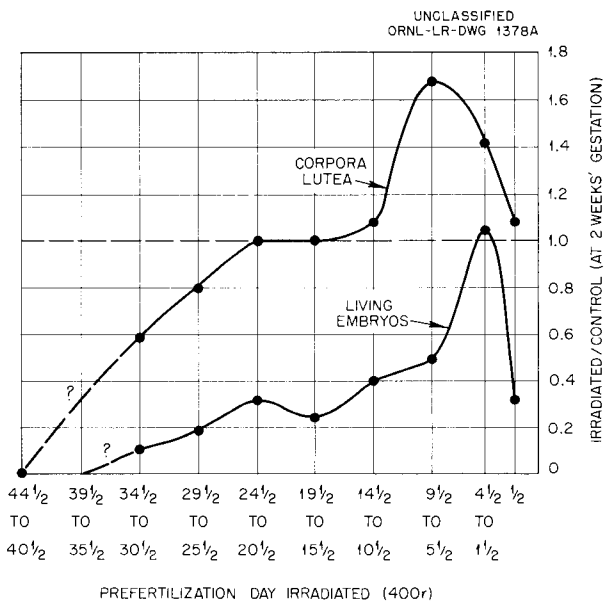

Fig. 23. Corpora Lutea and Living Embryos Observed in 2-Week Gravid (101 × C3H) F_1 Females Following Irradiation with 400 r at Various Intervals Before Copulation. The body anterior to the ovaries was shielded. All values are expressed as ratios of contemporary controls.

[4] G. D. Snell and F. B. Ames, *Am. J. Roentgenol. Radium Therapy* 41, 248-255 (1939).

[5] D. P. Murphy, *Surg. Gynec. Obstet.* 49, 440-446 (1929); 50, 588-593 (1930).

[6] J. M. Murray, *Am. J. Roentgenol. Radium Therapy* 25, 1-45 (1931); and others.

[7] Review by W. L. Russell, in *Radiation Biology*, ed. A. Hollaender, Vol. I, pp 825-859, McGraw-Hill, New York, 1954.

[8] L. B. Russell and M. H. Majar, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, pp 44-45.

TABLE 21. EFFECT OF PREFERTILIZATION IRRADIATION ON NUMBER OF CORPORA LUTEA AND SURVIVAL OF EMBRYOS TO 2 WEEKS OF GESTATION

	0 r		400 r		400 r/0 r	
Prefertilization day treated →	½	1½ to 4½[a]	½	1½ to 4½[a]	½	1½ to 4½
1. Number of females observed 2 weeks postcopulation	9	22	12	22		
2. Total number corpora lutea	91	227	134	322		
Mean number (per ♀) of:						
3. Corpora lutea	10.1	10.3	11.2	14.6	1.11	1.42
4. Living embryos	6.9	8.7	2.3	8.9	0.34	1.02
5. Resorbing bodies	1.6	0.9	(1.7)[c]	1.8	(1.07)[c]	2.00
6. "Preimplantation death"[b]	1.7	0.7	(7.2)[c]	3.9	(4.30)[c]	5.44
7. Living embryos/corpora lutea	0.68	0.84	0.21	0.61	0.31	0.72

[a]Separate data are available for treatments on prefertilization days 1½, 2½, 3½, and 4½, and indicate that there are no marked differences between these days.

[b]Estimated from the number of corpora lutea that are not represented by implants. That this probably does represent death of embryos is shown in Table 22.

[c]The apportionment of death between pre- and postimplantation may be biased in favor of the former, since death of all embryos of a litter often leads to abortion of all resorbing bodies before 2 weeks postcopulation.

TABLE 22. EFFECT OF PREFERTILIZATION IRRADIATION ON NUMBER AND CONDITION OF TUBAL EGGS OBSERVED WITHIN 24 HOURS OF OVULATION

	0 r		400 r	
Prefertilization day treated →	½	1½ to 3½	½	1½ to 3½
1. Number of tubal eggs counted	60	98	80	132
2. Mean number of eggs (per ♀)	10.0	9.8	10.0	12.0
3. Number of tubal eggs classified	57	95	72	127
4. Percentage normally fertile[a]	71.9	90.5	68.1	89.0
5. Mean number of fertile eggs (per ♀)[b]	7.2	8.9	6.8	10.7

[a]With two normal-appearing pronuclei as observed in physiological saline under dark-contrast-medium phase.

[b]Assuming distribution among lost eggs same as among classified eggs.

liberation. Tubal egg counts parallel corpora lutea counts in showing a significant increase for ovulations made 1½ to 3½ days postirradiation (Table 22, row 2).

To determine the importance of cause (2), tubal eggs were isolated within 24 hr after ovulation and examined in saline under dark contrast medium phase. They were classified as normally fertilized only if they had two clear pronuclei. (This is presumably a slight underestimate since a few normal eggs may have been in earlier developmental stages preceding the formation of the pronuclei and were thus classified with the questionable or unfertilized eggs.) Table 22 (row 4)

shows that the proportion of eggs normally fertilized is approximately the same in irradiated and control groups for copulations made 1½ to 3½ days following treatment and not significantly different for copulations made ½ day following treatment.

Thus the absolute number of fertilized eggs found in irradiated females is not significantly reduced for copulations made ½ day following irradiation and is actually *increased* for copulations made 1½ to 3½ days postirradiation. The results, therefore, definitely point to cause (3) for reduced litter size, namely, to increase in prenatal death. [Note: It should be repeated here that, at present, only ovulations during the first estrus following irradiation are considered. Obviously, after the third week postirradiation, cause (1) is important (see Fig. 23); and preliminary results indicate that cause (2) may also apply.] Because of the actual increase in fertilized eggs in irradiated females, radiation-induced mortality cannot be measured by comparing irradiated and control groups with respect to litter size but is measured by comparing them with respect to living embryos/corpora lutea ratios (corpora lutea being proportional to fertilized eggs). When this is done, it is apparent from Table 21 (row 7) that irradiation with 400 r causes appreciable death of embryos even when the litter size (as indicated by row 4) is, as is the case in one group, slightly higher in experimentals than in controls. The dissection of 2-week gravid uteri (Table 21) shows that most of this death takes place prior to implantation and some of it shortly after implantation.

It is legitimate to ask whether this early death of embryos may not be due to radiation effects on maternal physiology rather than to effects on the germ cells, especially since the ovarian tissue of the mother must, of necessity, be irradiated (even though anterior regions, including the pituitary, are shielded in these experiments). The most convincing evidence for the argument that the effect is directly on the female germ cells is the demonstration of chromosome damage in the early embryo. Numerous Feulgen-stained embryos may be found showing micronuclei following the first cleavage. These have also been found in living two-cell embryos. It is reasonable to assume that the micronuclei represent chromosome fragments produced in the course of the breakage-fusion-bridge cycle. As determined for other organisms, better suited for cytological investigations, this cycle is set up in cleavage when, by any one of several possible mechanisms, an irradiated germ cell contributes a broken chromosome to the zygote. Details of this interpretation are discussed for *Habrobracon* oocytes by A. R. Whiting.[9,10] Preliminary results of these studies make it appear possible that in the mouse, as in the wasp *Habrobracon*, the incidence of dominant lethals produced depends on the stage of the oocyte at the time of irradiation. A fairly high percentage of eggs ovulated ½ day after irradiation were probably undergoing the first meiotic division at the time of irradiation and several of these may have been in metaphase. It is the young resulting from these eggs which have the lowest survival ratio (Table 21, row 7).

The fact that micronuclei in early mouse embryos are also found following the irradiation of sperm ("Nebenkerne" of Brenneke[11]) strengthens the argument that death of embryos following prefertilization irradiation of females is indeed due to genetic causes and is not mediated by the maternal environment. Furthermore, the pattern of embryonic death following the irradiation of male germ cells[7,12,13] is similar to that following prefertilization irradiation of the mother. In both cases, death of embryos occurs before or shortly after implantation. If the radiation effect were on cytoplasm or maternal physiology, it would not be unreasonable to expect different results from the irradiation of the mother, since she contributes virtually all of the cytoplasm of the zygote and becomes the host of the embryo.

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[9] A. R. Whiting, *Am. Naturalist* 79, 193-227 (1945).

[10] A. R. Whiting, *Biol. Bull.* 89, 61-71 (1945).

[11] H. Brenneke, *Strahlentherapie* 60, 214-238 (1937).

[12] W. L. Russell, L. B. Russell, J. S. Gower, and C. W. Sheppard, *Record Genet. Soc. Amer.* 22, 98 (1953).

[13] W. L. Russell, L. B. Russell, and A. W. Kimball, *Am. Naturalist* 1954 (in press).

PATHOLOGY AND PHYSIOLOGY

PATHOLOGIC AND PHYSIOLOGIC EFFECTS OF RADIATION

A. C. Upton, Section Chief

T. T. Odell, Jr.
W. H. Benedict[1]
K. W. Christenberry[1]
D. M. Morris[2]
S. R. Tipton[1]
F. R. Conte[3]
G. F. Melville[3]
F. G. Tausche
W. D. Gude
B. Anderson
P. Ledford
F. F. Farbstein
C. B. Gurney[4]
P. W. Rueff, Jr.[4]

The Relative Biological Effectiveness of Fast Neutrons, X Rays, and Gamma Rays for Acute Lethality in Mice

A. C. Upton
F. R. Conte
G. S. Hurst[5]
W. A. Mills[5]
A. W. Kimball[6]
P. Ledford

Experiments on acute lethality have been carried out in preparation for studies of the late effects of fast neutron irradiation. Female RF mice 9 to 12 weeks old were exposed to fast neutrons in the Oak Ridge 86-inch cyclotron (Table 23), to 250-kvp X rays (Table 24), and to Co^{60} gamma rays (Table 25).

The animals were irradiated in the cyclotron within a lead chamber, the walls of which were 1 in. in thickness (this chamber occupied the position of box *B* described by Sheppard and Darden[7]); during exposure the mice were arranged in widely separated positions by means of lucite stanchions that permitted reproducible geometry (Fig. 24). Cyclotron radiations are expressed as surface doses in total rep (Table 23), as determined by tissue-equivalent chambers[8] and by means of Dupont film #510 and a proportional neutron counter similar in principle to that described earlier.[9] The gamma-ray contamination, estimated with the use of a Teflon chamber,[8] and Dupont film #510 was approximately 10% of the total dose in rep. The mean effective neutron energy was calculated to be 1.5 to 2.0 Mev, from data obtained with fission detectors. Studies of the neutron energy spectrum are being pursued currently, and further details of the dosimetry will be given in a later report.

Survival curves are shown in Fig. 25. The values for $LD_{50}/30$ days are given in Table 26, with the figures for relative biological effectiveness (RBE).

The observed RBE of neutrons is considerably lower than most earlier estimates for acute lethality in mice[10]; however, it approximates the value of 1.6 recently reported by Harris[11] for the RBE of 14-Mev neutrons in causing thymus and spleen weight loss in mice.

Uptake of Sulfate by Elements of the Blood and Bone Marrow of Rats

T. T. Odell
W. D. Gude
F. G. Tausche

These experiments were undertaken to study the uptake of S^{35}-labeled sodium sulfate by elements of the blood and the bone marrow. Rats were injected intravenously with 300 μc of $Na_2S^{35}O_4$ and were sacrificed at 6, 12, 18, 24, and 36 hr, and daily from the second through tenth days following injection. The blood was collected from the abdominal aorta at time of sacrifice and was separated into plasma, platelets, and erythrocytes by differential centrifugation. The platelets and erythrocytes were each washed twice with physiological saline, and the radioactivity of the platelets, erythrocytes, and plasma was determined. Autoradiograms were made of bone marrow smears and bone marrow sections to determine what cells had taken up radioactivity.

The initial decline in activity of the plasma was rapid until 18 hr; thereafter the decline was at a much slower rate (Table 27). The megakaryocytes took up sulfate rapidly, as seen in autoradiograms of the bone marrow taken 6 hr after injection (Table 28). Their peak activity occurred at 18 hours, and by two days it had declined to a low

[1] Consultant.

[2] Research Participant.

[3] U. S. Air Force.

[4] Temporary Employee.

[5] Health Physics Division.

[6] Mathematics Panel.

[7] C. W. Sheppard and E. B. Darden, ORNL-1559 (1953).

[8] The authors are indebted to Dr. H. H. Rossi of Columbia University for providing tissue-equivalent and Teflon chambers and assisting in their use, and to Dr. C. W. Sheppard for assistance in dosimetry.

[9] G. S. Hurst, *Brit. J. Radiol.* 27, 318 (1954).

[10] R. E. Zirkle, in *Radiation Biology*, ed. A. Hollaender, Vol. I, pp 315-350, McGraw-Hill, New York, 1954.

[11] P. S. Harris, LA-1629 (1954).

TABLE 23. MORTALITY OF RF MICE TO CYCLOTRON FAST NEUTRONS

Total dose (rep)		Geometrical position in lead box	Exposure time (min)	Dose rate (rep/min)		Fraction: Dead in 30 days/total exposed	Percentage dead in 30 days
A	B			A	B		
198	193	Middle center (2B)	2.1 ± 0.2	93.7	91.2	0/6	0.0
202	214	Middle right (3B)	2.1 ± 0.2	95.4	101.0	0/6	0.0
238	231	Middle center (2B)	2.5 ± 0.2	95.3	92.7	0/5	0.0
242	235	Middle right (3B)	2.5 ± 0.2	97.0	94.4	0/5	0.0
250	262	Top left (1C)	2.5 ± 0.2	100.0	102.7	0/9	0.0
		Bottom left (1A)	3.8 ± 0.2	62.2	70.1		
286	304	Bottom left (1A)	4.5 ± 0.2	63.5	67.5	3/24	12.5
316	330	Bottom center (2A)	4.5 ± 0.2	70.2	73.3	9/24	37.5
393	389	Top left (1C)	3.8 ± 0.2	106.0	98.2	16/24	66.7
		Middle center (2B)	3.8 ± 0.2	101.0	100.0		
		Middle right (3B)	3.8 ± 0.2	102.8	108.7		
447	473	Middle right (3B)	4.5 ± 0.2	99.0	104.8	24/24	100.0
499	517	Top center (2C)	4.5 ± 0.2	110.5	114.6	23/23	100.0
527	565	Top right (3C)	4.5 ± 0.2	116.8	125.2	25/25	100.0

Column A = total dose and dose rate measurements made with proportional counter + Dupont film #510 values.
Column B = total dose and dose rate measurements made with tissue-equivalent chamber values.

TABLE 24. MORTALITY OF RF MICE TO 250-KVP X RAYS

Total dose (r)	Dose rate (r/min)	Exposure time (min)	Fraction: Dead in 30 days/total exposed	Percentage dead in 30 days
440	79.8	5.51	3/24	12.5
480	79.8	6.02	5/24	20.8
520	79.8	6.52	11/24	45.8
560	79.8	7.02	18/24	75.0
600	79.8	7.52	24/24	100.0

Fig. 24. Lucite Stanchions Used for Exposing Mice in Cyclotron.

TABLE 25. MORTALITY OF RF MICE TO Co^{60} GAMMA RAYS

Total dose (r)	Dose rate (r/min)	Exposure time (min)	Fraction: Dead in 30 days/total exposed	Percentage dead in 30 days
643	62.0	10.37	3/10	30.0
704	62.0	11.35	2/10	20.0
765	62.0	12.34	8/10	80.0
771	62.4	12.36	16/24	66.6
820	62.4	13.14	19/24	79.2
827	62.0	13.34	7/10	70.0
868	62.4	13.91	21/24	88.0
916	62.4	14.68	24/24	100.0

TABLE 26. RELATIVE BIOLOGIC EFFECTIVENESS OF FAST NEUTRONS, X RAYS, AND GAMMA RAYS

Type of Radiation	LD_{50}/30 days	RBE
Fast neutrons (cyclotron)	362 rep (349–376)[a]	2.3 (1.63–3.51)[a]
250-kvp X rays	528 r (519–535)[a]	1.38[b] (0.90–1.87)[a]
Co^{60} gamma rays	730 r (712–735)[a]	1.00

[a]95% confidence intervals.
[b]RBE at the LD_{50} (the RBE varied slightly with the dose, being 1.33 at the LD_{20} and 1.48 at the LD_{80}).

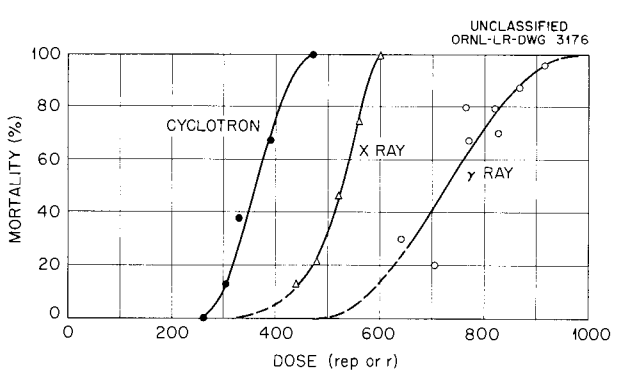

Fig. 25. Thirty-Day Mortality in Mice Exposed to Cyclotron Fast Neutrons, 250-kvp X Rays, and Co^{60} Gamma Rays.

level. The autoradiograms also showed activity associated with myeloid cells but not with the erythrocytes or their precursors. The platelets took up labeled sulfate; their activity reached its maximum 3 days after injection of the sulfate and then declined until 8 days when it leveled out at low values (Fig. 26). The activity of the erythrocytes paralleled that of the plasma, indicating no incorporation of sulfate.

The time relation of megakaryocyte and platelet radioactivities in these experiments supports the widely accepted view that platelets are derived from megakaryocytes. The curve of platelet activity indicates a mean life span of 4 to 5 days,

TABLE 27. RADIOACTIVITY OF PLASMA, PLATELETS, AND ERYTHROCYTES AFTER THE INJECTION OF S^{35}-LABELED Na_2SO_4

Time after injection	No. rats	Counts/min/200 μl of plasma		Counts/min × 10^7/av. RBC		Counts/min × 10^7/av. platelet	
		Mean	Range	Mean	Range	Mean	Range
6 hr	3	100,959	94,776–113,221	105	81.3–120	6.95	6.13–7.58
12 hr	2	36,232	35,352–37,111	42.1	41.1–43.1	5.33	4.60–6.06
18 hr	2	11,238	7,437–15,038	12.0	7.3–16.6	7.98	6.61–9.36
24 hr	3	9,098	7,411–10,284	9.3	7.3–12.0	8.42	6.59–10.8
36 hr	2	6,608	5,811–7,406	6.8	6.3–7.2	11.9	9.78–14.0
2 days	3	2,317	2,103–2,718	2.1	2.0–2.2	21.2	18.9–23.0
3 days	3	1,559	928–2,235	2.9	1.8–4.3	26.8	17.9–37.2
4 days	5	704	641–780	2.1	1.3–2.6	18.4	13.0–21.7
5 days	3	550	402–747	1.6	1.3–1.9	13.5	12.4–15.5
6 days	3	346	292–399	1.0	0.8–1.1	8.26	6.48–11.11
7 days	3	326	201–474	1.2	0.7–1.8	3.81	3.16–4.19
8 days	4	304	168–552	0.7	0.5–1.0	0.98	0.56–1.71
9 days	2	468	338–598	2.0	1.7–2.2	2.50	0.99–4.01
10 days	1	462		2.8		1.70	

TABLE 28. RADIOACTIVITY OF MEGAKARYOCYTES DETERMINED BY OBSERVATION OF AUTORADIOGRAMS OF BONE MARROW SECTIONS AND SMEARS

Time after injection (hr)	No. of rats	No. of observations[a]	Megakaryocyte activity[b]
6	3	36	0.66
12	2	24	0.98
18	2	21	1.70
24	3	27	1.25
36	2	18	0.81
48	3	27	0.23

[a]Observations were made on several slides from the same animal and every slide was studied three separate times.
[b]The activities of the megakaryocytes and neighboring areas were scored from 0 to 4 (in units of 0.5), and the background activity was subtracted. Scores made independently by two different examiners were in close agreement.

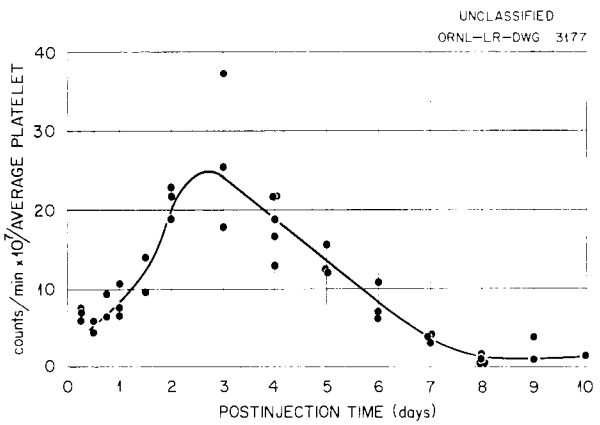

Fig. 26. Radioactivity of Blood Platelets After the Injection of S^{35}-Labeled Na_2SO_4. Each point represents the average of duplicate samples of a platelet suspension obtained from one rat.

which is in agreement with previous estimates.[12] The results also indicate the rate of turnover of sulfate by megakaryocytes.

The Lack of Injurious Effects of High Levels of Radioactive Sulfate in Rats

T. T. Odell B. Anderson
F. G. Tausche A. C. Upton
W. D. Gude

Rats were given single subcutaneous injections of $Na_2S^{35}O_4$ in doses ranging from 1 to 32 mc per rat in order to determine whether any of these levels might produce toxicity or radiation effects. The following blood cell counts were made at 5, 7, 10, 14, 20, and 27 days after administration of the sulfate: erythrocyte, platelet, total white blood cell, and a differential white blood cell count in which the percentages of lymphocytes, monocytes, and polycytes were observed. No significant difference in any of these counts was apparent at any of the sampling times. Two of the three rats in each group were sacrificed and autopsied 27 days following injection of the sulfate. Histological sections of the following organs were studied: lymph nodes, thymus, liver, spleen, kidney, adrenal, testis, intestine, skin, and bone marrow. No effects were observed in either the gross or histological material. The remainder of the rats will be autopsied 6 months after the injection of the sulfate.

Liver Damage and Hepatomas in Mice Following Intravenous Injection of Colloidal Radiogold

A. C. Upton W. D. Gude
W. T. Burnett[13]

The development of monocytic leukemia in two mice injected intravenously with colloidal radiogold in an earlier study prompted a reexamination of the late effects of this isotope. In this experiment colloidal Au^{198} (14) was injected intravenously into 1-month-old RF and 4-month-old C57BL female mice (Table 29). Nonradioactive colloidal gold (allowed to decay for 60 days) was similarly injected in comparable volumes into RF mice 1 month of age. The mice were placed in a 4π gamma chamber for estimation of total-body radioactivity immediately following and 5 days postinjection. The liver was found to take up 80% or more of the colloidal gold injected intravenously; the total doses given, 40 to 160 μc, were estimated, by the method of Marinelli et al.,[15] to deliver 850 to 3400 rep to hepatic cells. Mice were observed until natural death or were sacrificed *in extremis*.

The major late effects observed are presented in Table 29. Occurrence of liver damage was to be anticipated from earlier studies[16]; the high incidence of hepatoma, however, has not been hitherto reported. The tumors occurred late in life and only in mice with liver damage. Histologically, the hepatomas appeared benign, and metastases were not observed. They are presumed to be related to regenerative hyperplasia. Other hepatic changes included cholangiomatoid hyperplasia of bile duct epithelium, heterotopic ossification with formation of bone marrow, and additional degenerative changes similar to those described by Koletsky and Gustafson.[16]

The mice receiving 625 to 1350 μc developed leukopenia and died within 2 weeks. Histologic examination indicated marked atrophy of lymphoid and hemopoietic tissues and degenerative changes in the ovaries. After doses of 40 to 160 μc, longevity was reduced in proportion to the dose. The

[12] T. T. Odell, Jr. and F. G. Tausche, *Biol. Semiann. Rep. Feb. 15, 1954*, ORNL-1693, p 55.

[13] Of Enzymology Group.

[14] Abbott Laboratories, Inc.

[15] L. D. Marinelli, E. H. Quimby, and G. J. Hine, *Am. J. Roentgenol. Radium Therapy* 59, 260 (1948).

[16] S. Koletsky and G. Gustafson, *Lab. Invest.* 1, 312 (1952).

TABLE 29. LATE EFFECTS OF INTRAVENOUS RADIOACTIVE COLLOIDAL GOLD IN MICE

Treatment	Mean survival (months)	Cirrhosis (%)	Hepatoma (%)	Leukemia (%)	Mice	
					No.	Strain
Radiogold, 150 μc	21	86	29	0	21	C57BL
Radiogold, 625-1350 μc	⅓	0	0	0	7	C57BL
Noninjected controls	21	0	0	0	29	C57BL
Radiogold, 40 to 95 μc	20	25	25	25	8	RF
Radiogold, 103 to 160 μc	17	79	10	16	19	RF
Nonradioactive gold	22	0	0	27	30	RF
Noninjected controls	22	0	0	28	29	RF

incidence of leukemia was not elevated in the groups given radioactive gold.

The Effect of Thyroid Hormone on Survival of X-Irradiated Rats

T. T. Odell B. Anderson
F. G. Tausche

Hyperplasia and hypercellularity of the bone marrow are often associated with hyperthyroidism.[17] It seemed possible, therefore, that small amounts of thyroid substance administered to rats following X irradiation (LD_{50}/30 days) might stimulate the bone marrow and thus promote recovery. Consequently, three groups of rats, six in each group, were irradiated with 750 r of X rays, and the following treatments were begun immediately thereafter and continued for 38 days. One group was injected subcutaneously daily (except Sunday) with 0.5 cc of distilled water; the second group was injected subcutaneously with 4 μg of thyroxine per day in 0.5 cc of water; and the third group was fed by stomach tube 1 mg of desiccated thyroid per day in 1 cc volume.

White and red blood cell counts were made on half of the animals daily from the first through fifteenth days, except the third and tenth days, and on the twentieth, twenty-seventh, and thirty-ninth days. Differential counts of the white blood cells and platelet counts were also made at selected times. All extant animals were sacrificed on the thirty-ninth day, and the thyroids taken for sectioning.

No differences were found among the blood cell counts of the various groups. All six irradiated control rats recovered, whereas two of those receiving thyroxine and three of those receiving desiccated thyroid died within 15 days. These mortality rates are not considered to be significantly different in view of the small numbers of animals involved, yet they show that the treatments were not effective in improving survival, and they suggest that the treatments may actually have been deleterious.

A Transmissible Disease of Mice Characterized by Anemia, Leukopenia, Splenomegaly, and Myelosclerosis

A. C. Upton W. D. Gude
F. F. Farbstein

Discovery of this disease has been mentioned in earlier reports.[18] It was first encountered in 1950 in a female RF mouse used as breeder and previously untreated. Since then it has been serially transmitted in more than 11 passages and 30 subpassages (Table 30). The disease is characterized by a slowly progressive anemia, accompanied by erythropoietic splenomegaly and leukopenia. Terminally, there is myelosclerosis, hemosiderosis, and erythrophagocytosis in the reticuloendothelial system, with jaundice. Mice live 2 to 15 months after development of splenomegaly and anemia. The hematologic findings are summarized in Tables 31 and 32.

[17] A. P. Axelrod and L. Berman, *Blood* 6, 436-453 (1951).

[18] A. C. Upton and J. Furth, *Federation Proc.* 11, 430 (1952).

TABLE 30. TRANSMISSION OF DISEASE

Transmitted from mouse no.	Passage number[a]	Age at inoculation (days)	Recipient: Strain RF Injected	Strain RF Positive	Strain AK Injected	Strain AK Positive	Strain RF/AK[b] Injected	Strain RF/AK[b] Positive
RF/J 41	I	210	8	3				
RF/J 74	II C	45	5	5				
		60			7	2		
RF/J 102	III G	50	12	3				
		75			1	1		
RF 159	IV D	60					16	13
RF/AK 88	VI E	11					13	8
		20					6	0
		2	10	5				
RF/AK 105	IX A	14					13	3
		6			11	5		
		3	15	5				
RF/AK 128	XI A	5	12	4				
		5			12	12		
		4					9	9

[a]Roman numerals denote passages and capital letters denote subpassages.
[b]Later generations of hybrids of the RF and AK strains.

TABLE 31. HEMATOLOGIC FINDINGS

Affected mouse no.	Red blood cells ($\times 10^6$)	White blood cells ($\times 10^3$)	Hemoglobin (g %)	Hematocrit (%)	Mean corpuscular volume	Mean corpuscular hemoglobin	Reticulocytes (%)
RF-J 99	4.0	2.1	11.3	39	93	28	32
RF-J 98	3.6	2.3	9.7	39	107	27	28
RF/AK 50	2.4	3.6	4.8	15	61	20	42
RF/AK 77	2.1	5.2	5.9	18	86	28	40
RF/AK 58	1.7	2.4	4.3	14	80	25	21
RF/AK 55	1.7	1.6	4.5	16	92	26	31
Normals (6)	11.1	19.0	15.9	42	38	14	4

The pathogenesis of the disorder is unknown, but the available evidence points toward a viral etiology. Transmission has been routinely accomplished by intraperitoneal injection of involved spleen cells. The agent in splenic tissue survives lyophylization and is present in bacteria-free filtrates (Table 33). Microorganisms have not been found in smears or sections, nor has benefit resulted from treatment with terramycin, aureomycin, chloramphenical, neoarsphenamine, vitamin B_{12} and iron (Lederle's "perihemin liquid"), or splenectomy. New cases have not developed spon-

TABLE 32. DIFFERENTIAL BLOOD COUNTS

Affected mouse no.	Granulocytes			Lymphocytes		Monocytes	Normoblasts	Erythroblasts
	Poly.	Metam.	Eos.	Large	Small			
RF-J 41	28	3	2	15	48	0	3	1
RF-J 99	18	1	0	12	61	2	4	2
RF/AK 22	33	2	1	14	45	1	3	1
RF/AK 50	14	2	1	12	66	0	4	1
RF/AK 55	13	1	0	18	60	1	5	2
RF/AK 83	6	0	0	21	67	2	3	1
Normals (6)	20	1	2	12	63	2	0	0

TABLE 33. RESULTS OF ALL PASSAGES

Material injected	Total no. injected	Total positive	
		No.	%
Intact spleen cells[a]	633	300	47
X-irradiated spleen cells (10,000 r)	9	7	78
Lyophylized spleen cells	34	25	74
Frozen-thawed spleen cells	30	26	87
Centrifuged spleen cells			
Sediment	14	2	14
Supernatant	18	4	22
Filtered supernatant	23	6	26
Filtered supernatant, heated	8	0	0
Saline, glucose, or Tyrode's solutions	28	0	0
Noninjected	78	0	0

[a]The percentage of positive transmissions produced by intact spleen cells appears falsely low in comparison with that produced by other types of inocula because of many variables contained in the numerous passages, data of which are combined.

taneously or in controls caged with experimentally infected mice.

The disease simulates the myeloproliferative disorders of man and affords opportunities for study of the altered growth of hemopoietic derivatives. The natural mode of infection and the pathogenesis of the anemia, splenomegaly, and myelosclerosis remain to be determined.

[19]A. Gorbman, *Proc. Soc. Exptl. Biol. Med.* 66, 212 (1949).

[20]C. F. Winchester, C. L. Comar, and G. K. Davis, *Science* 110, 302-304 (1949).

The Suppression of I^{131} Release by Treatment with Thyroprotein in the Mouse and Thyroxine in the Chick

D. M. Morris T. T. Odell

It has become increasingly popular to thyroidectomize animals by injection of radioiodine instead of removing the thyroid by surgery, since a greater percentage of complete thyroidectomies can be obtained with less effort. However, the use of relatively high levels of radioactivity (200 μc of I^{131} in the mouse[19] and 6 mc per 100 g in the chick[20] entails the possible radiation

damage to other tissues of the body. The protection of personnel from the high levels of radioactivity that may be reached if large numbers of animals are used also becomes a problem, especially when dealing with the chick.

It is desirable therefore to achieve thyroidectomy with smaller levels of radioiodine. Gorbman[21] was able to destroy the thyroid of the mouse with as little as 30 μc of I^{131} by keeping the animals on a "low iodine diet" prior to the injection of radioiodine. Since the loss of iodinated materials from the thyroid is mediated in part by the animal's pituitary, it was thought that inhibition of the pituitary after uptake of iodine should delay the release of iodine from the gland.

Ten male mice (C3H), weighing 19 to 22 g, were injected with about 5 μc of I^{131} in 0.2 ml of saline. Half of these mice received subcutaneous injections of 0.2 ml of physiological saline immediately afterward and daily for the next 7 days, while the others received injections of 0.2 ml of thyroprotein (Parke, Davis & Co., 0.00125 g/ml) according to the same schedule. Immediately after the initial injections and on days 1, 4, 5, 6, 7, and 8, the radioactivity of the thyroid gland was measured by holding the neck region of the mouse over the opening of a well-type scintillation counter. Activity was recorded in counts per minute, and corrections were made for decay, the time of injection being used as zero time. The results are shown in Fig. 27.

Twenty-three 10-day-old White Leghorn cockerels weighing 115 to 130 g were injected subcutaneously with about 1 μc of I^{131} in 0.1 ml of saline. Twelve of them received at the same time, and every day (except the fifth) for 9 days, a subcutaneous injection of 0.2 ml of thyroxine solution (50 μg). The remaining birds received a similar series of injections of saline. On days 1, 4, 7, and 10 after the initial injection three animals of each group (two controls on day 10) were sacrificed, and the thyroids were placed in Bouin's fluid and counted in a well-type scintillation counter. Corrections were made for decay, and the data recorded in counts per minute are shown in Fig. 28.

In both cases the hormonal treatment noticeably delayed release of the injected I^{131} from the thyroid. Seven days after the injection of radioiodine the experimental animals retained about twice as much iodine in the thyroid as the con-

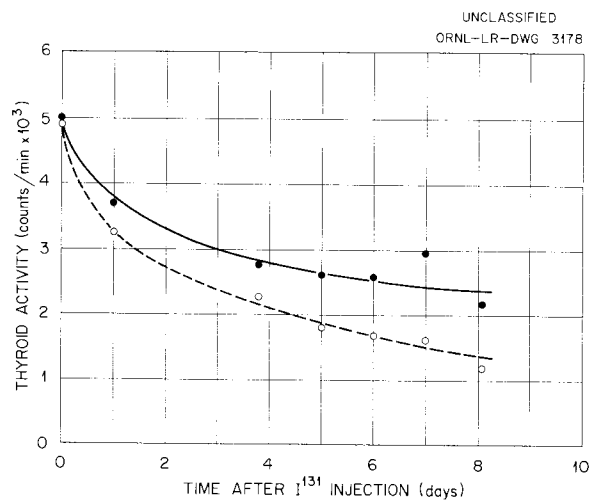

Fig. 27. Effects of Thyroprotein on the Radioactivity of the Thyroid Gland of the Mouse After the Injection of I^{131}. O ----; Saline injected; ●——, thyroprotein injected.

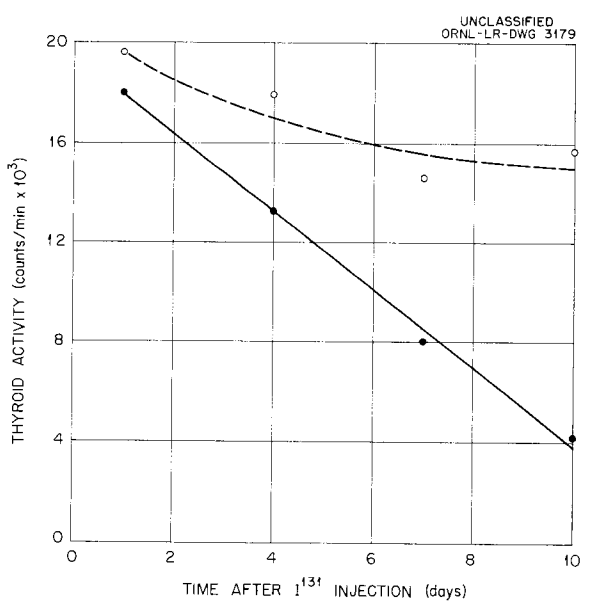

Fig. 28. Effects of Thyroxine on the Radioactivity of the Thyroid Gland of the Chick After the Injection of I^{131}. O ----, Saline injected; ●——, thyroxine injected.

[21] A. Gorbman, *Proc. Soc. Exptl. Biol. Med.* 80, 538-540 (1952).

trols. It is clear that retention of I^{131} at this relatively high level by the use of thyroprotein or thyroxine might make it possible to radiothyroidectomize with much lower levels of activity than have been commonly used. This method, coupled with pretreatment with a low iodine diet, might be particularly advantageous, especially in the chick.

A Comparison of Two Methods for Assay of Thyrotrophic Activity of a Pituitary Tumor

D. M. Morris

Burnett, Anderson, Gadsden, and Furth[22] have studied the thyrotrophic activity of pituitary tumor tissue by several assay procedures, using uptake of I^{131} by C57BL mice as one criterion of activity. This method proved more sensitive than those based on histological thyroid changes, but results were variable. In the present studies this variability was reduced by injecting C57 mice with thyroprotein prior to injection of the tumor.

Mice weighing 24 to 33 g were injected daily for 3 days subcutaneously with either 0.2 ml of thyroprotein solution (Parke, Davis & Co., 0.00125 g/ml) or 0.2 ml of saline. Twenty-four hours after the last injection, tumor extract was injected intraperitoneally, and 24 hr later, a tracer dose (5 to 15 μc) of carrier-free I^{131} was injected intraperitoneally. At sacrifice, 4 hr later, a section of trachea with the thyroid attached was removed and counted in a scintillation counter. Uptake of radioiodine was expressed as percentage of the injected dose.

The results of five experiments in which several different dose levels of thyrotrophin-containing tumor extract were used in saline-injected controls and in mice pretreated with thyroprotein are plotted in Fig. 29.[23]

These curves emphasize three points: (1) The ability of the thyroid to take up injected radioiodine is markedly reduced by pretreatment with thyroprotein under the conditions of these experiments. The average mean value for percentage uptake in all groups of saline-injected controls was 6.29 but that for the thyroprotein-injected controls was 0.66. This reflects suppression of the thyrotrophic activity of the animal's pituitary. (2) The thyroprotein-treated mice showed a significant response to the tumor extract injections, whereas the control series of saline-injected mice showed an erratic, nonsignificant response at all levels tested. The regression coefficient of the curve of the thyroprotein-treated mice is 4.5645, with a standard error of 0.3070; thus the slope differs from zero at a level of significance less than 1%. The regression coefficient of the curve of the control series equals 1.9421, with a standard error of 1.290, so that this slope does not differ

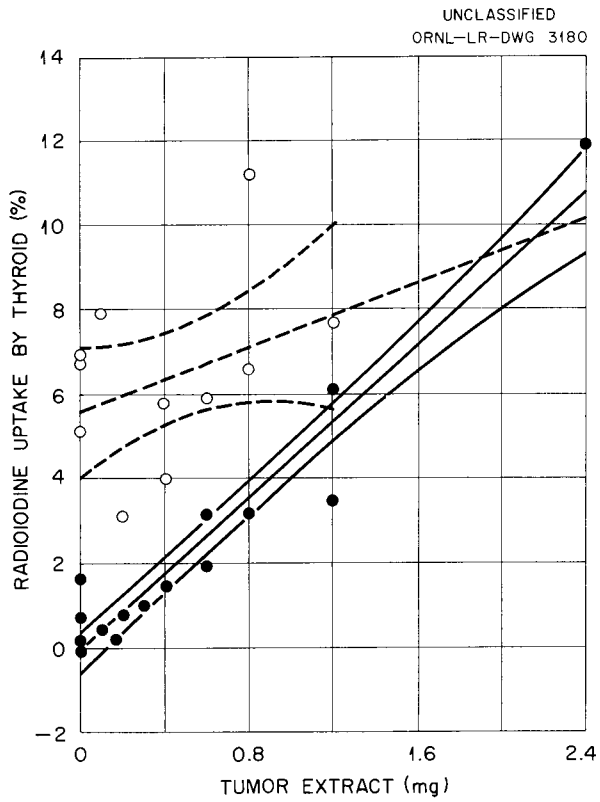

Fig. 29. Effect of Thyroprotein Treatment on Response to Graded Doses of Thyrotrophin-Containing Pituitary Tumor Extract as Shown by Thyroidal Radioiodine Uptake in Mice. O - - - -, Saline injected; ●——, thyroprotein injected. For each series, straight lines having a common slope were fitted by the least-squares method to each experiment. The observed mean uptakes for each experiment were then adjusted to an average intercept and the two series then plotted as in the figure. Confidence limits for each of the two curves have been plotted at the 95% level.

[22] W. T. Burnett, Jr., B. Anderson, E. L. Gadsden, and J. Furth, *Federation Proc.* 13, 21 (1954).

[23] Statistical analyses were done by A. W. Kimball, Mathematics Panel.

significantly from zero. (3) The confidence limits show that there is relatively little variability in the thyroprotein-treated series and marked variability in the saline-injected mice.

Vinisil as a Diluent in Making Bone Marrow Smears

W. D. Gude T. T. Odell

Preparation of satisfactory smears of bone marrow depends on the use of a suitable diluting fluid. Serum or plasma was found satisfactory by Endicott.[24] In this laboratory the plasma expander Vinisil[25] was found to be a suitable substitution for plasma or serum. It is convenient to use this fluid from the bottle as put up by the pharmaceutical company, thereby obviating the necessity of preparing plasma or serum from whole blood. Vinisil has been stored in a refrigerator for several months after opening without any apparent contamination. By mixing small portions of fresh bone marrow with Vinisil either on a glass slide or within a capillary pipette, satisfactory cell suspensions and smears are obtained which can be used for morphological study. Cells are adequately dispersed, and they can be stained readily with any of the standard stains such as Giemsa, Wright's, PAS, and methyl green-pyronin. This method has also been used in the preparation of autoradiograms of isotopically labeled bone marrow cells. Suitable smears can be obtained without excessive clumping, providing clear pictures of single cells actively labeled.

[24] K. M. Endicott, *Stain Technol.* 20, 25 (1945).

[25] The Vinisil (Abbott), a 3.5% solution of polyvinylpyrrolidone (PVP) in saline, was kindly supplied by Dr. A. E. Osterberg of Abbott Laboratories, Research Division, North Chicago, Illinois.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson
E. F. Phares
M. I. Dolin
Mary V. Long
Helen Sherwood
R. E. Koeppe[1]

DPNH-Oxidizing Enzymes of *Streptococcus faecalis*

M. I. Dolin

It is generally assumed that the respiration of streptococci passes through flavoprotein terminal oxidases, since the organisms contain flavins and hematin catalysts have not been found in members of this group. In addition, peroxide, which is a product of flavoprotein respiration, is sometimes but not always demonstrable following respiratory activity. The information that exists is based on studies of growing cultures or cell suspensions. This investigation was undertaken in an attempt to clarify the mechanism of hydrogen transport in streptococci by describing the reactions as catalyzed by well-defined enzyme systems. It was also felt that some light might be thrown on the general problem of the significance of flavoprotein respiration. Previous work from this laboratory[2,3] has demonstrated the existence in cell-free extracts of *Streptococcus faecalis*, strain 10C1, of a potent reduced diphosphopyridine nucleotide (DPNH) oxidase, cytochrome c reductase, and a cyanide- and azide-resistant DPNH peroxidase. The last enzyme is responsible for the absence of peroxide during DPNH oxidation by this organism and it was suggested that it also accounted for the peroxidations carried out by cell suspensions of other strains of streptococci[4,5] when oxidizable substrates are furnished.

Further work with the *S. faecalis* system indicated the existence of the following series of DPNH oxidases in essentially anaerobically grown cells:

By the use of ammonium sulfate and calcium phosphate gel procedures, coupled with zone electrophoresis on starch columns, it has been possible to demonstrate that reactions 1 to 4 and 6 are each catalyzed by separate enzymes. Attempts have not been made to separate completely the enzymes for reactions 1 and 3, although the fractionation properties, stability to storage, and sensitivity to ultraviolet irradiation suggest strongly that these activities are catalyzed by different enzymes. The relation of reaction 5, previously found in extracts of *Clostridium kluyveri*[7] to the other reactions is not known. It is not, however, a property of the purified peroxidase. This reaction has not been studied in detail in *S. faecalis*. It has been possible to show the flavoprotein nature of the enzymes for reactions 1 to 4 and 6 by employing cell-free extracts prepared from cells of *S. faecalis* that were grown in riboflavin-deficient synthetic medium. With such preparations, it can be demonstrated that reactions 1 and 3 require flavinadenine dinucleotide (FAD) whereas reactions 4 and 6 require either FAD or flavin mononucleotide (FMN). DPNH peroxidase, the enzyme-catalyzing reaction 2, has been purified over one hundredfold. It appears to be the first example of a well-defined peroxidase which is

[1] Research Participant.

[2] M. I. Dolin, *Arch. Biochem. and Biophys.* **46**, 483-485 (1953).

[3] M. I. Dolin, *Federation Proc.* **13**, 200 (1954).

[4] E. C. Greisen and I. C. Gunsalus, *J. Bacteriol.* **45**, 16-17 (1943).

[5] H. W. Seeley and P. J. Vandemark, *J. Bacteriol.* **61**, 27-35 (1951).

[6] 2,6-Dichlorophenol indophenol.

[7] M. M. Weber and N. O. Kaplan, *Bacteriol. Proc.* (1954), p 96.

$$\text{DPNH} + H^+ + O_2 \longrightarrow \text{DPN}^+ + H_2O_2 \quad (1)$$

$$\text{DPNH} + H^+ + H_2O_2 \longrightarrow \text{DPN}^+ + 2H_2O \quad (2)$$

$$\text{DPNH} + 2\ \text{cytochrome c}\ (Fe^{+++}) \longrightarrow \text{DPN}^+ + 2\ \text{cytochrome c}\ (Fe^{++}) + H^+ \quad (3)$$

$$\text{DPNH} + H^+ + \text{2,6-di-Cl-PIP}^6 \longrightarrow \text{DPN}^+ + \text{2,6-di-Cl-PIP}\cdot H_2 \quad (4)$$

$$\text{DPNH} + 2\ Fe\ (CN)_6 \longrightarrow \text{DPN}^+ + 2\ Fe\ (CN)_6 + H^+$$

$$\text{DPNH} + 2\ Fe^{++} \longrightarrow \text{DPN} + 2\ Fe^{++} + H^+ \quad (5)$$

$$\text{DPNH} + \text{menadione} \longrightarrow \text{DPN} + \text{reduced menadione} \quad (6)$$

not the typical hematin enzyme of plant and animal tissue. Present evidence suggests that it is a complex flavoprotein. It has been possible to reactivate vacuum-dried flavin-deficient cells with FAD. Cell-free extracts cannot be activated even with hot water extracts of active cells. These findings suggest that vacuum-dried preparations contain some component or synthetic system capable of forming the true coenzyme in the presence of FAD. The peroxidase is not inhibited by iron inhibitors, does not use typical substrates for hematin peroxidases, and actually increases in amount when cells are grown in the absence of any detectable inorganic or hematin iron. Furthermore, in the intact organism there are no spectroscopically demonstrable hematin components. In order to establish definitely the nature of the coenzyme, it will be necessary to prepare what can be characterized as a pure enzyme. Preliminary experiments indicate that the use of zone electrophoresis on starch blocks may make this purification possible. [See Fig. 30 for a typical separation of the peroxidase (fortyfold purified before electrophoresis) from the enzyme-catalyzing reaction 4.] The purified peroxidase utilizes ferricyanide and 2,6-dichlorophenol indophenol to only a very limited extent. The purified ferricyanide and indophenol enzyme does not utilize peroxide as an oxidant.

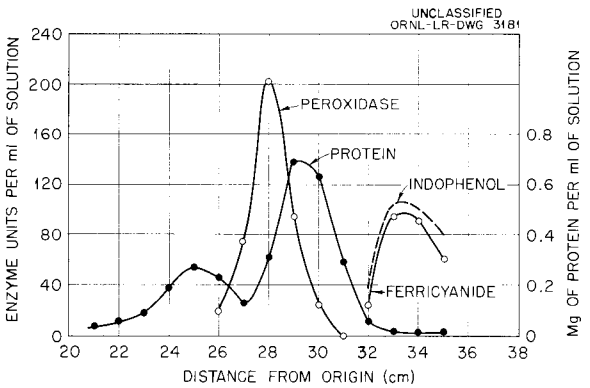

Fig. 30. The Separation of DPNH Peroxidase from the Indophenol and Ferricyanide Enzyme by the Use of Zone Electrophoresis on Starch Columns. One enzyme unit – Δ 0.01 density unites per min at 340 mμ in 3-ml Beckman cells with a 1-cm light path.

The demonstration of reactions 1 to 4 and 6 is shown in Figs. 31 to 34 and in Table 34. The table also illustrates the extent of the flavin deficiency that can be consistently produced in the growth experiments mentioned.

The menadione reductase and peroxidase (reactions 6 and 2) are the most interesting of the DPNH enzymes found so far in *S. faecalis*. Menadione may be entering into this reaction as a model of vitamin K. Actually, menadione itself has high vitamin K activity. The demonstration that menadione reductase is a flavoprotein suggests a biological function for the vitamin. It may be a physiologically important link in electron transport, functioning as the oxidant of reduced flavoprotein. As for the peroxidase, the presence of this enzyme in streptococci offers these organisms a competitive advantage over strict anaerobes. It enables the organisms to utilize oxygen as an

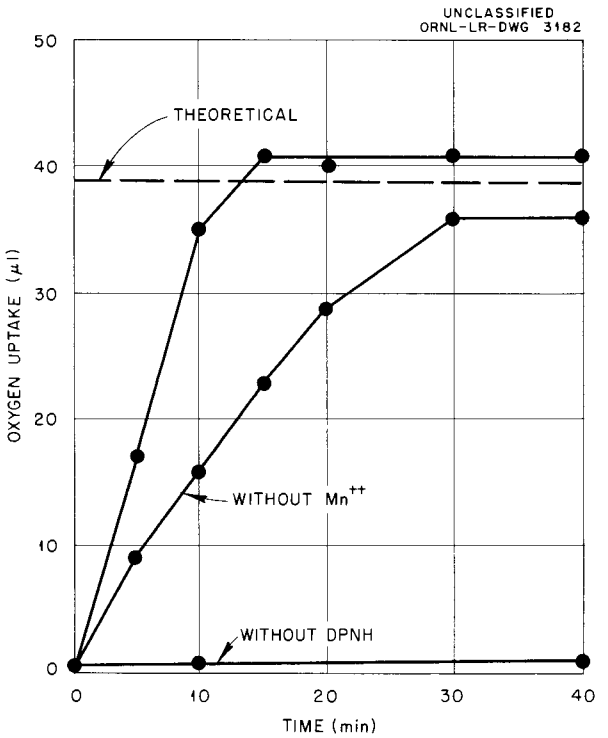

Fig. 31. Aerobic Oxidation of DPNH by Cell-Free Extract of *S. faecalis*. Potassium phosphate buffer, pH 6.0, 200 μmoles; DPNH, 3.44 μmoles; enzyme, crude cell-free extract, 3.7 mg protein; $MnSO_4$, 2 μmoles where used; H_2O to 2 ml; temperature, 37°C.

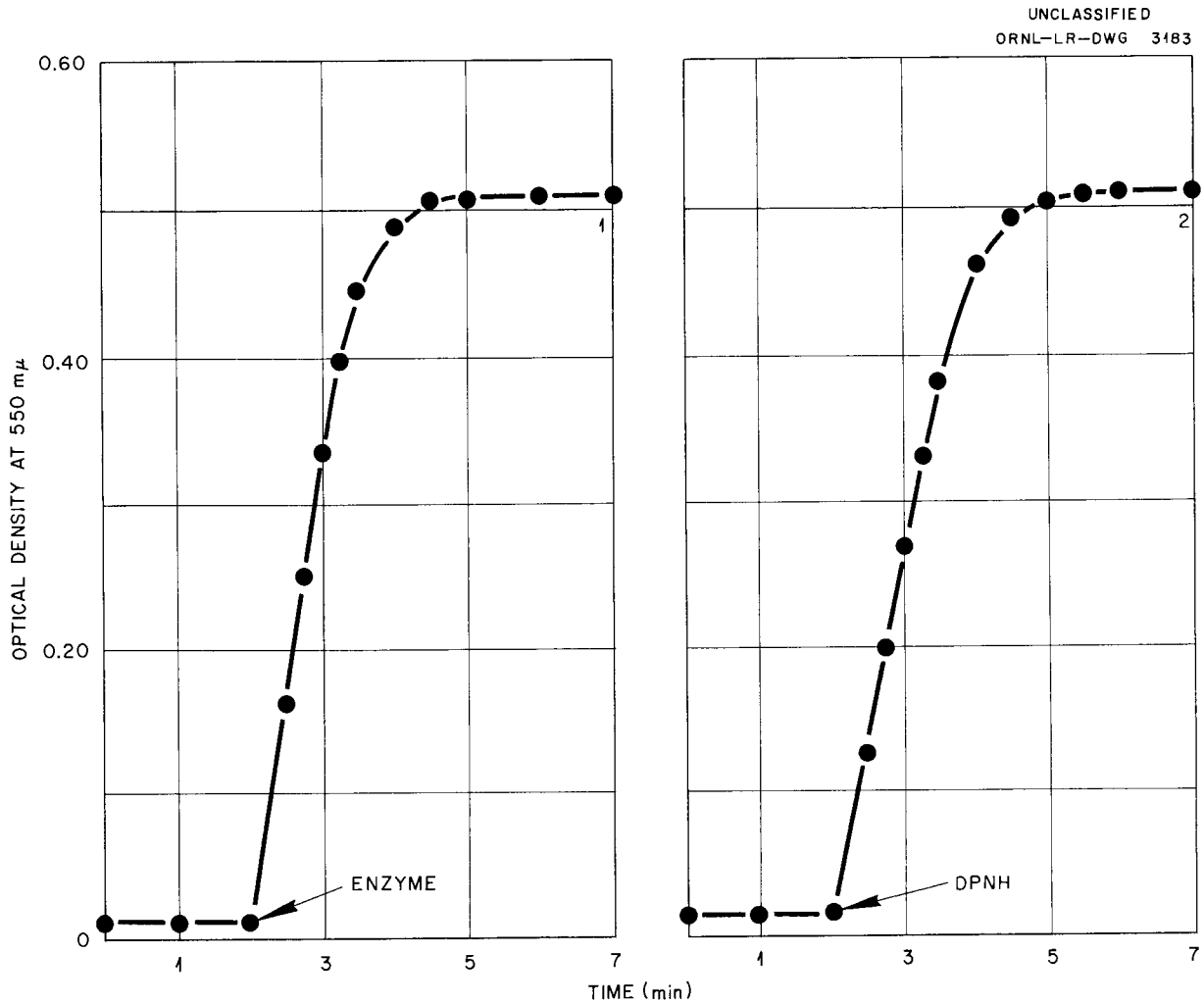

Fig. 32. Cytochrome c Reductase of *S. faecalis*. Tris(hydroxymethyl)aminomethane buffer, 0.5 *M*, pH 8.0, 0.2 ml; DPNH, 0.25 μmole; cytochrome c, 2.97×10^{-4} *M*, 0.3 ml; enzyme, crude extract, 0.26 mg of protein; H_2O to 3 ml; temperature, 23°C. The blank cuvette contained all additions except the starting reagent. Curve 1, reaction started with enzyme; curve 2, reaction started with DPNH.

alternative hydrogen acceptor without accumulating peroxide, even though the terminal oxidases are flavoproteins. Aerobically, pyruvate, the hydrogen acceptor in the lactic acid fermentation, is removed from its role as the obligate terminal hydrogen acceptor, and is then capable of being utilized oxidatively in a reaction which yields one substrate linked phosphorylation via acetyl-coenzyme A. Direct growth experiments with *S. faecalis* 10C1 show that, aerobically, there is a 40% increase in cell yield, compared to the yield obtained under anaerobic conditions. These growth experiments reveal the physiological importance of DPNH peroxidase.

Succinate Decarboxylase System

S. F. Carson E. F. Phares

The various functions of crude enzyme juices prepared from *Propionibacterium* and *Veillonella* cultures were described earlier.[8] It was demonstrated that the complete succinate decarboxylase system could be reconstituted by a combination of

[8] *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693.

the two juices. In addition it was shown that: (1) propionic enzyme juice specially prepared from *Propionibacterium* cells contained succinate-activating and -decarboxylating enzyme systems only; (2) specially prepared *Veillonella* enzyme

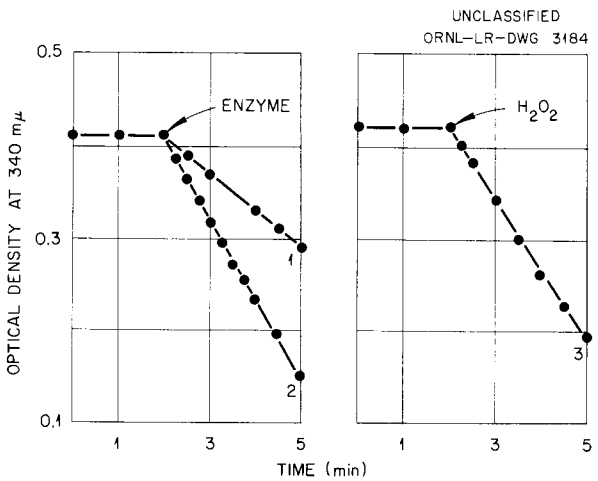

Fig. 33. DPNH Oxidation with H_2O_2 as Oxidant; Partially Purified DPNH Peroxidase of *S. faecalis*. Sodium acetate buffer, 0.1 *M*, pH 5.4, 1 ml; DPNH, 0.2 μmole; H_2O_2, 4 μmoles; enzyme is gel eluate at pH 7.5 containing 0.23 mg of protein per ml; H_2O to 3 ml; temperature, 23°C. Curve 1, 9.2 μg of enzyme protein; curves 2 and 3, 23 μg of enzyme protein.

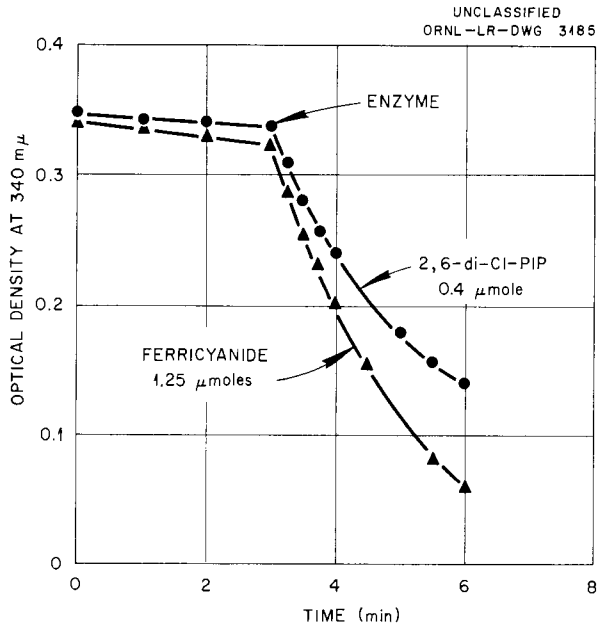

Fig. 34. Partially Purified DPNH Peroxidase; 2,6-Dichlorophenol Indophenol and Ferricyanide as Oxidants. Potassium phosphate buffer, pH 7.0, 200 μmoles; DPNH, 0.17 μmole; 2,6-dichlorophenol indophenol and ferricyanide as shown; enzyme, 23 μg of preparation used in Fig. 33; H_2O to 3 ml; temperature, 23°C. The data are corrected for dilution by the starting reagent and for the absorption of 2,6-dichlorophenol indophenol and ferricyanide at 340 mμ.

TABLE 34. FLAVIN REQUIREMENT OF MENADIONE REDUCTASE IN *STREPTOCOCCUS FAECALIS*, 10C1

Additions (μmole)	μmoles of DPNH oxidized/mg of enzyme protein/hr
Flavin-deficient extract, *S. faecalis*	0.88
Flavin + FAD, 6×10^{-4}	11.7
Flavin + FAD, 3×10^{-3}	25.6
Flavin + FAD, 6×10^{-3}	26.0
Flavin + FMN, 6×10^{-5}	12.9
Flavin + FMN, 6×10^{-4}	25.6
Flavin + FMN, 1.2×10^{-3}	26.3
FAD or FMN, 6×10^{-3}, w/o menadione	0
FAD or FMN, 6×10^{-3}, w/o enzyme	0

Tris(hydroxymethyl)aminomethane buffer, 0.5 *M*, pH 8.0, 0.2 ml; DPNH, 0.25 μmole; menadione, 0.3 μmole; FAD and FMN as shown; enzyme (see text) 90 μg of protein; water to 3 ml; temperature 23°C. DPNH oxidation was followed spectrophotometrically at 340 mμ in the Beckman spectrophotometer with 3-ml cuvetts with a 1-cm light path. Results were converted into DPNH concentration by means of the molecular extinction coefficient, 6.22×10^6 cm^2/mole.

juice contained the enzyme system which converts the C_1 fragment to CO_2, but neither of the two systems mentioned under (1).

Work has continued on the purification of the enzymes from these fractions by a number of different methods.

Fractionation Methods. Ammonium sulfate fractionation yielded increases in specific (enzyme) activity of three- to fourfold with only 10% over-all recovery of initial activity. Data for a typical ammonium sulfate fractionation of propionic juice are given in Table 35. In Table 36 data are given from a refractionation of the 30 to 55% ammonium sulfate fraction. In this latter fractionation, a total of 53% of the starting activity (of the 30 to 55% fraction) was recovered, and which amounted to only 8% of the total original activity present in the juice.

TABLE 35. AMMONIUM SULFATE FRACTIONATION OF PROPIONIC JUICE[a]

Percentage $(NH_4)_2SO_4$	Percentage recovery in fractionation	Specific activity[b]
0–30	8	0.4
30–55	60	2.3
55–100	32	3.6

[a]Protamine treated to remove nucleic acids; of the total activity originally present in the juice, 41% remained in the protamine supernatant, and 17% remained in the combined $(NH_4)_2SO_4$ supernatants.

[b]μl of CO_2 produced in 10 min; original juice = 3.3.

TABLE 36. REFRACTIONATION OF THE 30 TO 55% AMMONIUM SULFATE FRACTION

Percentage $(NH_4)_2SO_4$	Specific activity
30–37	0.2
37–45	2.4
45–55	3.1

Ammonium sulfate fractionations were also carried out in connection with the enzyme system which converts the C_1 fragment to CO_2. Table 37 contains data from a typical ammonium sulfate fractionation of *Veillonella* juice. A freshly prepared *Veillonella* juice was used for this fractionation (after such a juice has been stored at –30°C for 2 weeks, the only remaining activity is in the particle fraction).

TABLE 37. AMMONIUM SULFATE FRACTIONATION OF *VEILLONELLA* JUICE[a]

Percentage $(NH_4)_2SO_4$	Percentage recovery in fraction	Specific activity[b]
0–16	17	42
16–25	17	38
25–32	12	40
32–41	11	18
41–50	4	6
50–100	4	5

[a]Protamine treated; the protamine supernatant contained 72% of the initial activity.

[b]μl of CO_2 produced in 10 min; original specific activity = 13.3.

Other methods of fractionation have been tried. Solvent as well as gel fractionation procedures were not so good as ammonium sulfate. Solvent fractionation with acetone at –14°C completely denatured the *Propionibacterium* enzymes. Table 38 presents data from a calcium phosphate gel fractionation of propionic juice. Elution of the gel fractions with buffer yielded little protein or enzyme activity; elution with salt yielded much protein, but little enzyme activity.

TABLE 38. GEL FRACTIONATION OF PROPIONIC JUICE

Amount of gel added (ml)	Total protein (mg)	Specific activity[a]	Total activity[a]
0	648	2.1	1430
8	490	3.0	1440
13	413	3.3	1360
18	212	4.0	800
23	163	0.0	0

[a]μl of CO_2 produced in 10 minutes.

Starch Electrophoretic Separation. Although the ammonium sulfate fractionation method raised the specific enzyme activity, the over-all recoveries were too low. Therefore, the recently developed technique of starch electrophoresis has been investigated. The apparatus constructed consists of a 1000-volt DC regulated power supply, and

Tiselius type platinum electrodes connected to the ends of a potato starch column which has been equilibrated with a buffer. Crude cell extracts are dialyzed against buffer and placed near the cathode end of the column. The column is maintained at 5°C, while a 400- to 600-volt potential is applied through the column for 4 to 20 hours. At the end of this time, starch sections along the length of the column are cut out, protein eluted and prepared for testing of various enzyme activities.

In Fig. 35 data are presented from a typical starch electrophoresis separation carried out on a freshly prepared propionic juice. The juice was treated in the Spinco centrifuge at 100,000 *g* for 1 hr, and the supernatant was dialyzed at 0°C for 3.5 hr against 0.03 *M* phosphate buffer at pH 6.5. A large starch column, measuring 48 × 10 × 2 cm was used for this separation. Four hundred and eighty volts at 40 ma was applied for 15.5 hr; the starch column was maintained at 4°C.

The enzyme activities shown in Fig. 35 represent a more than 75% recovery of the original starting activity. Hence this is certainly the method of choice for continuation of separation and purification of the enzymes concerned with the succinic decarboxylase system.

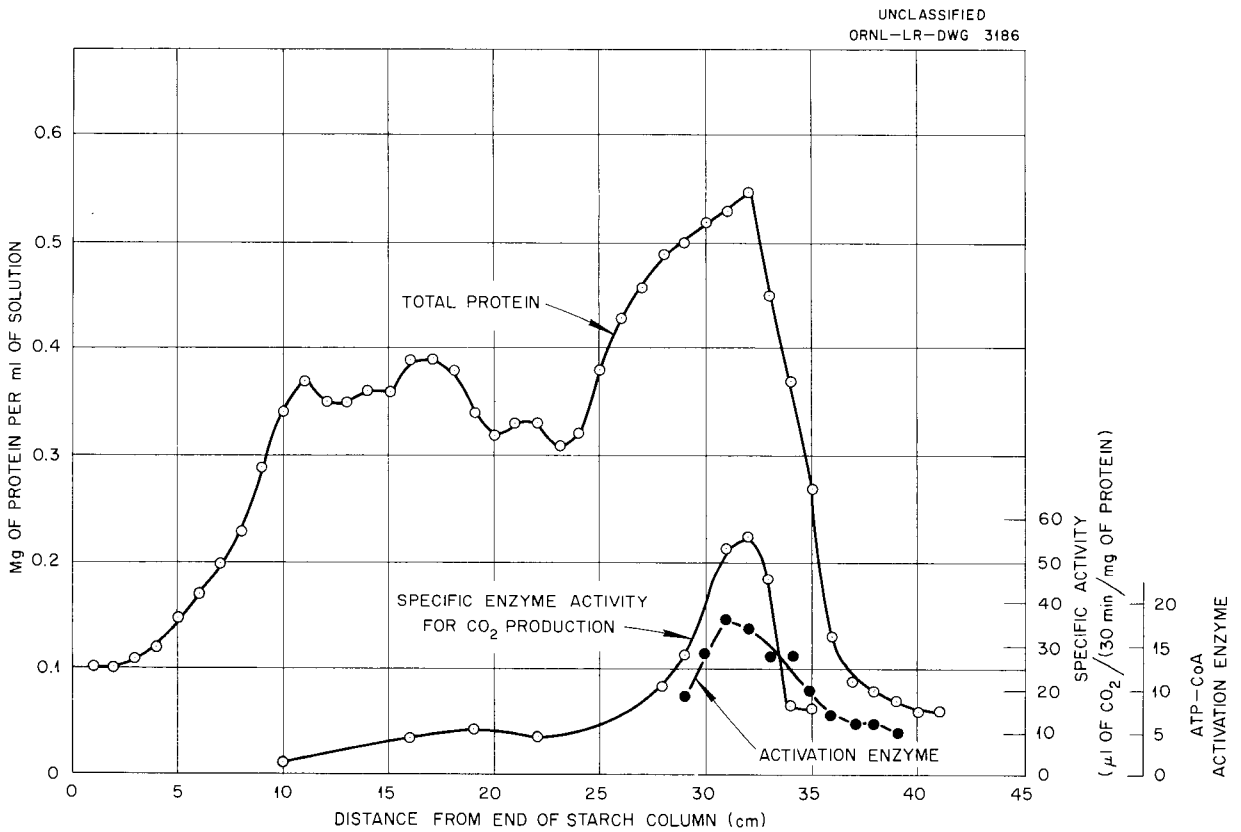

Fig. 35. Starch Electrophoresis of Succinate Enzymes.

BIOCHEMISTRY

STUDIES ON NUCLEIC ACIDS AND ENZYMES

W. E. Cohn E. Eavenson
D. G. Doherty M. H. Jones
J. X. Khym A. B. Ottinger
E. Volkin

Nucleic Acid Structure

W. E. Cohn J. X. Khym

The recent revision in our concept of the structure of ribonucleic acids (RNA) to a 3′,5′ polyribose phosphate instead of a 2′,3′ structure as originally postulated 20 years ago was brought about almost exclusively by the isolation of 5′ nucleotides from diesterase-degraded RNA and of 3′,5′-linked di- and trinucleotides from ribonuclease-degraded RNA. The ribonuclease products, in turn, owe a major share of their characterization to the same diesterase degradation which turned up the 5′ nucleotides. In connection with these studies, and as a means of determining chain length by end-group determination, phosphatases attacking only singly linked phosphate groups have been employed (prostatic, bone, barley, and venom phosphomonoesterases). Yet with respect to the action of both the diesterase and the monoesterase preparations on reasonably "native" RNA, questions arise as to the possibility of multiple enzymes or of multiple action, in particular, of a ribonuclease type of degradation giving rise to the ribonuclease end products or intermediate (cyclic) products which are then further degraded to give the products associated with these other preparations. In addition, there is the question of enzymatic resynthesis during the degradation, giving rise to an association of fragments which were not associated in the original polymer. Finally, questions have been raised as to the "native" character of the RNA used and of the possibility of a contamination of nuclease in it.

The problem of enzyme homogeneity and of mode of action can be approached by more detailed study of the time course or degradation and by the isolation of transient intermediates, as has been done for ribonuclease action by Markham and Smith. One method of isolating intermediates is by dialysis. Preliminary experiments on dialysis have been carried out with results that are difficult to interpret, in that a presumably high-molecular-weight RNA preparation (under sterile conditions) almost completely diffuses through Visking casing under the influence of dilute salt. The dialyzate seems to be a mixture of polynucleotides of various sizes, such as are met with in the ribonuclease digest, giving rise to the thought that the RNA, as isolated, contains nucleolytic enzymes as impurities. It is proposed to examine this possibility more closely and to develop ultrafiltration techniques in order to speed up the rate of separation of products from parent and enzyme molecules. Once a proper "control" is achieved (i.e., a nondialyzable, stable RNA), the search for the intermediates produced in enzyme degradation can begin.

Glycerol Phosphates (J. X. Khym, A. B. Ottinger). In the concluding phases of the work on the characterization of adenosine-2- and -3-phosphates,[1] two problems of new interest arose involving (1) the periodate oxidation of ribose phosphates and of ribitol phosphates, and (2) the concept of hydrogen bonding as a factor in the separation of phosphate esters on strong-base anion exchangers.

During the period when ribose-2- and -3-phosphates were being characterized little attention was given to periodate oxidation as a means for identifying structure because, at a first assumption, it would be expected that ribose-2- and ribose-3- as well as ribitol-2- and ribitol-3-phosphates would each reduce 2 moles of periodate. However, attention was attracted to a paper by Courtais and Ramet[2] wherein data were presented to show that a hexose esterified in either the 2, 3, or 4 position with phosphate upon oxidation with periodate would give the same dialdehyde phosphate compound, $OHC-CH(OPO_3H_2)-CHO$. The formation of this compound requires the reduction of 3 moles of periodate, and once the dialdehyde is formed, it reacts further to consume 4 additional moles of periodate. Thus for a 2,3,4 hexose phosphate, 7 moles of periodate would be required. On this basis it would be expected that ribose-2- and ribose-3-phosphates would react with 6 moles of periodate as well as ribitol-3-phosphate (produced directly from ribose-3-phosphate) since all of these compounds form the 3-carbon dialdehyde phosphate.

[1] W. E. Cohn and J. X. Khym, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, p 59.

[2] J. Courtais and M. Ramet, *Bull. Soc. Chim. Biol.* 27 610 (1945).

Inasmuch as ribitol-2-phosphate (obtained directly from ribose-2-phosphate) does not form a dialdehyde phosphate compound upon periodate oxidation, only 2 moles of periodate should be consumed. Periodate oxidations were carried out on the ribose compounds mentioned as well as ribose-5-phosphate and adenosine-5-phosphate, and data showing the results obtained are presented in Table 39.

TABLE 39. ACTION OF PERIODATE ON RIBOSE COMPOUNDS

Compound	Moles of periodate reacted per mole of compound	
	Calculated	Determined
Ribose-2-phosphate	6.0	5.9
Ribose-3-phosphate	6.0	6.3
Ribose-5-phosphate	3.0	2.9
Adenosine-5-phosphate	1.0	1.2
Ribitol-2-phosphate	2.0	2.3
Ribitol-3-phosphate	6.0	6.2

These studies offer further proof that the phosphate groups in ribose-2- and -3-phosphates were correctly assigned.

The concept of hydrogen bonding as a factor in the separation of the pentose phosphates has been fully described.[3] An extension of these studies has resulted in the separation of α- and β-glycerophosphates on anion-exchange resins. These isomers were separated in either acetate or chloride systems throughout the pH range 4 to 8. Owing to intramolecular hydrogen bonding, β-glycerophosphate is a stronger acid than its α isomer and should be eluted last from a column. This was the sequence found experimentally. The identity of each peak was determined by running pure α and β isomers separately through identical columns and by periodate oxidation studies of material eluted from the columns, since only the α isomer reacts with periodate.

Studies on Enzyme-Substrate Binding

D. G. Doherty E. Eavenson

The preceding semiannual report[4] described in detail the hypotheses that led to the discovery of nonoxidative carbon-carbon bond cleavage by the proteolytic enzyme, α-chymotrypsin. It is important that such a system be evaluated in terms of the usual kinetic constants and that some experimental evidence be obtained that such a splitting is effected by the same catalytic center that is responsible for the normal hydrolysis of amides and esters. The determination of the activity-versus-pH curve for α-chymotrypsin-ethyl-3-keto-5-(p-hydroxyphenyl) valerate revealed that maximum activity was obtained at pH 7.5 to 7.8 with a rapid decline on either side of that pH range. This pH response is similar to that for amide and ester hydrolysis and may indicate that the same groups on the enzyme surface are ionized to bind the substrate in both cases. The kinetic constants obtained from a series of rate determinations at various substrate concentrations were $K_s = 0.12\ M$ and $k_3 = 3.5 \times 10^{-3}$ moles/min/mg of protein N/milliliter. When competitive inhibitors for amidase activity were added, the rate of carbon-carbon bond hydrolysis was decreased; however, solubility difficulties precluded accurate measurement of K_I. Thus the evidence at hand seems to indicate that the same active center on the enzyme is responsible for both amidase and carbon-carbon bond activity.

Structure and Activity in Radiation-Protective Sulfhydryl Compounds

D. G. Doherty C. O. Doudney[6]
W. T. Burnett[5] E. Eavenson

The protection against radiation injury in mice afforded by β-mercaptoethylamine has been previously studied principally by Bacq et al., Straub and Patt, Rugh and Wang, and Langendorf; still, little is known about its mechanism of action. In addition, the instability, toxicity, and therapeutic index of this compound are not all that might be desired for a useful radiation-protective agent. Systematic chemical modification of β-mercaptoethylamine, possibly leading to a potentially useful compound and shedding some light on its action and the structure essential for protective effect, has been initiated as a cooperative project with W. T.

[3] J. X. Khym, D. G. Doherty, and W. E. Cohn, *J. Am. Chem. Soc.* 76, (1954) (in press).

[4] D. G. Doherty, L. A. Thomas, and E. Eavenson, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, pp 60-61.

[5] Enzymology Group.

[6] Radiation Protection Section.

Burnett, Jr. of the Enzymology Group and C. O. Doudney of the Radiation Protection Group. All compounds prepared in this study will be screened for protective action in both mice and bacteria and possible differing pathways of metabolism of protective compounds will be examined.

The synthetics program was initiated with the preparation of certain acetylated β-mercaptoethylamine derivatives and a series of mono- and diisothiouronium compounds. The effects of these compounds on bacterial recovery were presented by the Radiation Protection Group. The animal-testing program was carried out according to methods previously described by Burnett et al.[7] The 28-day LD_{50} of X rays of 8- to 10-week-old male mice of the C57BL strain and 12- to 14-week-old male mice of the 101 × C3H strain was determined. One derivative, S,β-aminoethylisothiouronium bromide HBr (250–450 mg/kg of body weight) given by intraperitoneal injection 10 to 15 min prior to total-body X irradiation raised the LD_{50} for the C57BL strain from ~600 to ~900 r and 101 × C3H strain from ~750 to ~1150 r. Similar results were obtained with β-mercaptoethylamine (250 mg/kg) confirming in this Laboratory the work of other investigators. Other derivatives (250 mg/kg) which did not decrease the radiation-induced mortality but enhanced the survival time include N-acetyl and N,S-diacetyl-β-mercaptoethylamine, bis(β-aminoethyl) disulfide and S,S-ethylenediisothiouronium dibromide. Little or no protection was furnished by β-carboxyethylisothiouronium bromide, S,S-methylenediisothiouronium dibromide, S,S-ethyldiisothiouronium dibromide, and 2-mercaptothiazoline. These preliminary results seem to indicate the essential nature of the free amino group for protective activity in β-mercaptoethylamine and its derivatives. It may well be that the structure of β-mercaptoethylamine is unique among protective agents having sulfhydryl groups. A preliminary report of this work will be presented at the American Chemical Society Meeting next month in New York.

Nucleic Acid Enzymology

E. Volkin M. H. Jones

Experiments have been described which indicated that bacteriophage $T4r^+$ contains a protein which is different from the so-called ghost or membrane protein and that this protein remains associated with the infected bacterium along with the deoxynucleic acid (DNA) of the phage.[8] The evidence consisted of analyses of the two proteins and a demonstration that labeled amino acids of the phage could be found in the bacterium. Since these conclusions are at variance with those previously advanced by Hershey,[9] other experimental approaches have been sought, and the ultracentrifuge is now being used to characterize the new component.

When the bacteriophage is lysed by osmotic shock and centrifuged at 80,000 *g*, the ghosts are completely centrifuged down in less than 10 minutes. The only other component to be seen is a single hypersharp peak corresponding in appearance and in sedimentation rate to DNA itself. If the suspension is treated with deoxyribonuclease (DNAse) before being centrifuged, one additional component is observed. This additional component sediments at a faster rate than the DNA itself but at a much slower rate than the ghosts. This additional peak is also observed if the ghosts are first removed from the lysed phage before the DNAse treatment. This eliminates the ghosts as a source of the new component. Similarly, highly purified $T4r^+$ DNA, completely free of protein, does not give rise to the new peak upon treatment with DNAse.

Enough of the new component for analysis can be prepared by DNAse treatment of an osmotically lysed suspension from which the ghosts have been removed at 5000 *g*. The new component is separated by centrifugation at 80,000 *g* for 45 minutes. Its spectral properties, phosphorus and nitrogen contents, and its biuret and ninhydrin reactions indicate it to be almost exclusively protein in nature. In the analytical ultracentrifuge, it appears as one major component, comprising 80% of the total material, with at least two smaller components. It is estimated to comprise 20 to 25% of the total phage protein. Its sedimentation rate is such that it probably would have been removed with the ghosts in Hershey's experiments with DNAse-treated lysed suspensions.

If the phage is allowed to adsorb on bacterial

[7] W. T. Burnett, Jr., A. W. Burke, Jr., and A. C. Upton, *Am. J. Physiol.* 174, 254-258 (1953).

[8] E. Volkin and M. H. Jones, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, pp 61-62.

[9] A. D. Hershey, *Cold Spring Harbor Symposia Quant. Biol.* 18, 135-139 (1953).

membranes (*Escherichia coli*), the components liberated into the supernatant are exactly comparable to those observed from osmotically lysed phage, both before and after the DNAse treatment. This experiment makes it quite probable that the new protein component enters the bacterium in normal infection along with the DNA.

It is planned further to characterize this protein and to endeavor to ascertain its mode of association with the DNA.

PLANT BIOCHEMISTRY

N. E. Tolbert F. B. Gailey[1] P. C. Kerr
A. R. Krall L. P. Zill B. L. McClanahan[2]

UNKNOWN COMPOUNDS IN PHOSPHORUS TRANSPORT IN PLANT XYLEM SAP

N. E. Tolbert H. Wiebe[3]
P. C. Kerr

Numerous investigations since the advent of radioactive phosphorus and sulfur have determined the rate and conditions for the movement of these two essential minerals from the soil, through the roots to the leaves. The work has involved detection of the radioactivity as a measure of phosphorus or sulfur movement. Previously it has been assumed that inorganic phosphate and sulfur were the forms in which these elements moved in the upward-flowing xylem sap of the plant stem.

This investigation was started to determine the form of phosphorus and sulfur transport from the roots to the leaves of higher plants. The results indicate that though inorganic phosphate is a major form of phosphorus being transported, there are in the xylem sap two organic phosphorus esters which in certain plants may account for as much as 20% of the transported phosphorus. Inorganic sulfate appears to be the only form of sulfur being transported.

Several species of plants have been used which were known to be capable of producing exudate (bleeding) when their stems or leaf bases were cut. Plants were grown in sand or soil in the greenhouse or in Hoagland nutrient solution in a constant-environment room of about 100 ft-c of light with a temperature of 75° to 77°F. The sand or soil was carefully washed from plants grown in these media and placed in 0.1 strength Hoagland nutrient solution or water before use. Radioactive phosphate or sulfate was placed in the water or nutrient solution and at the same time or later the plant tops were removed near the base with a sharp razor blade. As the exudate appeared on the cut stump it was removed by touching it to the origin point of a paper chromatogram. Thus for a given plant, the exudate could be transferred quantitatively, and this made it possible easily to carry out time-course studies.

Chromatograms were run on unwashed Whatman #1 or 3MM paper. They were developed in the first direction with the solvent phenol and water and in the second direction with butanol-propionic acid-water. After development, the chromatograms were briefly exposed (3 to 7 days) to no-screen X-ray film to establish the exact location of the P^{32}. These spots were cut out of the chromatogram sheet and the amount of radioactivity was determined by counting each spot.

Chromatograms of the exudate from about 45-day-old tomato plants and from young barley seedlings contained three radioactive phosphorus spots with the R_f characteristics shown in Table 40. The unknowns have also been observed in the exudate from cut-off sunflower and Black Valentine beans.

TABLE 40. R_f OF UNKNOWN COMPOUNDS

Compound	R_f in phenol-water	R_f in butanol-propionic acid-water
PO_4^{---}	0.25	0.25
Unknown (U_1)	0.9	0.3
Unknown (U_2)	0.35	0.2

The discovery of only three phosphorus-containing compounds in the exudate was somewhat surprising since the phosphorus was not all inorganic phosphorus nor was there the multiplicity of known organophosphorus compounds associated with respiration which may be found in whole tissue. Further experiments were run to determine how general a distribution these two unknown compounds might have. They have been found in large amounts in roots and leaves, and the results indicate that they may be important in growth as well as phosphorus transport. Thus it is especially important specifically to identify them and to inquire into the nature of their synthesis by the plant. A number of experiments have been designed with these ends in view, and the first of these are reported at this time.

[1]Research Participant.
[2]Temporary Employee.
[3]UT-AEC Agricultural Program.

The distribution of radiophosphorus between U_1 and U_2 (unknown 1 and unknown 2) in the exudate as compared to the P^{32} in inorganic phosphate depended on the time at which the exudate was collected following application of the P^{32} phosphate. This is shown by the data in Tables 41 and 42.

TABLE 41. DISTRIBUTION OF P^{32} IN UNKNOWNS OF EXUDATE FROM TOMATO STEMS

Time[a] (hr)	Total P^{32} in exudate[b] (counts/sec)	Percentage activity		
		PO_4^{---}	U_1	U_2
½	9.7	100.0	Trace	0
1	70.9	98.4	1.6	Trace
1½	154.9	97.2	2.8	Trace
2	193.2	95.0	4.8	0.2
3	302.7	90.3	9.3	0.4
4	207.1	87.4	12.3	0.3
6	229.5	79.4	20.3	0.3

[a]Time from simultaneous application of P^{32} to the roots and cutting off the stem.

[b]Amount of exudate collected was not quantitated and the total P^{32} is thus subject to wide variation.

TABLE 42. DISTRIBUTION OF P^{32} IN UNKNOWNS OF EXUDATE FROM BARLEY SEEDLING

Time (hr)	Total P^{32} in exudate (counts/sec)	Percentage activity		
		PO_4^{---}	U_1	U_2
½	190.4	99.2	0.8	Trace
1	776	98.8	1.15	0.06
2	5620	98.19	1.64	0.17
4	7407	97.68	2.09	0.23

Unknown 1 can also be detected in the guttation fluid from the tips of young barley leaves when P^{32} is applied to the roots, the guttate being chromatographed in the same manner as described for the exudate. The amount of U_1 and phosphate in the guttate is however much lower than in the exudate. The presence of U_1 in the guttate further indicates that U_1 is a constituent of the xylem sap.

As shown by the data in Tables 41 and 42, the percentage of P^{32} in U_1 and U_2 increased with the time after the radioactive phosphate was supplied to the roots. The percentage of P^{32} in U_1 and U_2 reached a constant maximum value in 4 to 8 hours. This slow increase in U_1 and U_2 has furnished valuable clues concerning the physiology of these two compounds. Barley seedlings have been placed into P^{32} phosphate nutrient solutions, the leaf bases cut off after various times, and the exudate collected. Results are described in Table 43 of one such experiment in which different leaves were cut off either at the time the P^{32} phosphate was given to the roots or 4 hr afterward.

TABLE 43. RATE OF *IN VIVO* LABELING OF U_1 AND U_2 WITH P^{32}

Time after P^{32} supply (hr)	Percentage of U_1 when leaf is cut at:		Percentage of U_2 when leaf is cut at:	
	$t = 0$	$t = 4$	$t = 0$	$t = 4$
¼	0		0	
½	1.22		0.20	
1	2.67		0.22	
2	1.50		0.27	
4	4.91		0.77	
4¼		4.693		0.91
4½		5.06		0.93
5	4.91	4.47	0.69	0.76
6	4.59	3.47	0.62	0.87
8	4.88	6.30	0.61	1.05

The amount of P^{32} in U_1 and U_2 of the exudate was dependent on the time of supplying P^{32} to the roots and not on the time at which the leaves were cut off. This implies that the incorporation of P^{32} phosphate into U_1 and U_2 in the roots was at a rate-limiting speed which was much slower than the transport of P^{32} phosphate in the xylem. This period of about 4 hr for barley seedlings before U_1 reaches maximum P^{32} activity is a measurement of the rate of enzymatic incorporation of P^{32} into

U_1 and indicates the rate of biological turnover for these two compounds.

If U_1 or U_2 were a wound compound formed on the cut stem surface, the percentage of total P^{32} in it should be the same regardless of when the leaf or stem was cut off. The data in Table 43 clearly indicate that such is not the case. The presence of U_1 in the guttate of leaves also indicates that it is not a wound compound. Further to test the possibility that these compounds were wound produced, the exudate from plants not labeled with P^{32} has been collected and mixed with P^{32} phosphate. Also, P^{32} has been added directly to the exudate on the stem. In addition, stem or leaf slices have been placed in P^{32} phosphate solution. In none of these cases were U_1 and U_2 formed in the amounts or within the time intervals which are now being considered. However, the appearance of some U_1 and U_2 under certain of the stated conditions was noted and is being further investigated.

FORMIC ACID METABOLISM IN BARLEY LEAVES

N. E. Tolbert P. C. Kerr

Formic-C^{14} acid has been fed to various plant tissues, especially barley leaves, in both the light and dark and in various gaseous atmospheres, and the compounds into which it was incorporated were analyzed by paper chromatography. Formic acid in animals is known to be incorporated into certain carbons of characteristic products for this 1-carbon substrate such as the beta carbon of serine. Besides inquiring whether plants metabolize formic acid in a similar way, the data have revealed that several new and as yet unknown compounds are formed from formic acid. In addition, the results have permitted a comparison of the labeling in photosynthetic products from formic acid fixation in the light with the labeling during $C^{14}O_2$ photosynthesis. At the levels used, formic acid has not been found to be inhibitory or toxic to plant metabolism.

The major products of formic acid fixation under some of the environmental conditions employed are listed in Table 44. The formate moved by transpirational pull into barley leaves standing in a dilute solution of $HC^{14}OOK$. Formate was then rapidly and completely fixed when the leaves stood in the light and in air or nitrogen. Some of the major products in the light were similar to the photosynthetic carbon dioxide products; e.g., sucrose, sugar phosphates, alanine, serine, and malic, glyceric, and aspartic acids. Since formic acid dehydrogenase is known to be present in plant leaves, a possible major pathway for formic acid fixation by the leaf was its conversion first to $C^{14}O_2$, which was then fixed photosynthetically. That this mechanism will account for a considerable amount of the formate fixation is indi-

TABLE 44. PRODUCTS FROM FORMIC-C^{14} ACID METABOLISM BY BARLEY LEAVES[a]

Products	Percentage of total C^{14} incorporated by leaf during indicated time in:							
	Light and air			Dark and air		Light and nitrogen		
	2 min	15 min	40 min	2 min	60 min	5 min	15 min	60 min
Glyceric acid	30	24.9	14.3	0	0.4	23.2	8.4	4.4
Alanine	Trace	1.7	3.9	0	2.4	17.1	11.3	21.9
Serine	40	35.3	7.4	50	28.2	3.7	7.4	3.3
Glycine	10	11.8	1.5	Trace	0	Trace	Trace	Trace
Aspartic acid	5	6.3	24.7	Trace	10.9	8.5	11.3	3.6
Phosphate esters	5	2.9	6.2	0	0	18.3	19.6	20.3
Sucrose	0	Trace	25.0	0	0	9.8	13.5	21.4
Unknown 1	Trace	Trace	1.7	Trace	28.2	Trace	4.8	8.1
Unknown 2	10	2.5	Trace	50	2.8	8.5	5.1	3.6

[a]Small amounts of many other compounds account for the percentage incorporation not listed.

cated by the degradation data in Table 45. In plants, it appeared that formic acid was being predominantly incorporated into the beta carbons of serine and into the carboxy carbon of glyceric acid. In animal tissue, serine has been shown to be labeled in the beta carbon by formate. Glyceric acid has been shown to be labeled in the carboxy group as the first step during photosynthetic carbon dioxide fixation. Thus it appears that formate in the plant was being incorporated rapidly by both pathways. The largest amount of formate was incorporated into the compounds listed in Table 44 by the leaves when kept in a nitrogen atmosphere, because there was then no other source of CO_2 for photosynthesis. The labeling in the glyceric acid was then predominantly carboxy, but the serine remained labeled heavily in the beta carbon.

The amount of fixation of formate in the dark was severely reduced compared to the light fixation. In the dark and in the air, formate was incorporated into serine as expected, but the amount was much less than in a comparable light period, whereas there was little incorporation via the CO_2 pathway. In the dark and in a nitrogen atmosphere the incorporation of formate even into serine was negligible. These data substantiate the idea that the fixation of formate requires considerable energy which may arise slowly from oxidative phosphorylation in the dark but much faster photosynthetically in the light.

Many unknown compounds were heavily labeled with formic acid in the barley leaf during periods of 12 or more hours. Two unknown compounds, U_1 and U_2, however were labeled by formic-C^{14} so rapidly under all conditions tested that further investigations of them have been made. U_2 with an R_f of 0.8 in phenol-water solvent was labeled as rapidly as serine (Table 44), but its pool size was very small. U_1 was labeled somewhat more slowly, but over a period of several hours a considerable amount of C^{14} from the formic acid appeared in it. Thus, with longer periods for formate incorporation, the percentage and amount of C^{14} rose in U_1, but the percentage in U_2 fell to a mere trace of the total fixation while the amount of C^{14} remained constant. This suggests that U_2 is an active intermediate in metabolism. Unknowns 1 and 2 were not labeled during comparable periods of $C^{14}O_2$ photosynthetic fixation, indicating that the formate was being incorporated into them directly. The area of the lower left-hand corner of the two-dimensional chromatogram where these compounds appeared does not correspond to the extension of any homologous series of sugars, amino acids, or organic acids.

METABOLIC CHANGES DURING GREENING OF ETIOLATED PLANTS

N. E. Tolbert F. B. Gailey

Plants germinated and grown in total darkness remain yellow in color and continue to elongate until the food supply of the seed is exhausted. If such etiolated plants are placed in the light, rapid elongation stops and the plants become green due to the formation of chlorophyll. The chlorophyll formation is a slow process, requiring 24 hr or longer for the plant to acquire a normal green color. The purpose of this project is to search

TABLE 45. SPECIFIC ACTIVITY[a] OF EACH CARBON ATOM OF GLYCERIC ACID AND SERINE

Compound	$NaHC^{14}O_3$; 30 min in light and air or light and N_2	$HC^{14}OOK$; 15 min in light and air	$HC^{14}OOK$; 15 min in light and N_2	$HC^{14}OOK$; 60 min in light and N_2
Serine COOH	4.2	1.2		3.0
$CHNH_2$	4.2	2.4		4.5
CH_2OH	3.6	2.4		6.0
Glyceric COOH		3.0	1.56	
acid CHOH		3.0	0.36	
CH_2OH		2.4	0.48	

[a]Expressed as counts/min/mg of $BaCO_3$.

for biochemical changes during greening of such etiolated plants. One enzyme, glycolic acid oxidase, has already been found to be formed within 24 hr after exposure of etiolated plants to light.[4] As measured by oxygen formation, photosynthesis does not commence for 6 to 8 hr after etiolated plants are placed in the light.[5] During this slow development of photosynthesis, all the enzymes associated with it must be brought into action. Experiments have been designed to determine whether these individual enzymes preexist in an etiolated plant or are activated by light or substrate during the greening period.

Etiolated 7-day-old wheat plants have been placed in white light and the rate of fixation of radioactive carbon dioxide and the rate of formation of chlorophyll have been measured at hourly intervals up to 32 hours. The products formed in the greening plants were quantitated by paper chromatographic analysis. The results indicate that, except for the protochlorophyll preexisting in the plant, the production of chlorophyll by the etiolated plant did not start until the leaves had been exposed to light for 3 to 4 hr. Photosynthetic $C^{14}O_2$ fixation did not become appreciable for about 2 hr after large-scale chlorophyll production began. The greening plant was able to exchange $C^{14}O_2$ with carboxy groups of organic acids associated with respiration during only the first 4 hr of exposure to light. This dark-type fixation was increased at least 100-fold during the first 8 hr of light and before considerable amounts of C^{14} were found in the normal photosynthetic fixation products. This increase of $C^{14}O_2$ fixation in the light into respiratory products as stimulated by photosynthetic reducing power from the newly forming chlorophyll is photosynthesis as judged by criteria usually employed to designate this process.

Detectable amounts of C^{14} label in the compounds associated with the path of carbon in photosynthesis became appreciable after 4 to 6 hr of light, and had greatly increased after 6 to 9 hours. The total amount of fixation continued to increase rapidly for the longer periods of illumination. The photosynthetic fixation into phosphoglyceric acid and hexose phosphates began after a time lag of 1 to 2 hr from the onset of rapid chlorophyll formation. Sucrose was formed and accumulated in these greening plants as soon as there were detectable amounts of labeled phosphoglyceric acid and hexose phosphates. However, ribulose diphosphate was not detectable for many hours afterward. Sedoheptulose phosphate was present in appreciable quantities as soon as phosphoglyceric acid and hexose phosphates were labeled. This indicates that the conversion of sedoheptulose to pentoses in the path of carbon in photosynthesis may be a limiting or adaptive step in the greening plant. The results also indicate that the first photosynthetic reducing power is used for building up products associated with the Krebs respiratory cycle, and not until the greening leaf is ready to store sucrose is CO_2 fixation channeled into phosphoglyceric acid and the hexose phosphate pathway usually thought of as the path of carbon in photosynthesis.

[4]N. E. Tolbert and M. S. Cohan, *J. Biol. Chem.* 204, 639-648 (1953).

[5]J. H. C. Smith, *Plant Physiol.* 29, 143-148 (1954).

A MANOMETRIC ACTINOMETER FOR THE ULTRAVIOLET SPECTRUM

L. P. Zill B. L. McClanahan

A projected study of the effect of ultraviolet radiation on biochemical processes and on the individual components of such processes required an evaluation, in absolute energy units, of the radiations in various lines or wave-length regions emitted by an ultraviolet radiation source. Such an evaluation is a prime requisite in the quantitation of observed effects with energy requirements. Since parts of the projected study involved the determination of respiratory and photosynthetic gas exchanges in a Warburg respirometer fitted with a quartz reaction vessel, it was highly desirable to effect an energy measurement under conditions closely comparable to those existing inside the Warburg flask. The difficulties involved for a physical measurement of radiation under such conditions are obvious. Photochemical reactions which indicate the effectiveness of a radiation source are known and certain of them have been studied extensively with a resultant critical understanding of the physical factors involved. Foremost among these reactions is the photosensitized decomposition of uranyl oxalate.[6] Such a system presents four marked advantages for the problem at hand: (1) The volume of actinometric fluid can be adjusted in volume and treated in a manner

[6]W. G. Leighton and J. Forbes, *J. Am. Chem. Soc.* 52, 3139 (1930).

identical with that of experimental runs. (2) The photochemical reaction produces CO and CO_2 in a stoichiometric ratio, thus permitting manometric measurements. (3) The accuracy of the manometric methods can be verified by titration of the oxalic acid with permanganate. (4) Very accurate quantum yields for this reaction have been determined for the major part of the ultraviolet spectrum.

Linearity of Manometric Response with Time of Irradiation

A standard Warburg apparatus equipped with an ultraviolet source in a position directly below the the quartz Warburg reaction vessels was used for a 2-hr observation of a linear production of gas from a solution of uranyl oxalate. Absorption of the CO_2 by KOH in the center well demonstrated the presence of the two previously known uranyl photosensitized reactions.[7]

$$H_2C_2O_4 \longrightarrow CO_2 + CO + H_2O \qquad (1)$$

$$H_2C_2O_4 \longrightarrow HCOOH + CO_2 \qquad (2)$$

It has been found that 33% of the gas phase consisted of CO, indicating that the decomposition of oxalic acid was almost equally divided between the two reactions. It is therefore obvious that the production of 3 *M* gas volumes represents the decomposition of 2 moles of oxalic acid. This factor must also be incorporated in the calculation of gas production from the titration data. Close correlation was obtained between the observed gas production and that calculated from the titration value of the same sample.

Although gas production by the actinometer fluid was negligible in total darkness a small, linear production was observed under the room lights. This could be circumvented by making determinations in darkness (except for the ultraviolet source) or, more conveniently, by applying a suitable correction factor. Contamination of the external surface of the quartz reaction vessel with grease or oil from any source must be scrupulously avoided, since these exhibit extremely high ultraviolet absorption with subsequent variable reduction of transmittance into the flask.

[7] A. H. Carter and J. Weiss, *Proc. Roy. Soc.*, A174, 351 (1940).

Effect of Shaking on Light Absorption

Accurate measurements of the gas evolution in a Warburg respirometer requires an equilibrium between the gas and liquid phases, which is obtained by constant shaking of the reaction vessel. For determination of any possible effect of randomization of the actinometer fluid thickness on light absorption, a comparison was made between the rate obtained with constant shaking and that obtained by exposing the actinometer fluid to ultraviolet light for 10 min without shaking followed by 5 min shaking without ultraviolet light to obtain equilibrium and subsequent repetition of this cycle to give a total of 60 min exposure to the radiation source. Identical rates were obtained in both cases, indicating either that no light absorption error was introduced or that there was a compensation of errors.

Total Absorption of Incident Light

Obviously, it is a stringent requirement that the actinometer fluid have a total absorption of the incident radiation. Since there is a severe limitation on the volume of actinometer fluid which can be placed in the Warburg vessel, it was necessary to ascertain the concentration of uranyl ion required for complete absorption in the thickness of the layer to be used. It was found that the concentrations previously employed were too low for total absorption. The reason for previous use of this lower concentration involved, in part, the titration method employed and therefore does not apply to this manometric method. A series of determinations were then made in which the uranyl ion concentration was increased (with a concomitant increase in oxalate to maintain a ratio of 1 uranyl ion to 5 oxalate ions) until a threshold value was obtained. When a 4-ml volume was used it was found that a minimum value of 0.5 *M* uranyl ion was necessary for complete light absorption.

Desensitization by Iodide Ion

Critical studies of the desensitization of this reaction by iodide ion have been made. Certain of these were repeated to check the validity of this manometric method and to demonstrate its applicability to other photochemical reactions which result in gaseous products. Changes in the production of CO and CO_2, and reaction rates were closely comparable to those previously obtained.

Calculation of Energy from Manometric Results

The volume of gas produced at standard temperature and pressure is obtained from the observed manometric change by the ordinary method. The number of moles of oxalic acid decomposed is obtained by the method described. Since the quantum efficiency of this reaction is 0.58 at 2537 A[6] the number of moles of oxalate is multiplied by the factor, 1.724, to obtain the total number of light quanta absorbed. Some 90% of the radiation from a low-pressure mercury source is in the resonance line of 2537 A, and therefore the energy of the quantum at this wave length and frequency may be used for the average energy without introducing an appreciable error. Direct measurement or calculation of the surface of the actinometric fluid may be carried out in order to express the value in energy units per unit of area.

PHOSPHORYLATED COMPOUNDS IN PLANTS

A. R. Krall L. P. Zill

The separation of sugar phosphates by ion exchange with the use of borate complexing has been reported by Khym and Cohn. In the present work a study has been made of the application of this procedure to the analysis of the phosphorylated compounds present in plants. Incorporation of P^{32} into these compounds, making it possible to record a column elution pattern automatically, facilitated a rapid survey of the effects of varying the environment on levels of the P^{32}-labeled materials and also provided a measure of the efficiency of different extraction methods used. In addition, double labeling of the plants with P^{32} and C^{14} was carried out as an aid in the identification of the carbon moieties of the phosphates and to determine the presence of nonphosphorylated compounds which are regarded as contaminants of the separated phosphates.

Paper chromatography of the individual peaks demonstrated that none contained less than three components. Compounds present in the original synthetic mixture separated by Khym and Cohn were identified in peaks obtained at similar elution volumes. Treatment of the individual peaks with phosphatase and subsequent paper chromatography of the carbon residues gave excellent separation. Several of these carbon skeletons could be identified by cochromatography with suspected compounds. Others represented complete unknowns. A minimum of 50 phosphates could be separated by the combined ion-exchange and paper-chromatography method. Variation in the percentage distribution of the activities of these compounds under varying conditions of phosphate incorporation can be determined.

NATURE OF THE OXIDATIVE SYSTEM OPERATIVE IN PHOTOSYNTHETIC CARBON DIOXIDE FIXATION

A. R. Krall

The essentiality of oxygen for the mediation of CO_2 fixation in the photosynthesizing plant has not been definitely proved. Since the green plant produces 1 mole of oxygen for each mole of CO_2 fixed, it is obvious that if such a requirement exists it may easily be satisfied by oxygen produced within the plant. Thus the requirement could make itself manifest only if a way were found to remove oxygen from its site of production as fast as it is formed or if inhibitors known to be specific for oxidative systems were found to inhibit photosynthetic carboxylation.

That oxygen is essential seems reasonable since the first stable product of photosynthesis is a phosphate ester and high-energy phosphate bonds are most efficiently formed by oxidative processes mediated by the cytochrome system. A schematic representation of a possible coupling between the water-splitting reaction of photosynthesis and the fixing of CO_2 is shown in Fig. 36.

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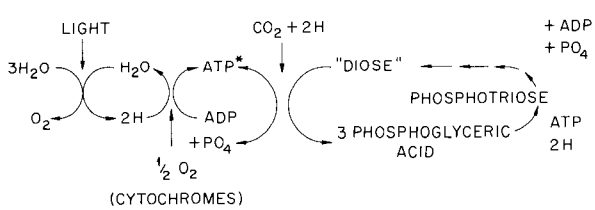

Fig. 36.

If the cytochrome system takes part in carboxylation reactions, cyanide should inhibit CO_2 fixation. Warburg first demonstrated this in 1919.[8] Carbon monoxide also should inhibit this system but its inhibition is reversible by light or by increasing amounts of oxygen, both a part of the

[8] O. Warburg, *Biochem. Z.* 100, 230-270 (1919).

photosynthetic machinery. If a CO inhibition were present, it would exhibit itself only by prolonging the period of lowered CO_2 fixation immediately following illumination. During this time levels of ATP and sugar phosphates intermediate between phosphotriose and "diose," which would be depleted by inhibition of oxidative activity in darkness, would be rebuilt to normal photosynthetic levels.

It has been reported that the photosynthetic fixation of $C^{14}O_2$ by barley leaves is inhibited by preexposing the leaves to N_2 in light or dark or by exposure to CO-O_2 mixtures in darkness.[9] Exposure to the latter mixture in light produced no inhibition. This pattern of inhibition suggests the participation of cytochrome oxidase in the carbon dioxide fixing mechanism. It has now been found that yellow light at 589 mμ is more effective in the reversal of the carbon monoxide inhibition than red light, whereas the same yellow light at the same intensity is only one-third as effective as the red light in causing barley leaves to fix $C^{14}O_2$ under steady-state photosynthetic conditions. The data presented in Table 46 show that the yellow light causes as much reversal of the inhibition in 3 min as the red light does in 10 minutes. The reversal by the yellow light proceeds at about the same rate as reversal of nitrogen-induced inhibition by air. The inhibition induced here by CO is almost certainly on cytochrome oxidase since reversal of a CO inhibition by light has been termed the most specific inhibition pattern known[10] and the specificity is further increased here by use of light of a frequency at which the photodissociation spectrum of the CO–enzyme complex shows a maximum.

It is seen that red light reverses the inhibition, but more slowly. Since the inhibition is not complete, red light is able to induce some oxygen formation in inhibited leaves. This oxygen is then able to compete with the carbon monoxide for a site on the enzyme and thus slowly and autocatalytically to reverse the inhibition.

Exposure to darkness also causes some inhibition. This inhibition is probably caused by lowered amounts of sugar phosphates in the 2-carbon regenerative cycle for CO_2 acceptor resulting from a lowered level of the reduced coenzymes and ATP necessary to produce these compounds. In the inhibitor-treated tissues these sugar phosphates are reduced still further in amount by a more nearly complete stoppage of high-energy phosphate bond generation. Conversely, exposure of the plant to higher concen-

[9] A. R. Krall and R. H. Burris, *Physiol. Plantarum* (in manuscript).

[10] W. O. James, Ann. Rev. *Plant Physiol.* 4, 81-101 (1953).

TABLE 46. EFFECT OF PRETREATMENT OF BARLEY LEAVES ON THE RATE OF PHOTOSYNTHETIC CO_2 FIXATION

Expt. no.	Pretreatment	Fixation (% of control)
1	Air-light	100
2	Air-dark for 40 min	41
3	95% CO, 5% O_2-dark for 40 min	11
4	Same as 3, then yellow light for 3 min	71
5	Same as 3, then yellow light for 10 min	83
6	94% CO,5% O_2, 1% CO_2-dark for 40 min then yellow light for 20 min	127
7	Same as 3, then red light for 3 min	41
8	Same as 3, then red light for 10 min	71
9	Same as 6, except red light for 20 min	140

1 g of barley was used in all cases and exposed to $C^{14}O_2$ for 45 sec after the pretreatment and before killing with hot ethanol-water solution.

trations of CO_2 in light should push the levels of intermediates in the acceptor generation cycle higher than in air and should enable the plant to fix CO_2 at a higher rate. This probably accounts for the larger amounts of $C^{14}O_2$ fixed in experiments 6 and 9 where the tissue was inhibited and allowed to recover in gas containing 1% CO_2. The higher level fixed in red light probably reflects the greater efficiency of absorption of red light by the leaves. It should be noted here that yellow light is able to mediate photosynthesis but at a lower rate than red light.

Since the data clearly indicate involvement of cytochrome oxidase and 2,4-dinitrophenol is known to inhibit $C^{14}O_2$ fixation,[11] it was thought that the inhibition of cytochrome oxidase probably stopped formation of high-energy phosphate compounds, which in turn would inhibit CO_2 fixation. Barley leaves were labeled with P^{32} and treated with various inhibitors. The extracts of these tissues were then separated on Dowex-1 columns[12] to determine distribution of the P^{32}. It was found that plants contained large amounts of label in ATP only when they were exposed to light in the presence of air. The amount of label in the sugar phosphates was sharply reduced when the leaves were placed in the dark and still more sharply reduced when the plants were exposed to conditions strongly inhibitory to CO_2 fixation. The most striking reduction in phosphate esters occurs in the amount of label present in phosphoglycerate. ATP never completely disappears, but phosphoglyceric acid is down to less than 1% of its normal level after treatment of the leaves with N_2 in light or in dark or with $CO-O_2$ in darkness.

[11] R. Newburgh and R. H. Burris, *Arch. Biochem. and Biophys.* 49, 98 (1953).

[12] J. X. Khym and W. E. Cohn, *J. Am. Chem. Soc.* 75, 1153 (1953).

PHYSIOLOGY

C. W. Sheppard M. L. Anderson J. N. Dent[2] J. Ginsburg[1]
N. G. Anderson J. R. C. Brown[1] P. J. Mathias E. C. Horn[1]

ERYTHROCYTES

P. J. Mathias C. W. Sheppard

Red cell acetylation studies have been continued with methods entirely similar to those described previously.[3] Essentially, the only change in the method has been the exclusion of adenosine triphosphate, fluoride, yeast, and coenzyme A from the synthetic system and the inclusion of preformed acetyl coenzyme A. Under these conditions, synthesis of small amounts of an acetylcholine-like substance has been repeatedly observed. The synthesis has been found to be dependent on the presence of choline during incubation and to be reduced by incubation in the presence of $CuSO_4$, suggesting that it is mediated by an enzyme similar to the choline acetylase of neural tissue. Under varying experimental conditions, synthetic rates of 1 to 9 $\mu g/g$ of powder/30 min of incubation have been observed. The results suggest that the red cell preparations are normally incapable of acetylating coenzyme A.

HISTONES

N. G. Anderson M. L. Anderson

A study of the methods of preparation and fractionation of nuclear basic proteins has been undertaken. Electrophoretic examination of several preparations obtained from fowl erythrocytes demonstrates consistently the presence of two major components and three minor ones. Fractionation by salting-out techniques and ion exchange have been studied. Paper electrophoresis proved unsuitable, since histones and protamines are strongly adsorbed on filter paper. Ion exchange on XE-64 resin has been only partially successful, due in a large part to the inability of histone to be subsequently eluted, either with strong acids or salts.

DISTRIBUTION OF LITHIUM IN AMPHIBIAN EMBRYOS

J. N. Dent

In the preceding semiannual report,[4] an autoradiographic technique for the detection of lithium in tissues was described. The development of the technique has been continued and improvements involving control of background, staining of sections, and adherence of sections to emulsions have been made. Preliminary observations indicated a uniform distribution of lithium throughout the embryo at the developmental stages examined (gastrula and neurula). Further observations indicate that, under certain conditions, there is a heavy cortical localization of lithium in the embryo.

THE EFFECT OF THE LITHIUM ION ON THE ELECTROLYTES OF THE AMPHIBIAN EMBRYO

J. Ginsburg J. N. Dent

Gustafson[5] has suggested that the primary effect of the lithium ion in producing its morphogenetic effect on the development of the amphibian embryo may be the displacement of potassium. In an earlier report,[6] was described the penetration of lithium ion into embryos of *Rana pipiens* at neural-fold stage as being rapid during the first 30 to 60 min and slower thereafter without reaching equilibrium by 16 hours. It was also noted that the amount of lithium accumulated by the embryo is dependent on the external concentration of the lithium ion, and it was further demonstrated that there are no gross movements of potassium in the presence of a strongly hypertonic solution of LiCl (0.15 *M*) which induced large fluid shifts and caused marked microcephalia and hypomorphoris in the subsequent development of the exposed embryos.

In more recent studies, measurements have been made of the lithium, potassium, and sodium concentrations in embryos, both intact and with jelly coats removed, and in separated jelly after various periods of exposure to 0.15 *M* LiCl. Fig. 37 shows the results of these experiments. After 2 hr in 0.15 *M* LiCl, the Li concentration in the jelly coat

[1] Research Participant.

[2] Temporary Employee.

[3] P. J. Mathias and C. W. Sheppard, *Proc. Soc. Exptl. Biol. Med.* 86, 69-74 (1954).

[4] J. N. Dent, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, p 67.

[5] T. Gustafson, *Rev. Suis. Zool.* 57, Suppl. 1, 77-92 (1950).

[6] J. Ginsburg and J. N. Dent, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, p 67.

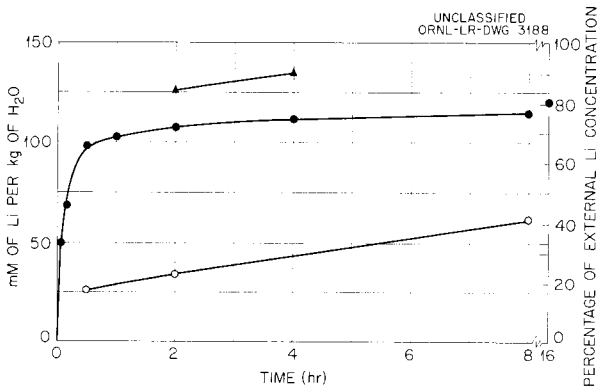

Fig. 37. Uptake of Lithium by Amphibian Embryos. ●, Li concentrations in intact embryos; O, Li concentrations in stripped embryos (i.e., embryos from which the jelly coat has been removed); ▲, Li concentrations in the jelly coat. The concentration of Li in the media was 0.15 *M*.

is 3.7 times that of the stripped embryos and the concentrations in the jelly and stripped embryos are 81 and 23%, respectively, of the Li concentration in the external medium. Thus the early phase of uptake of Li by intact embryos represents a rapid influx of Li into the jelly coat, and the later portion of the curve represents movement of Li into the inner capsule and embryonic cell mass.

The potassium concentration of the embryos remains constant when calculated as millimoles per gram of dry weight. Changes in concentration based on wet weight or water content can be explained by the loss of water from the embryos to the hypertonic LiCl solution. The loss of sodium from embryos exposed to LiCl cannot be explained as a quantitative replacement of sodium by lithium. The ratio of millimoles of lithium accumulated to millimoles of sodium lost in intact embryos is 6.3 after 9 hr in both stripped and intact embryos. Upon being transferred from 0.15 *M* LiCl solution to a 20% Holtfreter's solution, the Li moves out of the embryo rapidly at first and then more slowly, and Na and water enter the embryo, returning to near control levels within 16 hours.

Analysis of jelly-coat and stripped embryos raised in 20% Holtfreter's solution shows the K and Na concentrations of the jelly coat to be in equilibrium with those of the medium, whereas they are considerably more concentrated in the embryo proper. When placed in Na-free Holtfreter's solution, there is a loss of Na but no change in K concentration. Neither the Na nor K concentrations show changes in K-free Holtfreter's solution. Furthermore, it has been observed that, during the development of the embryos, there is a progressive increase in water content and Na concentration based on dry weight but little or no change in Na concentration based on water content. The reverse is true for K, which shows a constant concentration based on dry weight and a decreasing concentration based on water content. The amount of Na necessary to produce the increased dry weight concentration corresponds to the amount of Na present in the same volume of 20% Holtfreter's solution necessary to produce the increased water content of the embryos. Thus all the embryonic K, except the 5% in the jelly coat, is not freely diffusible, whereas the greater portion of the embryonic Na is free to leave the cells of the embryo, as well as the jelly coat, when the Na concentration in the external medium falls.

The uptake of Li seems to be best described as a rapid penetration into the jelly coat and a slower penetration into the embryo itself. There is no evidence to indicate a specific displacement of either K or Na since all changes in Na and K concentrations can be explained on the basis of fluid shifts or diffusion gradients.

BIOPHYSICS

MOLECULAR BIOPHYSICS AND RADIOLOGICAL PHYSICS

J. S. Kirby-Smith M. L. Randolph
D. L. Craig E. B. Darden, Jr.
T. L. Rapp[1]

Molecular Biophysics: Effects of Radiation on *Tradescantia*

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

Ultraviolet. Further investigation of the action spectrum for chromosome aberrations in ultraviolet-irradiated *Tradescantia* pollen has extended the short wave-length limit of the spectral range studied to 2350 A. Additional data for effects of other wave lengths in the 3650 to 2350 A region have also been accumulated. The action spectrum obtained from these measurements is illustrated in Fig. 38, along with the well-known nucleic acid absorption spectrum for comparison.

The original experiments in this project on the production of chromosome aberration by ultraviolet irradiation in air and in nitrogen revealed no differences in over-all aberration frequencies but did indicate some difference in the proportion of incomplete isochromatid aberrations produced under these conditions. These results have been confirmed by several additional experiments in which 1×10^6 ergs/cm of 2650 A ultraviolet radiation was used. In every instance, a significantly larger proportion of incomplete isochromatid aberrations has been observed for pollen irradiated in nitrogen than for material treated in air. These increases, approximately 45 to 65% incomplete, respectively, are in good agreement with the hypothesis that breaks produced in nitrogen are less likely to restitute than those induced in air. The additional experimental observation that equal numbers of chromatid aberrations are produced in pollen irradiated in air and in nitrogen is in complete disagreement with such a simple restitution theory. This apparent paradox can be resolved by an additional assumption that fewer breaks are initially produced in nitrogen than in air. The present experimental results thus support a tentative hypothesis in which (1) fewer breaks are produced in nitrogen than in air, and (2) the breaks induced in nitrogen are, in addition, less likely to rejoin than the ones produced in material irradiated in air. This argument contains implicitly the underlying assumption that at least some of the photochemical processes involved in oxygen and in nitrogen are quite different.

The search for possible photorecovery processes in ultraviolet-treated *Tradescantia* dry pollen has been extended, with negative results. Further study of photoreactivation in *Tradescantia* is now being carried out on growing pollen tubes instead of the dry material.

X Rays. A number of time–intensity experiments with X rays on dry *Tradescantia* pollen indicated no significant difference in the aberration frequencies for exchanges when the dose was given at rates from 4.5 to 240 r/minute. Preliminary experiments with growing pollen tubes irradiated 1 hr after sowing have shown a higher frequency of exchanges in the material irradiated at 240 r/min than in that irradiated at 10 r/minute. Preliminary experiments have been started on the effect of X rays on dry pollen at different temperatures.

Neutrons. Inflorescences and pollen have been irradiated in the cyclotron facility. Dose curves for chromosome aberrations in the flower buds and for chromatid aberrations in the pollen tube division have been obtained. The data for chromosome aberrations in which the more recently available cyclotron fast neutron dosimetry was employed lead to somewhat lower values of the relative biological effectiveness for cyclotron neutrons

[1]Temporary Employee.

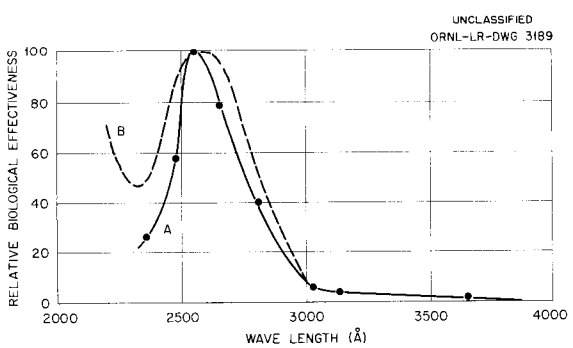

Fig. 38. Action Spectrum for Ultraviolet-Induced Chromosomal Aberrations. ----, isochromatid aberrations; ——, absorption spectrum of a nucleic acid.

TABLE 47. RELATIVE BIOLOGICAL EFFECTIVENESS IN *TRADESCANTIA*

Type of radiation	Pollen	Inflorescences
Co^{60} γ rays, 1.17 and 1.33 Mev	1.0	1
200-kv mean energy X rays (0.06 A)	1.5	1.5
60-kv mean energy X rays (0.20 A)	2.0	2.0
Fast neutrons, cyclotron facility highly degraded neutron spectrum approximately 1 Mev mean energy	8.0	10.0

than our previous cyclotron results. These figures, along with the data for pollen tube aberrations, are shown in Table 47. In some recent experiments, no differences in aberration frequencies have been detected for inflorescences irradiated under conditions of maximum hydration and almost lethal desiccation.

Paramagnetic Resonance

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

Microwave equipment for paramagnetic resonance studies at 25,000 megacycles has been assembled and placed in use. The sensitivity at this frequency is considerably better than that previously attained at X-band (9000 megacycles). Resonances in irradiated polymers reported by Schneider[2] have been detected and are being studied further. An attempt to observe resonances due to free radicals in chemical systems at room temperature and in aqueous solution is in progress.

RADIOLOGICAL PHYSICS

M. L. Randolph E. B. Darden

Chromatid aberrations in *Tradescantia* inflorescences have been obtained by irradiation with 2.5-Mev fast neutrons produced by the d-d reaction in the Cockcroft-Walton accelerator. The exposures were made for periods up to 2 hr with the flower buds at 5 cm from the target and covering about 20 cm^2. For convenience to other workers who may anticipate using the accelerator – comparison with X-ray effects indicated that the biological effect corresponded very roughly to that caused by 250-kvp X rays of intensity 0.5 r/minute. Longer exposures are obtainable and it is expected that greater exposure areas and dose rates will become available. (Biological evaluation has been performed by A. D. Conger and A. H. Johnston of the Cytology and Genetics Section.)

A comprehensive characterization of the fast neutron dose from the d-d reaction at the target of the accelerator has been undertaken. This study includes measurements of dose and flux intercomparisons, scattering measurements, distribution of flux around the target and estimates of the energy spread of the neutrons. Intercomparisons of measurements made in both the temporary and permanent facilities among three independent dose values have been found in rough agreement. The values were obtained by (1) measurements with a thimble-type polyethylene–ethylene ion chamber[3] calibrated both from its physical dimensions and versus the gamma rays of a standard radium source, (2) by measurements with a Hurst-type fast neutron dosimeter[4] calibrated versus a standard Po-B neutron source and by Rossi's dose to flux relation[5] to calculate the dose from the flux as measured by the associated particle method, and (3) by a Hanson-McKibben long counter[6] which was calibrated against two standard fast neutron sources. The ion-chamber dose values were higher than the other two values. Close attention is being given to refinement of all these measurements and elimination of possible sources of error.

After concrete block walls were erected around the temporary installation and a cone was used to shield the long counter from direct neutrons, neutron scattering estimates were made which should apply to the enclosed permanent facility. At about 1 meter from the target, it was found that the

[2] E. E. Schneider, *Nature* 168, 644 (1951).

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

[4] W. A. Mills and G. S. Hurst, *Nucleonics* 12, No. 4, 17 (1954).

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

[6] A. O. Hanson and J. L. McKibben, *Phys. Rev.* 72, 673 (1947).

scattered flux might comprise 40% of the total flux, but that in the vicinity where biological materials are to be placed, the scattered flux should comprise less than 1% of the total. Preliminary measurements in the permanent facility support these estimates. Scattering from the actual target assembly influences both the angular distribution and the energy of the neutrons. With the present bulky but efficiently water-cooled target, it is calculated that 20 to 25% of all neutrons generated are scattered, but that the energy spectrum is changed little. The ratio of flux in the forward direction to that at 90° (in the laboratory coordinates) for thin (gaseous) targets is reported as about 2.3 to 1.[7] This ratio is reduced when "thick" targets, necessary to obtain maximum yield, are used and is further reduced by scattering by the target assembly. Experimentally, with a drive-in target in the temporary facility, the ratio was about 1.6. Targets which will produce much less scattering are under consideration.

During this period the accelerator and all its accessories have been moved to the permanent well-shielded facility and successfully put into steady operation again. No serious faults have been found in the design or construction. In the new facility, the elaborate calibrations are being reestablished, deutero-paraffin targets tested, and biological exposures made.

A study of target yields is in progress. With a drive-in target on a thin silver disc cooled by a jet of water, a maximum yield of about 4.8×10^7 total neutrons/sec has been obtained with a 370-μa beam at 200 kv. This provides an extremely stable target. With thin (roughly 0.5 mg/cm^2) deutero-paraffin layers over the silver target, preliminary work has yielded about 2×10^8 total neutrons/sec (or about 7 rep/hr at 5 cm from the target) with a 300-μa beam at 250 kv. Experiments with zirconium deuteride targets are awaiting delivery of the zirconium deuteride buttons. Owing to the greater nuclear cross section of the d-t reaction, yields of 14-Mev neutrons from tritium targets may be 100 times as great as the yields from deuterium targets.

Detailed measurements have been made on the spatial distribution of radiation intensity inside the Co^{60} source installed in December 1953, at the U. S. Department of Agriculture Entomology Research laboratories in Orlando, Florida, for use in their screwworm control project. This work makes possible more effective utilization of the source in the sterilization of large quantities of screwworm pupae.

[7] W. A. Wenzel and W. Whaling, *Phys. Rev.* 88, 1149-1154 (1952).