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FOR PERIOD ENDING FEBRUARY 15, 1955

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BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

for Period Ending February 15, 1955

Alexander Hollaender, Director
Edited by E. J. Slaughter

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75. D. Schwartz
76. D. Billen
77. K. C. Atwood
78. A. C. Upton
79. E. F. Oakberg
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88. R. C. von Borstel
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BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

PUBLICATIONS AND LECTURES

Publications. During the period covered by this report, 47 papers and 32 abstracts and book reviews have appeared in the open literature. In addition, one project report has been issued. The complete papers are listed below; abstract references, when available, are printed under the titles of lectures given before professional societies.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Alexander, M. L.	Mutation rates at specific autosomal loci in the mature and immature germ cell of *Drosophila melanogaster*	*Genetics* 39, 409–428 (1954)
Atwood, K. C.	Aberration frequencies in irradiated populations (Comm. to ed.)	*Am. Naturalist* 88, 379–380 (1954)
_____	Survival and mutations in *Neurospora* exposed at nuclear detonations	*Am. Naturalist* 88, 295–314 (1954)
von Borstel, R. C.	Differential response of meiotic stages in *Habrobracon* eggs to nitrogen mustard	*Genetics* 40, 107–116 (1955)
_____	Feulgen-negative nuclear division in *Habrobracon* eggs after lethal exposure to X rays or nitrogen mustard (Comm. to ed.)	*Nature* 175, 342–343 (1955)
Bridgman, J., and R. F. Kimball	The effect of X rays on division rate and survival of *Tillina magna* and *Colpoda* sp. with an account of delayed death	*J. Cellular Comp. Physiol.* 44, 431–445 (1954)
Cohn, W. E.	The influence of ion-exchange chromatography on our concept of the structure of ribonucleic acid	*Chemical Specificity in Biological Interactions* (Harvard Memoirs, No. 3) ed. F. R. N. Gurd, Academic Press, New York, pp. 75–89 (1954)
_____	The separation of nucleic acid derivatives by chromatography on ion-exchange columns	*Nucleic Acids; Chemistry and Biology*, ed. E. Chargaff and J. N. Davidson, Vol. 1, Academic Press, New York, pp. 211–241 (1955)
Conger, A. D.	Radiobiological studies with *Tradescantia* at nuclear test detonations	*Am. Naturalist* 88, 215–224 (1954)
Cormier, M. J., and B. L. Strehler	Some comparative biochemical aspects of cell-free bacterial luminescence	*J. Cellular Comp. Physiol.* 44, 277–289 (1955)
Cormier, M. J., and J. R. Totter	Flavinmononucleotide-dependent menadione reductase coupled to cytochrome c reduction in cell-free extracts of *Achromobacter fischeri* (Comm. to ed.)	*J. Am. Chem. Soc.* 76, 4744 (1954)

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AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Darden, E. B., E. Maeyens, and R. C. Bushland	A gamma-ray source for sterilizing insects	*Nucleonics* **12** (10), 60–62 (1954)
Dent, J. N., E. L. Gadsden, and J. Furth	On the relation between thyroid depression and pituitary tumor induction in mice	*Cancer Research* **15**, 70–75 (1955)
Dolin, M. I.	Diacetyl oxidation by *Streptococcus faecalis*, a lipoic acid system	*J. Bacteriol.* **68**, 15–25 (1954)
———	The flavin requirement for DPNH-menadione reductase in *Streptococcus faecalis*	*Biochim. et Biophys. Acta* **15**, 153–154 (1954)
Furth, J., J. N. Dent, W. T. Burnett, Jr., and E. L. Gadsden	The mechanism of induction and characteristics of pituitary tumors induced by thyroidectomy	*J. Clin. Endocrinol. and Metabolism* **15**, 81–97 (1955)
Furth, J., and A. C. Upton	Leukemogenesis by ionizing irradiation	*Acta Radiol.*, Suppl. **116**, 469–476 (1954)
Furth, J., A. C. Upton, K. W. Christenberry, W. H. Benedict, and J. Moshman	Some late effects in mice of ionizing radiation from an experimental nuclear detonation	*Radiology* **63**, 562–569 (1954)
Giles, N. H., and C. A. Tobias	Effect of linear energy transfer on radiation-induced chromosome aberrations in *Tradescantia* microspores	*Science* **120**, 993–996 (1954)
Ginsburg, J. M., and W. S. Wilde	Distribution kinetics of intravenous radiopotassium	*Am. J. Physiol.* **179**, 63–75 (1954)
Gude, W. D., and T. T. Odell, Jr.	Vinisil as a diluent in making bone marrow smears	*Stain Technol.* **30**, 27–28 (1955)
Hollaender, A.	Preface: Radiation Biology, Vol. I	*Radiation Biology*, Vol. I, McGraw-Hill, New York, pp. v–vi (1954)
———	Biology Division Semiannual Progress Report for period ending August 15, 1954	Project Report ORNL-1776
Khym, J. X., and W. E. Cohn	The ion-exchange separation of the 5′ ribonucleotides and deoxyribonucleotides	*Biochim. et Biophys. Acta* **15**, 139 (1954)
Khym, J. X., D. G. Doherty, and W. E. Cohn	Ribose phosphates: production from nucleotides, ion-exchange separation, and characterization	*J. Am. Chem. Soc.* **76**, 5523 (1954)
Kimball, R. F.	The role of oxygen and peroxide in the production of radiation damage in *Paramecium*	*Ann. N. Y. Acad. Sci.* **59**, 638–648 (1955)
———	The effects of radiation on protozoa and the eggs of invertebrates other than insects	*Radiation Biology*, ed. A. Hollaender, Vol. II, McGraw-Hill, New York, pp. 285–331 (1955)
Lovelace, L. R.	Chromosome shattering by ultraviolet radiation (2650 A)	*Proc. Natl. Acad. Sci. U.S.* **40**, 1129–1135 (1954)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Mickey, G. H.	Visible and lethal mutations in *Drosophila*	*Am. Naturalist* **88,** 241–255 (1954)
Odell, T. T., Jr., F. G. Tausche, and J. Furth	Platelet life span as measured by transfusion of isotopically labeled platelets into rats	*Acta Haematol.* **13,** 45–52 (1955)
Parker, D. R.	Radiation-induced exchanges in *Drosophila* females	*Proc. Natl. Acad. Sci. U.S.* **40,** 795–800 (1954)
Pomper, S., and K. C. Atwood	Radiation studies on fungi	*Radiation Biology*, ed. A. Hollaender, Vol. II, McGraw-Hill, New York, pp. 431–453 (1955)
Russell, W. L., L. B. Russell, and A. W. Kimball	The relative effectiveness of neutrons from a nuclear detonation and from a cyclotron in inducing dominant lethals in the mouse	*Am. Naturalist* **88,** 269–286 (1954)
Schwartz, D.	Studies on the mechanism of crossing over	*Genetics* **39,** 692–700 (1954)
Stapleton, G. E.	The influence of pre- and posttreatments on bacterial inactivation by ionizing radiations	*Ann. N. Y. Acad. Sci.* **59,** 604–618 (1955)
Strehler, B. L., and M. J. Cormier	Isolation, identification, and function of long-chain fatty aldehydes affecting the bacterial luciferin-luciferase reaction	*J. Biol. Chem.* **211,** 213–225 (1954)
———	Kinetic aspects of the bacterial luciferin-luciferase reaction *in vitro*	*Arch. Biochem. and Biophys.* **53,** 138–156 (1954)
Swanson, C. P., and A. H. Johnston	Radiation-induced pycnosis of chromosomes and its relation to oxygen tension	*Am. Naturalist* **88,** 425–443 (1954)
Tipton, S. R., and G. S. St. Amand	The effects of X rays on the respiratory metabolism of eggs and embryos of the grasshopper, *Chortophaga viridifasciata*	*Physiol. Zool.* **27,** 311–317 (1954)
Totter, J. R., and A. N. Best	The metabolism of formate-C^{14} by rabbit bone marrow *in vitro*	*Arch. Biochem. and Biophys.* **54,** 318–329 (1955)
Upton, A. C., J. Furth, and K. W. Christenberry	Late effects of thermal neutron irradiation in mice	*Cancer Research* **14,** 682–690 (1954)
Upton, A. C., and W. D. Gude	Physiologic and histochemical changes in connective tissue of rat induced by total body irradiation	*Arch. Pathol.* **58,** 258–264 (1954)
Volkin, E.	The linkage of glucose in coliphage nucleic acids	*J. Am. Chem. Soc.* **76,** 5892–5893 (1954)
Whiting, A. R.	The effect of oxygen on the frequency of X-ray-induced mutations in *Habrobracon* eggs	*Genetics* **39,** 851–858 (1954)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Wolff, S., and R. C. von Borstel	The effects of pre- and postirradiation centrifugation on the chromosomes of *Tradescantia* and *Vicia*	*Proc. Natl. Acad. Sci. U.S.* **40,** 1138–1141 (1954)
Zelle, M. R., and A. Hollaender	Effects of radiation on bacteria	*Radiation Biology*, ed. A. Hollaender, Vol. II, McGraw-Hill, New York, pp. 365–430 (1955)
———	Monochromatic ultraviolet action spectra and quantum yields for inactivation of T1 and T2 *Escherichia coli* bacteriophages	*J. Bacteriol.* **68,** 210–215 (1954)

Publications in Press. The number of publications now in press includes 66 papers, 47 abstracts and book reviews, and the proceedings of the 1954 Symposium on Genetic Recombination. These proceedings will be published in the usual form – a supplement to the Journal of Cellular and Comparative Physiology. This publication has been delayed by the change in managing editors of the journal but should be off the press by late May. The table of contents follows:

SYMPOSIUM ON GENETIC RECOMBINATION

Introduction – Alexander Hollaender
Rollin D. Hotchkiss. Bacterial transformation
Norton D. Zinder. Bacterial transduction. Four figures
A. H. Doermann, Martha Chase, and Franklin W. Stahl. Genetic recombination and replication in bacteriophage. Three figures
Joshua Lederberg. Recombination mechanisms in bacteria. One figure
J. D. Watson. Biological consequences of the complementary structure of DNA. (Abstract and discussion)
David D. Perkins. Tetrads and crossing over. Seven figures
Edward Novitski. Genetic measures of centromere activity in *Drosophila melanogaster*. Two figures
Drew Schwartz. Studies on crossing over in maize and *Drosophila*. Four figures
Maurice Whittinghill. Crossover variability and induced crossing over. Seven figures
Hampton L. Carson. Variation in genetic recombination in natural populations. Three figures
A. H. Sturtevant, Karl Sax } Evaluation of recombination theory
Alexander Weinstein. Unraveling the chromosomes. Eight figures
Norman H. Giles. The oxygen effect on radiation-induced chromosome aberrations: breakage-versus-recombination hypotheses
C. P. Swanson. The oxygen effect and chromosome breakage. One figure
William K. Baker and Elizabeth S. Von Halle. Evidence on the mechanism of the oxygen effect by use of a ring chromosome. Three figures
Discussion of the last three papers. Two figures

Scientific Society Lectures and Traveling Seminars. Participation in programs of professional societies and in the Traveling Seminar program is represented by the following lectures.

Anderson, N. G.	Department of Botany, University of North Carolina, Chapel Hill	Concepts of origin of life

_____	Department of Zoology, Duke University, Durham, N. C.	Centrifugal techniques
Anderson, N. G., and M. L. Anderson	Am. Soc. Zool., Chapel Hill, N. C.	High-resolution differential centrifugation [Abstr., *Anat. Record* **120**, 709 (1955)]
Atwood, K. C.	Division of Medical Physics, University of California, Berkeley	The nuclear localization of radiation damage in *Neurospora*
Atwood, K. C., F. Mukai, and T. H. Pittenger	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Gainesville, Fla.	*Neurospora* techniques for large-scale studies of recessive lethal mutation [Abstr., *Genetics* **39**, 957 (1954)]
Baker, W. K.	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Gainesville, Fla.	Chromosame assaciation and segregation in polysomic *Drosophila* males [Abstr., *Genetics* **39**, 957 (1954)]
_____	Florida State University, Tallahassee	Gene action and gene position
_____	Department of Biology, University of Florida, Gainesville	As above
_____	Biological Science Departments, University of Georgia, Athens	As above
Benedict, W. H., K. W. Christenberry, and A. C. Upton	Intern. Cangr. Ophthalmol., New York City	Ocular effects of ianizing radiation
_____	Am. Acad. Ophthalmol., New York City	As above
Benedict, W. H., and A. C. Upton	Assoc. Res. Ophthalmol. (with Sou. Med. Assoc.), St. Louis, Mo.	Adenocarcinoma of Harder's gland in mice
von Borstel, R. C.	Department of Zoology, Duke University, Durham, N. C.	Effects of radiation on egg nucleus and cytoplasm of the parasitic wasp, *Habrobracon*
_____	Botany Department, University of Narth Carolina, Chapel Hill	As above
Cohn, W. E.	Biology Seminar and Graduate School Seminar, Medical College of Virginia, Richmond	Ion-exchange chromatography and the structure of nucleic acids
Conger, A. D.	University of Arkansas, School of Medicine, Little Rock	Radiobiological studies at atomic bomb detonations
_____	Radiol. Soc. N. Amer., Los Angeles, Calif.	The effect of oxygen on radiosensitivity of mammalian cells
Doherty, D. G., and W. T. Burnett, Jr.	Am. Chem. Soc., New York City	A study of the protective effect of a series of β-mercaptoethylamine derivatives on X-irradiated mice [Abstr. of Papers, 126th Natl. Meet. Am. Chem. Soc., p. 9C (1954)]
Dolin, M. I.	Department of Biochemistry, Duke University, Durham, N. C.	DPNH peroxidase

Green, J. A.	Am. Soc. Zool., Chapel Hill, N. C.	Ovarian weight and responsiveness to gonadotrophin during the estrous cycle of mice [Abstr., *Anat. Record* **120**, 714–715 (1955)]
Hollaender, A. Paper read by N. E. Tolbert	Joint session – AAAS, Am. Soc. Plant Physiol., and Western Soc. Naturalists, Berkeley, Calif.	Report on Congrès International de Photobiologie, Amsterdam, August 1954 (Introduction to Symposium on Photochemical Effects in Biological and Biochemical Systems)
_____	Natl. Bureau of Standards, Washington, D. C.	Presentation of the Niels Finsen Medal of Comité International de Photobiologie to Dr. W. W. Coblentz
_____	Natl. Bureau of Standards, Washington, D. C.	Some biological effects of ultraviolet and X irradiation
_____	Indiana University, Bloomington	Some studies on the basic mechanism of radiation damage in biological materials
_____	Research Department of Eli Lilly & Company, Bloomington, Ind.	As above
_____	Department of Botany, University of North Carolina, Chapel Hill	Radiation effects on bacteria
_____	Department of Zoology, Duke University, Durham, N. C.	As above
Khym, J. X., A. B. Ottinger, and W. E. Cohn	S. E. Section Am. Chem. Soc. Birmingham, Ala.	The hydrogen bond as o factor in the ion-exchange separation of the pentose phosphates and of the glycerol phosphates [Abstr., *Anal. Chem.* **26**, 1856 (1954)]
Khym, J. X., L. P. Zill, and W. E. Cohn	S. E. Section Am. Chem. Soc. Birmingham, Ala.	Relation between structure and elution position for the saccharide-borate complexes [Abstr., *Anal. Chem.* **26**, 1856 (1954)]
Kimball, R. F.	Department of Zoology, University of Maryland, College Park	The role of oxygen and peroxide in radiation damage to cells, especially *Paramecium*
_____	Biology Department, Emory University, Atlanta, Ga.	As above
_____	Biology Department, Agnes Scott College, Decatur, Go.	Some aspects of the induction of genetic changes by radiation
Kimball, R. F., and N. Gaither	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Gainesville, Fla.	Lack of on effect of a high dose of X rays on aging in *Paramecium aurelia*, variety 1 [Abstr., *Genetics* **39**, 977 (1954)]
Krall, A. R.	Am. Inst. Biol. Sci. (Botan. Soc. Amer.), Gainesville, Fla.	Further studies on the oxygen requirement of photosynthetic carbon dioxide fixation in barley

Parker, D. R.	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Gainesville, Fla.	Role of the Y chromosome in induced detachment of attached-X chromosomes in *Drosophila melanogaster* [Abstr., *Genetics* **39**, 986 (1954)]
Pittenger, T. H., and K. C. Atwood	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Gainesville, Fla.	The relation of growth rate to nuclear ratio in *Neurospora* heterocaryons [Abstr., *Genetics* **39**, 987 (1954)]
Roberts, H. S.	Soc. Am. Zool., Chapel Hill, N. C.	The mechanism of cytokinesis in neuroblasts of *Chortophaga viridifasciata* (De Geer) [Abstr., *Anat. Record* **120**, 726–727 (1955)]
Russell, L. B.	University of Oklahoma, Norman	Effects of radiation on the prenatal development of mammalians
_____	The University of Tennessee Medical School, Memphis	As above
W. L. Russell	Conference on Genetics, Argonne National Laboratory, Chicago, Ill.	Part in round table discussion "Mutation rate Studies"
_____	University of Oklahoma, Norman	Genetic hazards of radiation
_____	The University of Tennessee Medical School, Memphis	As above
Sheppard, C. W.	Department of Experimental Medicine, Vanderbilt University, Nashville, Tenn.	Some physical considerations in the field of circulatory mixing
Tolbert, N. E.	Joint session – AAAS, Am. Soc. Plant Physiol., and Western Soc. Naturalists, Berkeley, Calif.	Biochemical changes during greening of etiolated plants
_____	Soc. Gen. Physiol., Western Section (AAAS), Berkeley, Calif.	An unknown phosphate ester abundant in plants
_____	Am. Inst. Biol. Sci. (Am. Soc. Plant Physiol.), Gainesville, Fla.	1. Formate metabolism in plants
_____		2. Organophosphorus compounds of the xylem sap
_____	University of Oklahoma, Norman	Plant metabolism studies with C^{14}-labeled substrates
_____	University of Utah, Salt Lake City	Biochemical changes during greening of etiolated plants
_____	Radiation Laboratory, University of California, Berkeley	Informal discussion on "Excretion of glycolic acid by *Chlorella*"
_____	Department of Zoology, University of Texas, Austin	Plant metabolism studies with C^{14}-labeled substrates
Totter, J. R.	Biochemistry and Physiology Departments, Indiana University, Bloomington	The use of bioluminescence in enzyme studies
Upton, A. C.	Tenn. State Medical Soc., Morristown, Knoxville, and Johnson City, Tenn.	Radiation sickness and internal radiation problems

———	Tenn. Soc. Med. Technol., Knoxville	Acute radiation injuries
———	Lions Club, Knoxville	As above
———	Argonne National Laboratory, Symposium on Radiological Physiology, Chicago, Ill.	Hemorrhage and related effects
Upton, A. C., and J. Furth	Third Intern. Congr. Clin. Pathol., Washington, D. C.	ACTH-secreting pituitary tumors induced in mice by whole-body irradiation [Abstr., *Am. J. Clin. Pathol.*, **24**, No. 8 Suppl. (1954)]
Wilbur, K. M., C. B. Kenaston, N. Wolfson, A. Ottolenghi, and M. E. Gaulden	Am. Soc. Zool., Chapel Hill, N. C.	Inhibition of cell division by ultraviolet irradiated methyl linolenate [Abstr., *Anat. Record* **120**, 708–709 (1954)]
Wolff, S.	Beta Beta Beta, Biol. Soc., Spring Hill College, Spring Hill, Ala.	The effects of radiation on hereditary material
———	School of Veterinary Medicine, Tuskegee Institute, Ala.	As above
Wolff, S., and R. C. von Borstel	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Gainesville, Fla.	The effect of pre- and postirradiation centrifugation on the chromosomes of *Tradescantia* and *Vicia* [Abstr., *Genetics* **39**, 1001 (1954)]
Zill, L. P., and A. R. Krall	Am. Inst. Biol. Sci. (Am. Soc. Plant Physiol.), Gainesville, Fla.	Phosphorylated compounds in plants: separation into groups by ion-exchange chromatography

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

Dr. L. M. N. Duysens	Department of Botany, University of Illinois, Urbana	Studies on oxidation-reduction reactions in photosynthesizing cells by means of sensitive absorption spectrophotometry
Dr. Melvin M. Green	University of California, Berkeley	Pseudo alleles in *Drosophila*
Dr. Daniel M. Brown	University Chemical Laboratory, Cambridge, England	Problems in nucleic acid chemistry
Dr. J. R. Preer	Department of Zoology, University of Pennsylvania, Philadelphia	The cytoplasmic factor, kappa, in *Paramecium*
Dr. Edwin S. Lennox	Department of Bacteriology, University of Illinois, Urbana	Genetic transduction in strains of *E. coli*
Dr. Franz Moewus	University of Heidelberg; presently, Columbia University, New York	Function and biosynthesis of sex hormones in *Chlamydomonas*
Dr. D. V. Whittaker	College of Medicine, University of Cincinnati, Cincinnati	The biological occurrence of physiologically active homologs of acetylcholine

Dr. Alfred Glücksmann	Strangeways Research Laboratory, University of Cambridge, England	The pathological effects of beta radiation on the skin
Dr. Michael Evenari	Hebrew University, Jerusalem, Israel	The influence of light upon seed germination
Dr. Joseph Leighton	National Cancer Institute, National Institutes of Health, Bethesda, Md.	The growth of some normal and neoplastic tissues in sponge matrix tissue culture
Dr. J. B. Thomas	Biophysical Research Group, Der Rijks University, Utrecht, The Netherlands	On the substructure of the chloroplast
Dr. E. G. Butler	Department of Biology, Princeton University, Princeton, N. J.	The effects of ultraviolet radiation on regenerative activity in urodele limbs
Dr. Ray D. Owen	Division of Biology (Kerckhoff Laboratories), California Institute of Technology, Pasadena	Genetic determination of serological specificities
Dr. E. W. Caspari	Department of Biology, Wesleyan University, Middletown, Conn.	Genes and mitochondria
Dr. E. P. Cronkite	Brookhaven National Laboratory, Upton, New York	(Classified)
Dr. Bruce Wallace	Long Island Biological Association, Cold Spring Harbor, New York	Studies on irradiated populations of *Drosophila melanogaster*
Dr. James D. Ebert	Department of Zoology, Indiana University, Bloomington	Some aspects of protein biosynthesis in development
Dr. H. S. Kaplan	National Institutes of Health, Bethesda, Md.	Biological studies on the factor in bone marrow (and spleen) responsible for hematopoietic recovery in irradiated mice
Dr. Peter Alexander	The Chester Beatty Research Institute, The Royal Cancer Hospital, London, England	Physico-chemical methods of protecting against ionizing radiations
Dr. Paul K. Stumpf	National Institutes of Health, Bethesda, Md.	The role of glycolic acid in fatty acid oxidation
Dr. James S. Dinning	Department of Biochemistry, University of Arkansas School of Medicine, Little Rock	Vitamin E and nucleic acid turnover

Education. Participation by Biology Division members in education projects during the period has been represented by the following:

1. Dr. Mary Esther Gaulden was granted leave of absence for the Fall 1954 Quarter to serve on the instructoral staff of the University of North Carolina, Chapel Hill.
2. Dr. W. L. Russell took part in the Fourth Regional Symposium on the Uses of Atomic Energy; he lectured at the Duke University session.
3. A course in radiological physics is being given for the benefit of recent members of the Division staff not yet well acquainted with the fundamentals of radiation physics and dosimetry. Dr. J. S. Kirby-Smith is in charge of the course.

Eighth Annual Biology Research Conference. Plans for the eighth in the series of spring conferences sponsored by the Biology Division of Oak Ridge National Laboratory and supported by the Atomic Energy Commission are in the final stage. The conference, which will be held in Gatlinburg on April 4–6, 1955, will present a symposium on "Enzyme and Protein Structure." Members of the Biochemistry Section of this Division have been active in arranging the program, especially D. G. Doherty and W. E. Cohn. The program follows.

First session – Chairmen: S. Spiegelman, University of Illinois, and H. B. Bull, State University of Iowa
- "The Nature of the Protein Molecule" – F. Haurowitz, Indiana University
- "Mechanism of Protein Synthesis" – H. Borsook, California Institute of Technology
- "Incorporation of Labels into Proteins" – P. C. Zamecnik, Harvard University
- Discussers: F. W. Putnam and S. Spiegelman, University of Chicago, and R. B. Roberts, Carnegie Institution of Washington

Second session (evening) – Round-table discussion on "Nucleoproteins"

Third session – Chairmen: W. E. Cohn, Oak Ridge National Laboratory, and A. L. Dounce, University of Rochester
- "Studies on the Denaturation of Proteins" – W. Kauzmann, Princeton University
- "Recent Chemical Studies on Enzymes" – H. Fraenkel-Conrat, University of California
- "Macromolecular Protein Structure" – J. T. Edsall, Harvard University
- "Incorporation of Labeled Amino Acids into Enzymes" – S. F. Velick, Washington University
- Discussers: B. Vennesland, University of Chicago; A. L. Dounce, University of Rochester; H. B. Bull, State University of Iowa; R. M. Herriott, Johns Hopkins University

Fourth session – Chairmen: R. B. Roberts, Carnegie Institution of Washington, and H. Borsook, California Institute of Technology
- "Molecular Geometry in Enzyme Catalysis" – D. E. Koshland, Brookhaven National Laboratory
- "Kinetic Effects of the Ionization of Groups in the Enzyme Molecule" – R. A. Alberty, University of Wisconsin
- "Energetics of Enzyme-Substrate Complex Formation" – D. G. Doherty, Oak Ridge National Laboratory
- Discussers: G. E. Perlmann, Rockefeller Institute for Medical Research; E. Ellenbogen, University of Pittsburgh

Speakers at Professional Meetings, Spring 1955. From March 1 to July 1 of this year, 51 papers are scheduled to be delivered by Biology Division members before 16 professional groups.

At the *Sixth National Research Council Conference on Radiation Cataracts* in Washington, D. C., A. C. Upton will be the principal speaker in a discussion also authored by K. W. Christenberry and G. S. Melville. Three papers will be given at the *American Chemical Society* in Cincinnati, two by A. R. Krall and one by M. J. Cormier; also attending will be L. P. Zill and J. X. Khym. D. G. Doherty will represent the Biology Division at the *Eighth Annual Biology Research Conference*, held this year in Gatlinburg. J. N. Dent, R. Auerbach, and W. D. Gude will give papers at the *American Association of Anatomists* in Philadelphia. The program of the *Federation of American Societies for Experimental Biology*, San Francisco, will list papers to be given by N. E. Tolbert, J. R. Totter, A. C. Upton, N. G. Anderson, and W. E. Cohn (two papers will be given by Dr. Cohn); E. Volkin will also attend this meeting. D. M. Morris will present a paper at the *American Association for Cancer Research*, which meets with the *Federation* in San Francisco. At the *Association of Southeastern Biologists* in Charleston, South Carolina, four papers will be given on Biology Division research, by E. C. Horn, C. W. Hinton, W. St. Amand, and G. St. Amand. M. E. Gaulden, who is secretary of the *Association*, will also attend.

Seven papers will be presented at the *Society of American Bacteriologists* in New York City by the following investigators: S. F. Carson, G. E. Stapleton, D. Billen, C. O. Doudney, A. J. Sbarra, J. F. Christman, and M. I. Dolin. Dr. Alexander Hollaender, who is coauthor of three of the papers, will attend

the meeting. While in New York, Dr. Hollaender will take part in the discussions at the National Research Council *Conference on Basic Mechanisms of Radiobiology*, at Bear Mountain Inn.

New York City will also be the place of meeting for the *Radiation Research Society*. Sixteen papers on work from this Division will be presented; one of the papers will be presented by a member of another division since the work was a cooperative effort. Biology Division speakers are: R. F. Kimball, W. T. Burnett, Jr., T. T. Odell, S. Wolff, A. R. Whiting (consultant), R. C. von Borstel, W. St. Amand, A. C. Upton, G. S. Melville, M. L. Randolph, K. C. Atwood, and L. B. Russell. Three demonstration papers will be presented by a group composed of A. Hollaender, G. E. Stapleton, D. Billen, C. O. Doudney, A. J. Sbarra, W. T. Burnett, Jr., and A. W. Kimball (Mathematics Panel). Dr. C. C. Congdon, who joined the Biology Division this winter, will attend the meeting and present a paper on work done in another laboratory.

A. D. Conger will give a paper in the *Symposium on Ascites Tumors*, sponsored by the *New York Academy of Sciences*, in New York City. W. H. Benedict (consultant) will speak at the joint meeting of the *American Medical Association* and *Association for Research in Ophthalmology* in Atlantic City. At the *Brookhaven Symposium No. 8*, on *Mutations*, W. L. Russell, J. S. Kirby-Smith, and W. K. Baker will represent this Division. At the newly organized *American Nuclear Society*, Pennsylvania State College, another talk by Dr. Hollaender will be given.

CYTOGENETIC EFFECTS OF RADIATION

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

Effects of X Rays on Nuclear Behavior at Conjugation in *Paramecium aurelia*

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

Conjugation in *Paramecium aurelia* involves a number of steps of which the following are important for this report, (1) meiosis of the two diploid micronuclei to give eight meiotic products, (2) entrance of one product into the paroral cone and disintegration of the rest, (3) formation of the male and female gamete nuclei by mitotic division of the cone nucleus, (4) penetration of the male gamete nucleus into the other conjugant and fusion with female gamete nucleus, and (5) formation of a new nuclear apparatus (micronuclei and macronucleus) from the synkaryon so formed in each conjugant. These processes involve a series of divisions and movements of the nuclei which must proceed in an orderly manner in the great majority of pairs. Two previous reports[2,3] have dealt with the disturbances in this process caused by high doses of X rays and by haploidy of the original micronuclei. The present report extends the analysis further, especially with regard to the combined effects of radiation and micronuclear haploidy.

The irradiated animals of stock 90 origin were mated with unirradiated ones of stock CD origin. After conjugation, the clones derived from two members of the pair were cultured separately and their phenotype determined for growth and survival and for some of the gene markers in which stocks 90 and CD differ. Only the clones from the CD member will be considered in this report. These clones can be divided into three categories: (1) hybrid clones with normal growth, (2) clones with normal growth and with nuclei from CD only, and (3) clones with poor growth. Most of the clones in this latter category were probably hybrid although this could be demonstrated for only a part of them by direct test. Clones in category (1) and most of those in (3) must have received a male gamete nucleus from the stock 90 member of the pair whereas clones of category (2) did not. Three kinds of clones of stock 90 origin were used, clones with two diploid micronuclei, clones with two haploid micronuclei, and a clone with one diploid and two haploid micronuclei.

The frequency of clones in the three categories is shown in Fig. 1 for crosses involving these three kinds of clones given different doses of X rays. Most hybrid clones from the cross of diploid CD with haploid 90 grew poorly (Fig. 1B) as has already been reported.[3] This is a result of the serious genic imbalance occasioned by the fusion of grossly deficient meiotic products from the haploid micronuclei with normal meiotic products from CD. Some failure of male gamete nuclei to function (category 2 clones) also occurs in this case, which suggests that deficiencies in the genome of the meiotic products can interfere with their behavior. However, a high dose of radiation

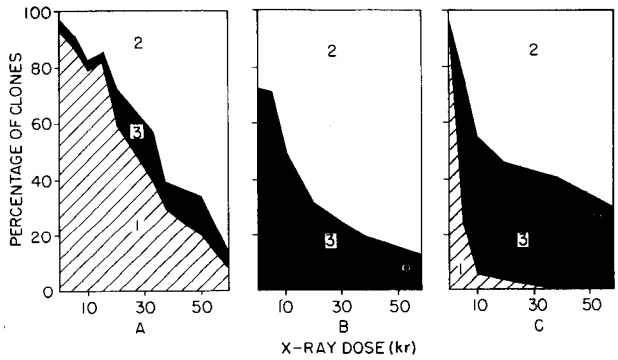

Fig. 1. (A) Cross of stock CD with irradiated, diploid stock 90. Proportion of clones of CD cytoplasmic origin in the three categories: (1) hybrid clones with good growth, (2) clones with good growth and nuclei from CD only, and (3) poorly growing clones. **(B) Same as A for crosses of stock CD with an irradiated haploid clone of stock 90 origin. (C) Same as B for crosses of stock CD with an irradiated clone with both haploid and diploid micronuclei.**

[1]Research Associate.

[2]R. F. Kimball, N. Gaither, and M. K. King, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1614, pp. 11-12.

[3]R. F. Kimball, N. Gaither, and M. K. King, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, p. 11.

to the diploid causes a greater failure of the male gamete nucleus than in the haploid control whereas most of the hybrid clones are still viable. Thus the production of deficiencies by the radiation cannot account for all the failure of the male gamete nucleus from the irradiated diploid. The frequency of failure of the male gamete nucleus increases more rapidly with X-ray dose in the haploid than in the diploid. This suggests that the portion of the radiation effect not caused by deficiencies is either produced more effectively in the haploid micronucleus or interacts with the deficient genome from meiosis to produce more effect.

Figure 1C shows the result of competition between meiotic products from diploid and haploid micronuclei for entrance into the paroral cone. In the unirradiated material, the diploid almost always succeeds and so gives rise to the gamete nuclei. However, as the dose increases, the competitive advantage passes to the haploid so that at high doses the only contribution seems to be from these nuclei. The competitive disadvantage of the haploid nuclei in the controls is probably to be attributed to the genetic deficiency of their meiotic products. Radiation-induced deficiencies might bring the diploid nucleus to equality with the haploid nuclei but could hardly put it at a disadvantage.

Thus two different tests for an effect of radiation on nuclear behavior have shown that radiation-induced deficiencies can account for, at most, only part of the effects. However, the two tests must be for different kinds of nuclear behavior since the test for competition for entrance into the paroral cone shows that the haploid is less affected than the diploid by X rays, whereas the test for male gamete nucleus formation shows the reverse.

Form of Dose Curve for Mutations in *Paramecium*: Evidence from Fractionation and Intensity Studies with X Rays and from the Dose Curve for Fast Neutrons

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

Kimball[4] has shown that the number of mutations per micronucleus induced by radiation in *Paramecium aurelia* can be calculated from the relation $m = 2(\ln P_0 - \ln P)$ where P_0 and P are the fractions of exautogamous clones with normal growth for the controls and treated animals respectively. However, it was found that the relation of m to dose was nonlinear for beta particles from an external P^{32} source. The simplest explanation of this nonlinearity would seem to be that it resulted from two-hit chromosome aberrations which, on theory, should be included in the events detected. However, a fractionation experiment failed to give any evidence for such aberrations and so an alternative hypothesis, the occurrence of many mutations with such small effects that several were needed to affect growth appreciably, was adopted and some evidence for it presented. Powers[5] pointed out that in his material a linear relation of m to X-ray dose was found, and our more recent work has also shown a more nearly linear relation with X rays though the relation still departs appreciably from linearity.

The problem of the nonlinear component was reopened and several new experiments were performed. Several new fractionation and intensity experiments were carried out with X rays and, in contradiction to our earlier findings, an intensity effect was found (Table 1). This suggests that two-hit aberrations are an appreciable component of the total effect.

If the nonlinear component for X rays was mainly two-hit aberrations, then on analogy with other organisms, it should be greatly decreased or nearly eliminated for neutrons. Therefore, a neutron dose curve was obtained with the 86-inch cyclotron. Dr. C. W. Sheppard of the Physiology Group and Mr. Morris Slater of the Biophysics Group gave valuable advice and assistance in carrying out the irradiations. A description of the facility and methods of measurement have been given elsewhere.[6] The dose was given at approximately 100 rep/minute.

The results are shown in Fig. 2 with two other curves for comparison. The curve for beta particles is from the previously published data[4] whereas the X-ray curve was obtained a few weeks before the neutron curve. The beta-ray dose rate was about 200 rep/min and was given at constant intensity with time varied. The X rays were from the Maxitron 250 machine operated at 250 kvp with 3 mm of Al filtration (hvl, 0.55 mm of Cu). Time was held

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

[5] E. L. Powers, *J. Cellular Comp. Physiol.* 35, Suppl. 1, 168 (1950).

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

TABLE 1. EFFECT OF FRACTIONATION AND INTENSITY OF X RAYS ON MUTATION INDUCTION IN *PARAMECIUM AURELIA*

Procedure	Dose (r)	Number of treated or control animals	Percentage of normal exautogamous clones
Single dose given at 1331 r/min	2663	63	55.6
Three fractions with 3 min rest periods, each fraction given at 488 r/min	2657	63	62.0
Control	None	21	98.1
Single dose at 1476 r/min	2952	120	46.7
Six fractions with 5 min rest periods, each fraction given at 99 r/min	2962	60	57.1
Control	None	20	99.2
Single dose at 1412 r/min	2823	63	36.4
Single dose at 77 r/min	2823	63	48.8
Control	None	21	89.7

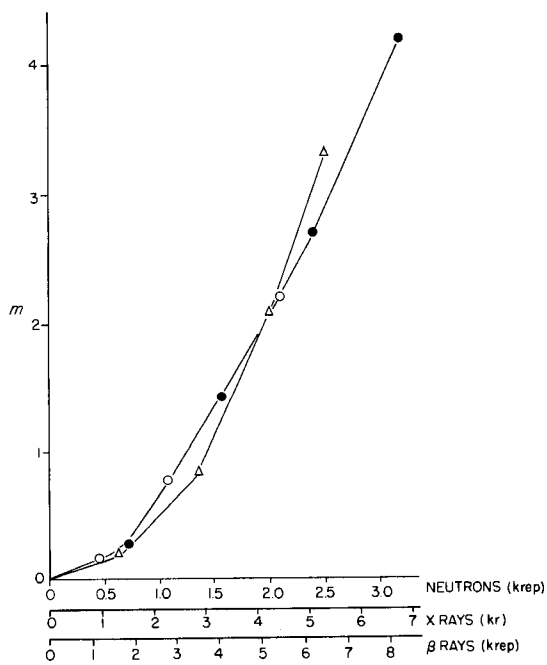

Fig. 2. The function m (see text) plotted against dose for fast neutrons, X rays, and β particles. Dose scales which have been used make the values for $m = 2$ coincide. The points are weighted averages of the values from several different experiments. O, neutrons; ●, X rays; Δ, β particles.

constant at 3 min and intensity was varied by changing the tube current. In order to compare the departures from linearity in the three curves, the dose scales were chosen so that the point for $m = 2$ coincided for all three curves. In comparison to X rays, the relative biological effectiveness (RBE) for neutrons was 2.1 and for beta rays, 0.8. However, neither RBE should be taken very seriously since the curves were not determined in simultaneous experiments and there may have been errors of dosimetry, especially for the beta particles. However, it is fairly safe to assume that the RBE for fast neutrons compared to X rays is greater than 1 for mutations in *Paramecium* as detected by our method.

The main point for our present purpose is that the neutron curve shows about the same departure form linearity as the X-ray curve. The beta-ray curve apparently departs appreciably from the form of the other two. Since the X-ray curve was obtained by varying the intensity, the nonlinearity from two-hit aberrations should have been maximized. Therefore the close agreement of the two is difficult to explain on the two-hit aberration hypothesis for the nonlinearity. This explanation would be possible only if, unlike *Tradescantia*, the probability of a single proton track producing

two breaks is very small. However, all the chromosomes in the micronucleus are concentrated in a very small ring. Thus the probability of a single track traversing several chromosomes must be quite high. This evidence, therefore, suggests that very little of the nonlinear component is the result of two-hit aberrations.

Thus the experiments lead to conflicting interpretations, and no final explanation is possible. Either the probability of two breaks by a single event is really not very different for neutrons and for X rays or the intensity experiments must be interpreted in some other way than by two-hit aberrations. A further complication in explaining the nonlinear dose curve is introduced by the evidence of selective effects of deficiencies on nuclear behavior during sexual processes.[7]

A Method of Fusion Analysis to Explain How Oxygen Causes an Increase in Chromosomal Aberration Production

A. D. Conger A. H. Johnston

It has been clearly demonstrated that, for the same dose of radiation, more chromosomal aberrations are produced by irradiation in the presence of oxygen than in its absence. There has been considerable disagreement in the literature as to the mechanisms by which oxygen achieves this effect. The number of aberrations, i.e., the visible products, resulting from a given dose of radiation is dependent on two unseen events: (1) the number of primary breaks produced by the radiation and (2) the fraction of these primarv breaks which fuse in such a way that aberrations are formed. It is known that a large fraction of the primary breaks re-fuse ("restitute") in their original way, and are therefore undetectable; the fraction that do not so re-fuse result in the visible aberrations. The first factor can be called breakage rate, the second fusion rate. It is obvious that oxygen could cause its increase in aberration yield by increasing breakage rate, decreasing fusion rate, or a combination of both.

In the absence of any measurement of these primary factors, it is understandable how disagreement over the mechanism of the "oxygen effect" on chromosomes could arise and persist. We have developed a method for *Tradescantia* by which the fusion of breaks can be measured. If fusion were known, as well as the aberration yield, a solution for the relations among the three related events – breakage, fusion, and aberrations – would be possible. The result of applying the fusion analysis to the influence of oxygen on chromatid types of aberrations in *Tradescantia* shows that: (1) both kinds of primary breaks, chromatid and isochromatid, are increased equally by oxygen, (2) the fusion of isochromatid aberrations is the same in the presence of oxygen as in its absence, i.e., the same proportion of breaks result in aberrations, and (3) the fusion of chromatid breaks is twice as great in oxygen as in its absence, i.e., in oxygen only half as many breaks result in aberrations as in nitrogen. The analysis also explains how each of the three principal types of aberrations – chromatid deletions, isochromatid deletions, and chromatid exchanges – can have different air/nitrogen ratios, and predicts for each a ratio that agrees with the experimentally observed ratios.

Inflorescences of *Tradescantia paludosa* (Sax clone #3) were irradiated in oxygen, air, or nitrogen with 250-kvp X rays filtered through ½ mm of Cu plus 3 mm of Al (hvl, 1.45 mm of Cu) at intensities of about 450 r/minute. The gas treatment was given for 10 min before, and during the irradiation. Acetocarmine smear slides were made 22-24 hr later for chromatid aberrations and 4 days later for chromosome aberrations, and frequencies of various types of aberrations were scored at metaphase of microspore mitosis. All scoring was done under code and by one person (ADC).

Derivation of Fusion Rates. Only three types of aberrations are produced in any significant numbers by irradiation in prophase, and these aberrations arise from the only two kinds of breaks, chromatid and isochromatid, that can occur. Figure 3 is a diagram of the fusion behavior of the two kinds of breaks producing the three kinds of aberrations. The fusion analysis is possible because, by careful observation, the various fusion types illustrated can be distinguished in the visible aberrations. In addition to the fusion terms of Fig. 3, the following are necessary:

f = fraction of chromatid breaks that result in chromatid deletions seen at metaphase, or,

$$f = \frac{\text{total chromatid deletions}}{\text{total chromatid breaks}},$$

z = same definition, for isochromatid breaks.

[7] R. F. Kimball, N. Gaither, and M. K. King, this report.

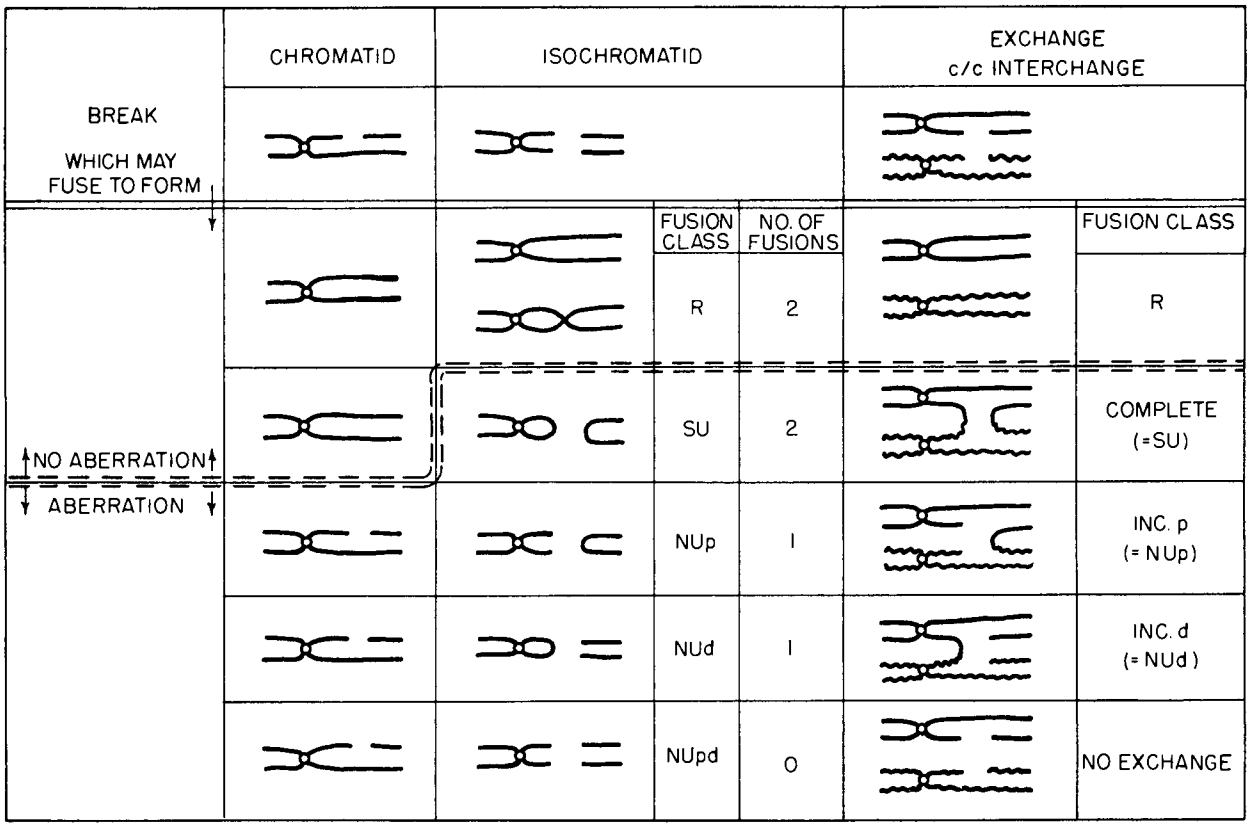

Fig. 3. Fusion fates of chromatid and isochromatid breaks. R, restitution (therefore not seen as aberration) – 1-f and 1-z; SU, sister union; NUp, nonunion, proximal; NUd, nonunion, distal; NUpd, nonunion, proximal and distal; Comp, complete; Inc, incomplete.

As can be seen, the problem is one of accounting numerically for all the fates of a break, including the unseen restitution event. For isochromatid breaks, magnitude of the R (restitution) event can be estimated in several ways. An isochromatid break which fuses at both ends, if it does so at random, will produce exact restitution, crisscross restitution, and SU in the ratio 1:1:1, the ratio of R:SU will be 2:1. Most conceivable factors should favor SU over R, making the ratio more nearly 1:1. Lea and Catcheside,[8] in fast neutron experiments, were able to estimate dosimetrically the number of chromosomal "hits" and hence the number of primary breaks produced by a given dose, and thus to solve directly for f and z; the ratio of R:SU was 1:1. A third and independent method allows estimation of a minimum value for R for chromatid aberrations, and is derived from intensity experiments. The basis for this method is that chromatid deletions are independent of intensity, whereas chromatid exchanges increase with intensity, the excess exchanges being derived from the R class of fusion. The ratio of R:SU events from this estimate is also about 1:1 as for the other two methods, and this ratio will therefore be accepted. Since it is known that the invisible R fusion event is numerically about equal to the SU event, z for isochromatids can now be expressed in the fusion terms of Fig. 3 as:

$$z = \frac{SU + NUp + NUd + NUpd}{2SU + NUp + NUd + NUpd} = \frac{\text{total isochromatid aberrations}}{\text{total isochromatid breaks}}$$

where $R \approx SU$.

[8] D. E. Lea and D. G. Catcheside, *J. Genet.* 44, 216-245 (1942).

The derivation of f for chromatid deletions is a bit more complex, since, as can be seen on Fig. 3, all the chromatid deletions look exactly the same, and no direct fusion analysis is possible. However, the fusion behavior of chromatid breaks can be observed in chromatid/chromatid (c/c) interchanges (Fig. 3), which display the same fusion classes that are found in isochromatid deletions, and there is no reason to suppose that those chromatid breaks which, by accident, participate in an exchange are any different from those which do not. It is apparent that there must be a relation between fusion in isochromatid breaks and in chromatid breaks, and the relation can be tested by comparing fusion of isochromatids and c/c interchanges as follows:

$$\frac{NUp + NUd}{SU + NUp + NUd}$$

for isochromatids with

$$\frac{Inc.p + inc.d}{Comp. + inc.p + Inc.d}$$

for c/c interchange or, I_i with I_c.

It will be shown in the experimental data that $I_c = I_i$ from irradiation in air or in oxygen. Now, since the ratios of all fusion classes to one another are known for isochromatids and it is also known that the isochromatid and chromatid fusions are equal, f can be expressed in fusion terms as

$$f_{air} = \frac{NUp + NUd + NUpd}{2SU + NUp + NUd + NUpd} .$$

However, in nitrogen, it is found that c/c interchanges are about twice as incomplete as in air, or $I_{c_{N_2}} \approx 2\, I_{c_{air}}$, so that the proper expression for f in nitrogen will be

$$f_{N_2} = f_{air} \times \frac{I_{c_{N_2}}}{I_{c_{air}}} .$$

With f and z known, it is possible to solve for the relation among breakage, fusion, and aberrations, for isochromatid and chromatid deletions. The only remaining unknown is fusion for c/c interchanges. The yield of c/c interchanges is clearly dependent on chromatid fusion, and will be greater as chromatid fusion increases. In the derivation of f, the proportions of chromatid breaks which fuse (or are capable of fusing) at both ends, only one end, or neither end were measured. By examination of all possible pair combinations that can be made from the different fusion types of chromatid breaks, and rejection of those pairs which will not form an exchange, and then assignment to each fusion type its measured frequency, it can be shown that the yield of exchanges is related to fusion as

$$\epsilon = (1 - f)(1 - f) + 4(1 - f)(2/5\, f) + 2(2/5\, f)(2/5\, f) .$$

Experimental Data. The combined fusion data for isochromatid and exchange aberrations produced by X irradiation in air or oxygen and nitrogen in three different experiments are given in Table 2. The aberration data show that the proportion of isochromatids in each of the fusion classes is approximately the same for air and nitrogen, whereas the fraction of chromatid exchanges which are incompletely fused is about twice as great in nitrogen as in air. It has been shown previously that aberration yield is approximately the same in air as in oxygen, and it is found here that the same is true for fusion also; for simplicity, the term "air" will be used to describe irradiation in air or oxygen, and "nitrogen" will be used for anoxia.

The fusion constants (Table 3), z, f, and ϵ in air and in nitrogen have been extracted from Table 2, and the ratios of these in air (oxygen is equivalent) and in nitrogen are compared. We are inclined to attach greatest weight to the values from the last experiment, since, by that time, we were most experienced in the observation of aberration fusion types. The ratios of the air/nitrogen fusion values is almost exactly 1:1 for z, 1:2 for f, and 0.87:1 for ϵ. This means that for the same number of "primary breaks" produced, equal numbers of isochromatid aberrations, twice as many chromatid deletions, and 0.87 times as many chromatid exchanges will result in nitrogen as in air.

It can now be shown that when the derived fusion values are applied to experimental data, there is satisfactory agreement between the observed air/nitrogen aberration yields, and the ratio predicted on the basis of the fusion values.

According to the definition of the fusion values,

TABLE 2. ISOCHROMATID AND c/c INTERCHANGE FUSION DATA

Experiment	Gas	Total cells	Isochromatids					c/c Interchanges			Incompleteness		z for isochromatids		f for chromatids	
			SU	NUp	NUd	NUpd	Total	Comp.	Inc.	Total	I_i for isocds.	I_c for inter-changes	Nos.	z	Nos.	f
Feb. 22, 1954	O_2	500	120	13	14	4	151	87	20	107	0.184	0.186	151/271	0.557	31/271	0.114
	N_2	300	78	8	14	3	113	57	31	88		0.352	113/181	0.625	1.9 f_{air}	0.216
Apr. 1954	O_2	575	166	16	15	7	204	72	20	92	0.157	0.217	204/370	0.551	38/370	0.103
	N_2	700	101	19	13	3	136	35	16	51		0.314	136/237	0.573	1.45 f_{air}	0.149
Aug. 24, 1954	Air	788	107	7	14	6	134	66	13	79	0.164	0.165	134/241	0.556	27/241	0.112
	N_2	700	98	13	9	6	126	60	26	86		0.302	126/224	0.562	1.83 f_{air}	0.205

TABLE 3. AVERAGE VALUES FOR FUSION CONSTANTS IN AIR AND IN NITROGEN, EXTRACTED FROM TABLE 2

Experiment	z for isochromatids			f for chromatids			ϵ for c/c interchanges		
	In air or O_2	In N_2	Ratio, N_2/air	In air or O_2	In N_2	Ratio, N_2/air	In air or O_2	In N_2	Ratio, N_2/air
Feb. 22, 1954	0.56	0.62	1.1/1	0.11	0.22	2/1	0.88	0.76	0.87/1
Apr. 13, 1954	0.55	0.57	1.04/1	0.14	0.19	1.45/1	0.84	0.99	0.94/1
Aug. 24, 1954	0.56	0.56	1/1	0.11	0.21	1.9/1	0.88	0.77	0.88/1
Accepted value	0.56	0.56	1/1	0.11	0.22	2/1	0.88	0.76	0.87/1

the relation among breaks, fusion, and aberrations is

$$c = f \cdot b_c \ ,$$

$$i = z \cdot b_i \ ,$$

$$e = b_c \cdot (\epsilon X)^n$$

where c is the number of chromatid aberrations, i is the number of isochromatid aberrations, e is the number of c/c interchanges, b_c is the number of chromatid breaks, b_i is the number of isochromatid breaks, and X is the dose in roentgens. The only unmeasured quantities are b_c and b_i, which, of course, can be solved for both in air and in nitrogen, since all other quantities are measured in both gases.

Because of the nonlinear increase in exchanges, it is necessary to have dose-response data. Table 4 is composed of data from these experiments, and Table 5 is a compilation of the ratios of aberration yields in air and in nitrogen from data in the literature. Table 6 presents a comparison of the observed and expected aberration ratios in air and in nitrogen; the expected ratios are calculated on the basis of the expressions given previously, the fusion values determined in these experiments being used. It can be seen that the observed and expected ratios are in good agreement with one another even though the air/nitrogen ratios vary from experiment to experiment. Only the c/c interchanges show any significant disagreement between observed and expected values from one another.

TABLE 4. YIELDS OF CHROMATID ABERRATIONS FROM X IRRADIATION IN AIR AND IN NITROGEN

(Aug. 24, 1954, 250-kvp X rays, hvl, 1.44 mm of Cu, 450 r/min. Slides made 22-24 hr later)

Gas	Dose (r)	No. of buds	No. of cells	No. of aberrations per cell for:		
				Chromatids	Isochromatids	c/c Interchanges
Air	22	3	200	0.125	0.055	0.005
	44	4	188	0.133	0.090	0.064
	88	3	200	0.30	0.215	0.095
	133	4	200	0.40	0.315	0.235
Nitrogen	88	4	200	0.310	0.045	0.015
	177	3	150	0.480	0.093	0.093
	265	2	150	0.814	0.253	0.127
	354	3	200	0.985	0.325	0.250

TABLE 5. CHROMATID ABERRATION YIELDS AND RATIO OF YIELDS IN OXYGEN, AIR, AND NITROGEN[a]

Experiment	Gas	Chromatids		Isochromatids		c/c interchanges	
		b	Ratio, air/N_2	b	Ratio, air/N_2	n	Ratio at $X = 100$ r
Riley, Giles, and Beatty,[b]	O_2	0.0023		0.0060		1.68	
Table 1	He	0.0018	1.28/1	0.00233	2.58/1	1.77	4.5/1
Swanson and Schwartz,[c]	Air	0.00555		0.00335		1.52	
Table 1 at 100 kvp, 24 hr	N_2	0.0053	1.05/1	0.00155	2.16/1	1.62	3.0/1
Swanson[d]	Air	0.00734		0.0042			
	N_2	0.00455	1.63/1	0.0011	3.8/1		
This report, Table 4	Air	0.00316		0.00236		1.60	
	N_2	0.00288	1.10/1	0.00086	2.74/1	1.76	5.6/1

[a]From least-squares fits to expressions:

$Y = bX$ for chromatids and isochromatids

$Y' = b'X^n$ for c/c interchanges

where

y = aberrations per cell

X = X-ray dose, r.

[b]H. P. Riley, N. H. Giles, and A. V. Beatty, *Am. J. Botany*, 39, 592-597 (1952).

[c]C. P. Swanson, 1955, *Genetics*, in press.

[d]C. P. Swanson and D. Schwartz, *Proc. Natl. Acad. Sci. U. S.*, 39, 1241-1250 (1953).

TABLE 6. COMPARISON OF OBSERVED AND EXPECTED RATIOS OF ABERRATION YIELDS IN AIR AND IN NITROGEN. OBSERVED RATIOS EXTRACTED FROM TABLE 5. EXPECTED RATIOS CALCULATED FROM FUSION DATA (see text)

Experiment		Air/N_2 ratios for:		
		Chromatids	Isochromatids	c/c Interchanges at dose 100 r
Riley, Giles, and Beatty[a]	Observed	1.3/1	2.6/1	4.4/1
	Expected	1.3/1	2.6/1	2.2/1
Swanson and Schwartz[a]	Observed	1.05/1	2.2/1	3.0/1
	Expected	1.08/1	2.2/1	1.8/1
Swanson[a]	Observed	1.6/1	3.8/1	
	Expected	1.9/1	3.8/1	
This report, Table 4	Observed	1.1/1	2.7/1	5.3/1
	Expected	1.3/1	2.7/1	1.8/1

[a]See footnotes b, c, and d in Table 5.

However, since the calculated yield of exchanges is, of course, extremely sensitive to a change in the power, n, a quantity that is not measurable with great accuracy, such deviations from observed values are not surprising.

Anaphase Abnormality in the Ehrlich Ascites Carcinoma

W. St. Amand

A substrain of the Ehrlich ascites carcinoma maintained in this laboratory has shown a very high frequency of anaphase abnormality. The influence of: (1) time after inoculation, (2) age of host animal, and (3) sex of host animal have been determined.

The frequency of abnormal anaphases as a function of time after inoculation was tested in conjunction with factors 2 and 3. An initial decrease in the frequency of abnormal anaphases was followed by a linear or near-linear increase. At the end of the transfer generation, stabilization was indicated.

Twenty C_3H male mice were used to test the effect of host age (Fig. 4). Those in the young series were 4 months old; those in the old series were 9 months of age. All animals were inoculated with 0.2 ml of 10-day-old peritoneal fluid.

The curves shown in Fig. 4 are based on 8800

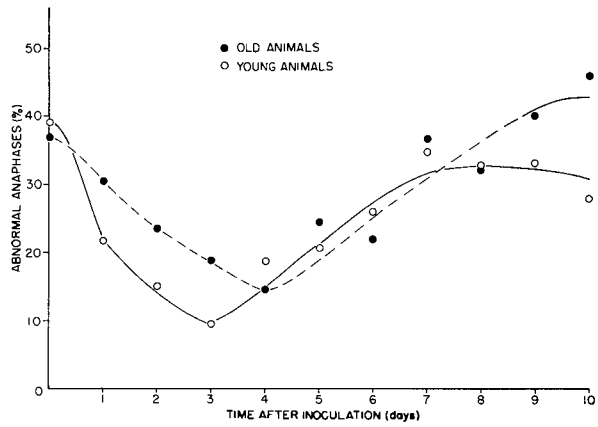

Fig. 4. Anaphase abnormality in old and in young mice as a function of time after inoculation. ●, old animals; O, young animals.

anaphases. Initial decrease of abnormality was more rapid in the young animals; the minimum occurred earlier and was at a lower level. Stabilization was at a lower frequency in young than in old animals.

All animals in the host sex test were C_3H mice 82-88 days of age, inoculated with 0.2 ml of 7-day fluid which had been pooled and diluted with heparinized Tyrode's solution. The curves (Fig. 5)

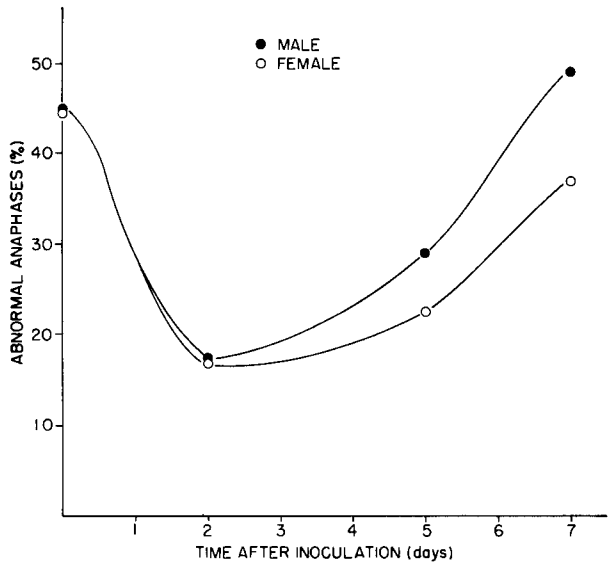

Fig. 5. Anaphase abnormality in male and in female mice as a function of time after inoculation. ●, male; O, female.

are based on 2800 anaphases. Stabilization at a lower level in the female than in the male is indicated.

It is difficult to explain the significance of the variations shown in Figs. 4 and 5. Changes of this type appear not to be unusual occurrences in tumors when, for example, a change is made in the host mouse strain. At the time the variability occurred, however, the tumor had been maintained in C_3H mice for 40 transfer generations. More recent observations indicate an abnormality frequency of about 10% and there is no evidence of wide fluctuations. It must be concluded that the data were obtained during a temporary phase of instability.

The Effect of X Rays on Anaphase Abnormality and Mitotic Index in the Ehrlich Ascites Carcinoma

W. St. Amand

This experiment was performed to characterize the response of ascites tumor cells after irradiation *in vivo*. The aminals used were male C_3H mice 7 months of age which had been inoculated 6 days previously with 0.2 ml of peritoneal fluid. Irradiation was with a Maxitron 250 X-ray machine operating at 250 kvp, 30 ma (filtration: 0.5 mm of Cu, 3 mm of Al). Samples of peritoneal fluid were taken just before treatment and at 3, 6, 12, 18, and 24 hr after irradiation.

In Fig. 6, mitotic index is shown as a function of time after treatment. The curves in this figure are based on 116,000 cells. In the 100-r curve, mitotic index is at a minimum about 3 hr after treatment. This is followed by a compensatory rise in mitotic activity. Mitotic index in the 200-r series decreases at about the same rate as with 100 r but reaches a minimum at about 6 hours. With 400 r, mitotic activity is at a minimum at about 12 hr, and very little recovery is seen at the end of 24 hours.

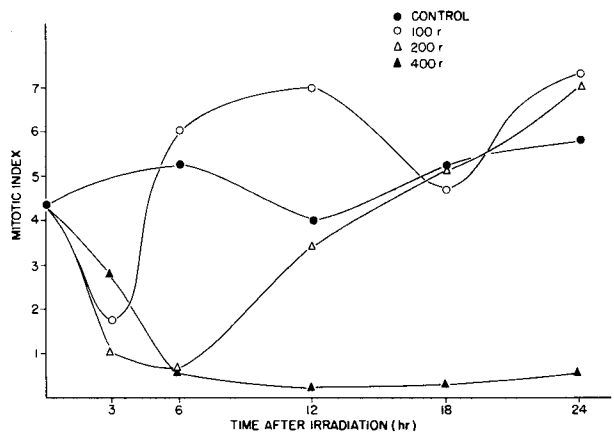

Fig. 6. Mitotic index in the Ehrlich ascites carcinoma after irradiation with 100, 200, and 400 r of X rays. ●, control; O, 100 r; Δ, 200 r; ▲, 400 r.

Normal anaphase frequencies are given in Fig. 7. These curves are based on 4500 anaphases. At all dose levels, normal anaphases decrease to a minimum and subsequently increase toward control level. The minimum is attained at 3 hr after 100 r, at 6 hr after 200 r, and at 12 hr after 400 r.

The minima of anaphase normality were found at periods of minimum mitotic activity. This could well lead to the conclusion that a causal relation exists between mitotic inhibition and anaphase abnormality. An earlier study with the grasshopper neuroblast, in which known mitotic stages were irradiated, yielded curves much like those in Figs. 6 and 7. In the grasshopper it is known, however, that the relation of chromosome breakage to mitotic inhibition is not a causal one. The relation of anaphase abnormality to mitotic index in the tumor

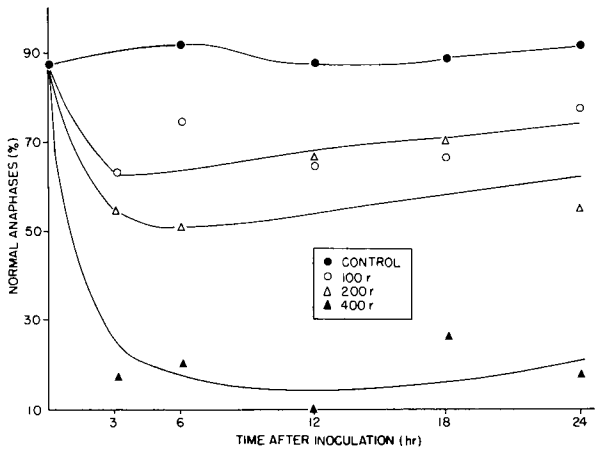

Fig. 7. Anaphase normality in the Ehrlich ascites carcinoma after irradiation with 100, 200, and 400 r of X rays. ●, control; O, 100 r; Δ, 200 r; ▲, 400 r.

is probably like that found in the neuroblast, and causality cannot be assumed.

Despite the deviations of the tumor cell population from "normal," the response to X rays of the Ehrlich tumor is identical to that of other materials.

The Effect of Oxidative Metabolism and ATP Formation on Rejoining of Chromosome Breaks

S. Wolff H. E. Luippold

Previous work from this laboratory has shown that in the seed, *Vicia faba*, the rejoining of radiation-induced chromosome breaks is inhibited by blocking cytochrome oxidase. This blocking was effected by postirradiation treatment with cold, KCN, or mixtures of 95% CO + 5% O_2 in the dark (but not in the light). It was also shown that removal of oxygen by placing the seed in a 4- to 5-mm vacuum did not prevent rejoining.

Experiments have been continued that elucidate the actual enzymatic mechanism of rejoining. These are presented in Table 7. The experimental procedure is the same as that reported previously and consists essentially of irradiating the soaked seed *in vacuo* with 600 r to produce breaks that ordinarily will rejoin within 30 minutes. Immediately after this irradiation the seeds are subjected to treatment with an inhibitor to prevent rejoining. Seventy-five minutes later a second dose of radiation is administered to determine by the numbers of 2-hit chromosome aberrations whether or not the broken ends formed by the first irradiation have rejoined.

As can be seen from the table, placing the seed in nitrogen immediately after the first irradiation does not inhibit rejoining. Thus the degree of anaerobiosis attained in this manner is no more effective than that achieved by 4-5 mm of vacuum. However, if the vacuum is combined with treatment of 2×10^{-3} M $Na_2S_2O_4$, which is a very efficient binder of intracellular oxygen, the breaks do indeed remain open for the 75-min duration of the experiment. This indicates that inhibition of cellular respiration, whether by enzyme inhibition (cytochrome oxidase inhibition, low temperature, etc.)

TABLE 7. THE EFFECT OF RESPIRATORY INHIBITORS ON CHROMOSOME REJOINING

Treatment immediately after dose 1 of 600 r *in vacuo* at time t	Dose 2 at t = 75 min (r)	No. of 2-hit aberrations per cell		
		Observed	Expected value	
			With rejoining	Without rejoining
N_2(Pyrogallol)	400 (Air)	0.167	0.195	0.380
$Na_2S_2O_4$ + vacuum	600 (Vac)	0.400	0.184	0.365
$Na_2S_2O_4$ + vacuum	600 (Vac)	0.407	0.184	0.365
1.5×10^{-5} M DNP	400 (Air)	0.240	0.195	0.380
1.5×10^{-4} M DNP	400 (Air)	0.377	0.195	0.380
3.0×10^{-4} M DNP	400 (Air)	0.367	0.195	0.380
1.5×10^{-3} M DNP	400 (Air)	Lethal		

or simply by complete oxygen removal, prevents normal rejoining of breaks.

It may also be observed in the table that treatment with 2,4-dinitrophenol (DNP) inhibits the repair of chromosome breaks. Inasmuch as DNP uncouples respiration from oxidative phosphorylation and thus prevents the formation of adenosine triphosphate (ATP), it is hypothesized that the role of respiration in the repair of chromosome breaks is to provide ATP as a source of energy to be utilized in the synthesis of those chemical bonds that join the previously broken ends.

To test this hypothesis, a series of dose intensity experiments were performed, the rationale behind this being that at low intensities of radiation some of the breaks formed rejoin before the full dose has been administered. Consequently, a given dose at low intensity results in fewer breaks being open simultaneously than would the same dose at higher intensity. In Fig. 8 it can be seen that 600 r, when administered in air to the seed, yields the same number of aberrations at 200 r/min as at 5 r/minute. This means that the chromosomes of *Vicia faba* seed irradiated with 600 r in air do not rejoin for at least 2 hours. If, however, these seeds are soaked in 2×10^{-3} M ATP for 30 min previous to irradiation, then an intensity effect is noted when the dose is given at 10 r/minute. This indicates that breaks which ordinarily stay open for 2 hr, if treated with ATP, rejoin in 1 to 2 hours.

[9]K. C. Atwood and F. Mukai, *Biol. Quar. Prog. Rep. Nov. 10, 1951*, ORNL-1167.

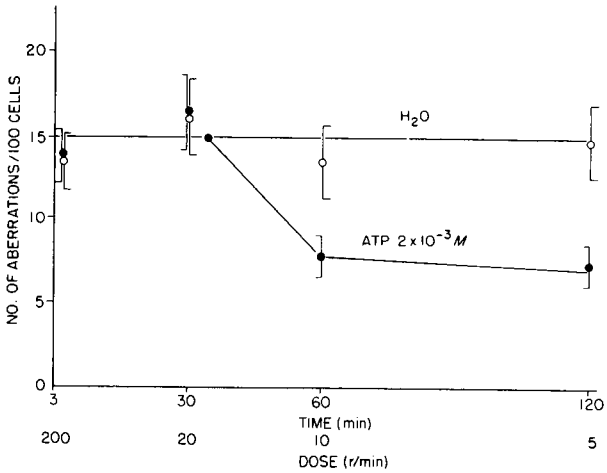

Fig. 8. Effect of varying the intensity of radiation on yield of 2-hit chromosomal aberrations. All points received 600 r.

This evidence that ATP is able to enter the cells of the seed is of interest per se, since previously ATP has never been shown to penetrate cell membranes. Preliminary tests utilizing the firefly luminescence assay for ATP indicate that root tips soaked in ATP solutions have more ATP in their interior than those that have been soaked only in water. The water itself in which beans have been soaked overnight exhibits the presence of small amounts of ATP. Consequently, it appears that in *Vicia* seed an organism has been discovered in which ATP can cross cell membranes.

Survival Curve Analysis of a Two-Component Heterokaryon, When the Distribution of Nuclear Number Is Taken into Account

K. C. Atwood

The expected relation between the survival curves of a two-component heterokaryon on minimal and supplemented media according to the nuclear inactivation hypothesis has been previously derived in an approximate way by treating the conidial population as though it consisted entirely of binucleate cells.[9,10] The experimental results conform closely to expectations based on this oversimplified derivation, even when extended to the more complex case of a triple heterokaryon with trinucleate cells.[11] In fact, however, each conidial population contains cells with several different numbers of nuclei. The frequency distribution of nuclear number has been determined for a large number of cases by direct counts on stained preparations, the results of which are shown in Fig. 9. It was decided to work out the theoretical survival curves on supplemented and on minimal media for a typical case with an average of 2.5 nuclei per conidium.

The survival for the total population is

$$S_t = \sum_{n=1}^{5} C_n S_n \qquad (1)$$

on supplemented, and

$$S_t = \sum_{n=2}^{5} C_n S_n \qquad (2)$$

[10]K. C. Atwood and F. Mukai, *Am. Naturalist* 88, 295-314 (1954).

[11]K. C. Atwood and T. H. Pittenger, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693.

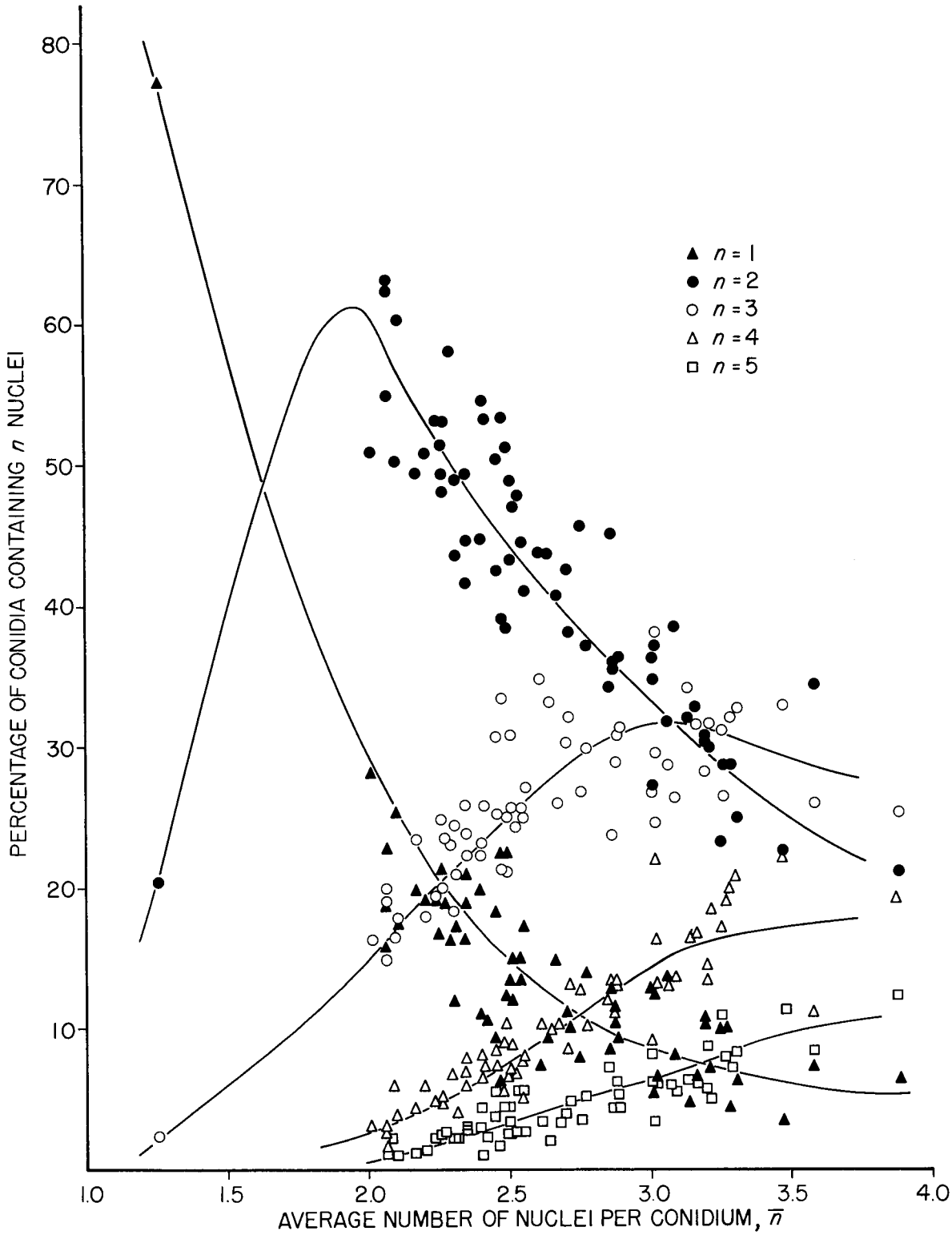

Fig. 9. Frequency distribution of nuclear number in conidial populations. The percentage of conidia containing exactly n nuclei is plotted against the average number of nuclei per conidium, $\bar{n}$ for n values from 1 to 5.

on minimal medium, where C_n is the fraction of conidia containing n nuclei and S_n is the surviving fraction thereof. In Eq. 1

$$\sum_{n=1}^{5} C_n = 1$$

and in Eq. 2

$$\sum_{n=2}^{5} C_n = 1 ,$$

if numbers greater than 5 are ignored.

On supplemented medium, for any n,

$$S_n = 1 - (1 - e^{-kD})^n . \quad (3)$$

On minimal medium

$$S_2 = e^{-2kD} \quad (4)$$

and

$$S_3 = 2e^{-2kD} - e^{-3kD} . \quad (5)$$

For the (1,3) combination

$$S_{4(1,3)} = 3e^{-2kD} - 3e^{-3kD} + e^{-4kD} \quad (6)$$

and for the (2,2) combination

$$S_{4(2,2)} = 4e^{-2kD} - 4e^{-3kD} + e^{-4kD} . \quad (7)$$

Similarly,

$$S_{5(1,4)} = 4e^{-2kD} - 6e^{-3kD} + 4e^{-4kD} - e^{-5kD} \quad (8)$$

and

$$S_{5(2,3)} = 6e^{-2kD} - 9e^{-3kD} + 5e^{-4kD} - e^{-5kD} . \quad (9)$$

It is assumed that the nuclear proportions are equal, and that nuclear types are randomly distributed in heterokaryotic cells so that

$$C_{4(1,3)} = (4/7)C_4$$

and

$$C_{5(1,4)} = (1/3)C_5 .$$

Plots of kD versus S_nC_n on minimal medium are shown in Fig. 10A. A typical set of experimental points fit well to S_t when $k = 3.1 \times 10^{-5}/r$. This shows that the commonly observed distribution of nuclear number leads to a survival curve of the observed form. In Fig. 10B, S_nC_n on supplemented medium is plotted against kD. The corresponding set of points from the same experiment is plotted, with the same constant, and agreement with S_t is rather good.

By use of a mutant stock which produces solely uninucleate conidia, it has been shown that equation (3) does not hold for $n = 1$; thus the curve for uninucleate conidia in Fig. 10B is empirical, again with the same k value. The uninucleate conidia are much more sensitive than the nuclei of multinucleate conidia. It is obvious, then, that the presence of one or more extra nuclei in a conidium will, even if these nuclei have received dominant lethal injury, prevent a large portion of the lethal effect of X rays on a given nucleus.

An Accurate Approximation for the Nuclear Ratio in Heterokaryons From Conidial Plating Data

K. C. Atwood T. H. Pittenger
A. W. Kimball[12]

The difficulty of determining the nuclear ratios of *Neurospora* heterokaryons has seriously hindered their potential usefulness in many areas of genetics and radiation biology. Methods have been proposed by Atwood and Mukai[13] and later by Prout *et al.*[14] which utilize data from the plating of macroconidia on differential media. Since these iterative methods require laborious computation, a simpler approximation was developed based on reasonable assumptions. The relative frequency of nuclei of the first type is given by

$$p \approx \frac{r(1 - r) + a(\bar{n} - 2r)}{\bar{n}(1 - r)}$$

where r is the proportion of heterokaryotic conidia; a, the proportion of homokaryotic conidia of the

[12]Mathematics Panel.

[13]K. C. Atwood and F. Mukai, *Biol. Quar. Prog. Rep. May 10, 1952*, ORNL-1297, pp. 25-28.

[14]T. Prout, C. Huebschman, H. Levine, and F. J. Ryan, *Genetics* 38, 519-529 (1953).

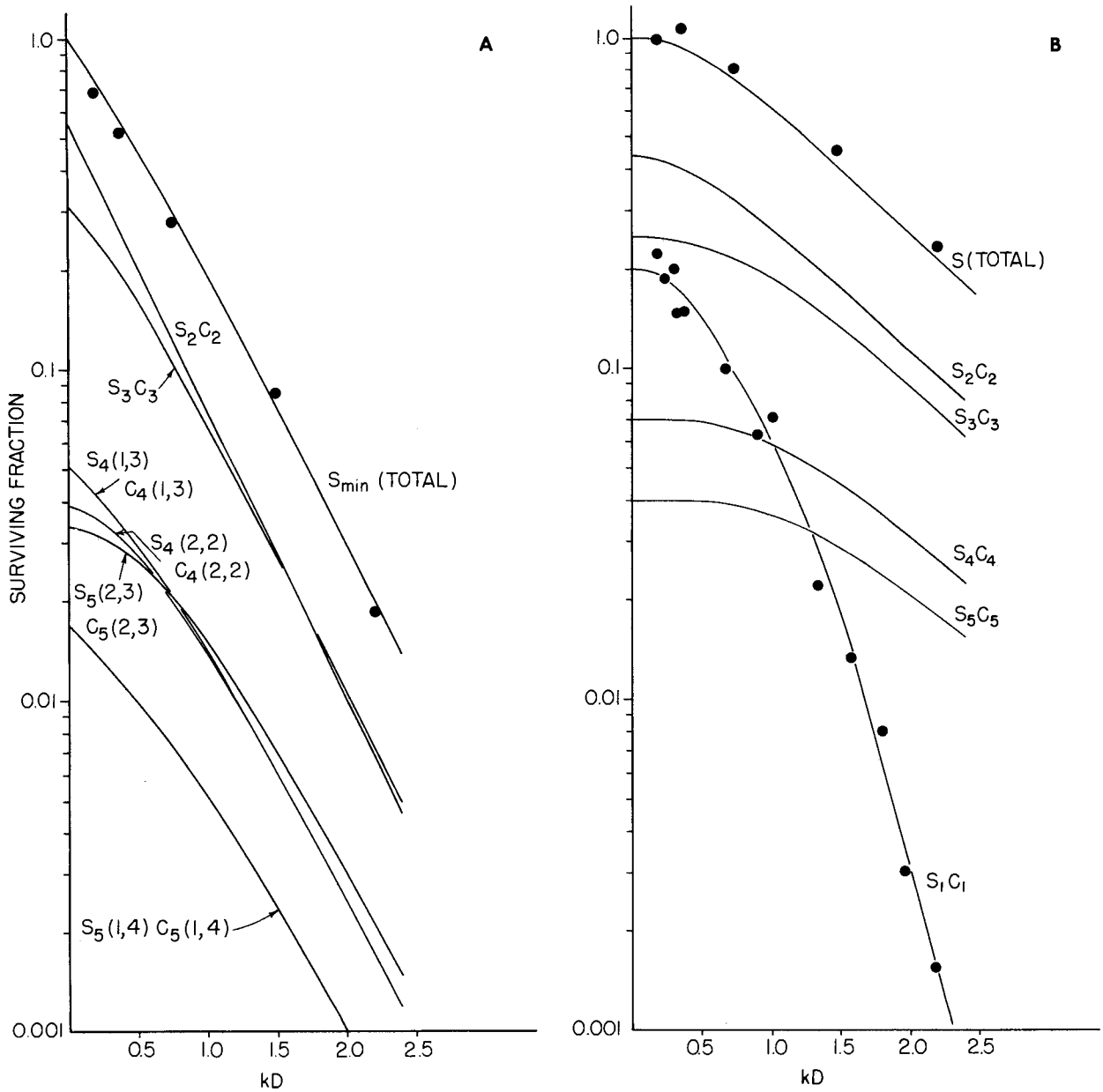

Fig. 10. (A) Survivals for the various number classes, S_nC_n, on minimal medium, plotted against kD. The theoretical survival curve for the total population, S_t, is obtained by summing the theoretical S_nC_n curves for each n value. The points are experimental with $k = 3.1 \times 10^{-5}/r$. $C_2 + C_3 + \ldots + C_5 = 1$, for heterokaryotic conidia; $S_2 = e^{-2kD}$; $S_3 = 2e^{-2kD} - e^{-3kD}$; $S_4(1,3) = 3e^{-2kD} - 3e^{-3kD} + e^{-4kD}$; $S_4(2,2) = 4e^{-2kD} - 4e^{-3kD} + e^{-4kD}$; $S_5(1,4) = 4e^{-2kD} - 6e^{-3kD} + 4e^{-4kD} - e^{-5kD}$; $S_5(2,3) = 6e^{-2kD} - 9e^{-3kD} + 5e^{-4kD} - e^{-5kD}$; $k = 3.1 \times 10^{-5}$. **(B) Similar plots of S_nC_n on supplemented medium.** Points near S_t are from the same experiment as in Fig. 10A and with the same constant. Points for the uninucleate conidia are experimental, obtained with a uninucleate stock. $C_1 + C_2 + \ldots + C_5 = 1$; $S_2 = 1 - (1 - e^{-kD})^2$; $S_3 = 1 - (1 - e^{-kD})^3$; $S_4 = 1 - (1 - e^{-kD})^4$; $S_5 = 1 - (1 - e^{-kD})^5$; S_1, empirical; $k = 3.1 \times 10^{-5}$.

first type; and $\bar{n}$, the average number of nuclei per conidium. The bases for this approximation are, first, the known presence in each heterokaryotic cell of at least one nucleus of each type; and second, the assumption that the ratio of types among nuclei in excess of those of known type equals the ratio of the two homokaryotic conidial types. Since deviations from a random distribution of the nuclear types into conidia of each number class are always in the direction which produces an excess of homokaryotic conidia, this assumption is indirectly justified. When p values computed from the approximation are compared with those obtained from the same data by the iterative methods the agreement is remarkably close, as shown in Table 8. The nature of the bias introduced by the approximation was then examined in more detail. It is clear that for true values of a and r the approximate formula gives the true p whenever p is 0, $\frac{1}{2}$, or 1. This result holds regardless of the mechanism controlling the nuclear constitution of conidia, i.e., whether it is a random process or one which favors the formation of homokaryotic cells. The expected values for a and r were computed according to the randomness hypothesis for values of p varying by intervals of 0.1 from 0 to 1. These values were then substituted in the approximate formula for p and the results are given in Table 9. The comparison is made for two values of $\bar{n}$ which, in general, represent the extremes of average nuclear number encountered in cultures grown on minimal medium. Although the formula is slightly biased for values of p other than 0, $\frac{1}{2}$, and 1, it should be noted that the bias changes direction at $p\frac{1}{2}$, and does not exceed 3.19% at any point. Since the approximate formula is not sensitive to departures from random distribution in the direction observed, it is probably more accurate, in general, than the iterative methods.

TABLE 8. COMPARISONS OF p ESTIMATED BY DIFFERENT MEANS

Experiment	Iterative method of Atwood and Mukai	Approximate formula	Iterative method of Prout *et al.*
a	0.555	0.556	0.557
b	0.468	0.463	0.578
c	0.654	0.659	0.715
d	0.886	0.864	0.875
e	0.858	0.846	0.860
f	0.775	0.763	0.813
g	0.876	0.861	0.878

TABLE 9. COMPARISON OF p VALUES FOR THE RANDOM HYPOTHESIS AND THE APPROXIMATE FORMULA FOR THE SAME a AND r VALUES

$\bar{n} = 2.34$		$\bar{n} = 3.16$	
Random hypothesis p	Approximate p	Random hypothesis p	Approximate p
0	0	0	0
0.10	0.1063	0.10	0.0932
0.20	0.2056	0.20	0.1781
0.30	0.3025	0.30	0.2681
0.40	0.4004	0.40	0.3753
0.50	0.5000	0.50	0.50000
0.60	0.5996	0.60	0.6247
0.70	0.6975	0.70	0.7319
0.80	0.7944	0.80	0.8219
0.90	0.8937	0.90	0.9068
1.00	1.0000	1.00	1.0000

Nuclear Migration in *Neurospora* Heterokaryons

T. H. Pittenger K. C. Atwood

The mycelium of *Neurospora crassa* is subdivided by septa having perforations which allow the passage of various cytoplasmic constituents. It has been assumed in the past that nuclei are able to migrate through these perforations; in fact, in closely related genera nuclear migration has been demonstrated during the processes leading to the formation of the zygote; nuclei of one mating type migrate within the mycelium of the opposite mating type.[15] However, the extent to which nuclei migrate within their own mycelium during growth has not been previously demonstrated. The use of genetically marked nuclei in unisexual heterokaryotic systems of *Neurospora crassa* provides a unique

[15] E. S. Douding and A. Bakerspigel, *Can. J. Microbiol.* 1, 68-78 (1954).

method for investigating this phenomenon, and such experiments have been undertaken in conjunction with other studies involving irradiation of continuously growing mycelium.

The procedure was to shield the mycelial frontier of growing heterokaryons and to X-ray the mycelia at various distances behind the growing frontier. If nuclei with radiation-induced recessive mutations migrate into the unirradiated portion of the mycelium and continue to multiply they must eventually be incorporated and detected in conidia formed at the ends of the growth tubes. The original distances migrated could be roughly determined by irradiating at different distances behind the mycelial frontier and seeing in which cases radiation-induced mutations failed to appear in the conidia.

The heterokaryon (*arg* + *amy,meth*) used in these experiments and the methods for determining recessive mutations in the nuclei of the *amy,meth* components have previously been described.[16] Heterokaryons were grown in special horizontal growth tubes 150 mm long and 20 mm in diameter. The growth tubes were longitudinally split and the top half had ports at each end for aeration and conidial sampling. The bottom halves of the tubes were completely filled with 2% minimal agar medium and inoculated at one end. After the heterokaryons had grown approximately 75 mm, the tops of the tubes were removed. Lead shields 5 mm thick were used to cover the mycelial frontier so that in separate tubes the first 3, 5, and 10 mm of the mycelium were shielded. The mycelia in these tubes, in addition to mycelium and conidia not shielded, were given 50,000 r of X rays. The tops of the tubes were then replaced and the mycelium allowed to complete its growth, which continued in apparently normal fashion after irradiation. Conidia formed under the distal aeration ports were removed and plated on minimal medium. Approximately 300 conidia from each growth tube were analyzed for recessive mutations in the *amy,meth* nuclear component.

Dry, unshielded heterokaryotic conidia given 50,000 r were checked for recessive mutations and 12.3% contained such mutations in the amy,meth nuclear component. In the growth tubes 3.2, 4.2, 1.4, and 2.7% recessive mutations were found where 0, 3, 5, and 10 mm respectively of the mycelial frontier had been shielded. The percentages of recessive mutations found in the four tubes were not significantly different. Comparable unirradiated cultures yield a mutant frequency of the order of 10^{-4}. It is apparent from these results that nuclei with induced recessive mutations migrate at least 1 cm into the nonirradiated portion of the mycelium, continue to multiply, and are eventually incorporated into conidia at the ends of the growth tubes. Similar experiments are in progress to determine the maximum distance over which nuclear migration can occur.

It is interesting to note that the incidence of recessive mutations from irradiated growing mycelium is three to four times as small as from conidia receiving the same dose. This may be caused in part by elimination of some of the recessive mutations during growth, and in part by differential sensitivity of nuclei in conidia and mycelium.

Accumulation of Mutant Nuclei in Irradiated Growing Mycelium

T. H. Pittenger K. C. Atwood

The rate of mutations induced in the growing mycelium and recovered from the end of a horizontal growth tube after a prolonged period of growth appeared to be lower in the mycelium than in conidia. This raised the question of whether the mutations are eliminated during growth, or would continue to accumulate on repeated irradiation. Continued accumulation would signify a greater resistance to recessive lethal mutation among nuclei in the mycelium than among those in the conidia. This question was investigated with the standard heterokaryon, *arg* + *amy,meth*, in 400-mm growth tubes.

Three growth tubes received the following treatment: tube 1, inoculated with pellet of irradiated conidia, no further irradiation; tube 2, inoculated with unirradiated conidia, irradiated twice during growth; tube 3, inoculated with irradiated conidia, irradiated four times during growth.

Each irradiation was 50,000 r as previously described, and several centimeters of growth was allowed between successive doses. A sample of the conidia irradiated in the dry state and used as the inoculum in tubes 1 and 3 was plated on minimal medium and assayed for recessive lethals. The surviving fraction of this sample was 0.031. The conidia from the distal ends of the growth tubes were plated on differential media to determine the

[16] K. C. Atwood and F. Mukai, *Biol. Quar. Prog. Rep. Nov. 10, 1951*, ORNL-1167, pp. 29-33.

heterokaryotic fraction, and colonies from the minimal plates were isolated and tested for recessive lethals. The results are shown in Table 10.

These results permit several conclusions. First, the heterokaryotic fractions from the growth tubes are high, showing that dominant lethal nuclei are not carried through the growth period. It is known from numerous previous experiments that dominant lethality reduces the heterokaryotic fraction to less than 0.1 when the surviving fraction on minimal medium is of the order of 0.03.[17] Second, the comparison of recessive lethal frequency in the distal end of tube 1 with that of its conidial inoculum reveals very little elimination of mutations during the 40 cm of growth; a χ^2 test shows the difference to be of doubtful significance, $0.1 > P > 0.05$. Some loss is to be expected, since previous experiments have shown that about 20% of X-ray-induced recessive lethals cause autonomous nuclear retardation, and these would certainly be selected against in a growing culture.[18] Third, a dose of 50,000 r induces more recessive lethals in conidia than in mycelium. The proportion, j, of mutant nuclei accumulated after repeated doses is given by

$$j = 1 - (1 - x)^n \qquad (10)$$

where x is the proportion of mutants obtained with a single irradiation and n the number of irradiations. Thus, for tube 2,

$$x = 1 - (1 - j_2)^{1/2} , \qquad (11)$$

or $x = 0.049$ for the observed value of j_2. For tube 3

$$j_3 = 1 - (1 - j_1)(1 - x)^4 . \qquad (12)$$

By substitution of values of j_1 and x from tubes 1 and 2 into Eq. 12 an expected value of 0.245 is obtained, not significantly different from the observed j_3 value of 0.215, $P \approx 0.20$. Since this crude analysis ignores the errors of j_1 and j_2, it is obvious that a minimum χ^2 method with all three j values would give a better fit, and that the x value of 0.049 is maximal. The maximal x value is significantly different from the j_1 value at the 5% level, supporting the conclusion that more mutations were induced in dry conidia than in the mycelium. This result is more surprising because the mycelium is fully hydrated, and conidia are, in general, more sensitive when hydrated than when dry. The anomaly may be brought about in part by the presence of resistant prophase nuclei in the mycelium, whereas the nuclei in conidia are in a resting stage. Experiments are in progress to explore the upper limit of accumulation, dose-effect curves in growing mycelium, and chronic irradiation during growth.

The Control of Nuclear Ratios in *Neurospora* Heterokaryons

T. H. Pittenger K. C. Atwood
A. W. Kimball[19]

In a recent study,[20] forced heterokaryons with extreme nuclear ratios were synthesized by the use of inocula containing disproportionate numbers of the two types of homokaryotic conidia. The achievement of experimental control of such ratios is of particular importance because it permits quantitative measurement of the effects of varying the

[17] K. C. Atwood and F. Mukai, *Am. Naturalist* 88, 295-314 (1954).

[18] K. C. Atwood and F. Mukai, *Radiation Research* 1, 125 (1954).

[19] Mathematics Panel.

[20] T. H. Pittenger and K. C. Atwood, *Genetics* 39, 987 (1954).

TABLE 10. X-RAY EFFECTS IN MYCELIA AND CONIDIA

	Total dose (r)	Percentage of recessive lethals	Total isolates	Heterokaryotic fraction
Tube 1	50,000	7.7	300	0.46
Tube 2	100,000	9.6	323	0.46
Tube 3	250,000	21.5	349	0.63
Dry conidia	50,000	12.3	162	<0.10[a]

[a] Known from previous experiments.

gene dosage over a continuous range. In order to provide a firm basis for such experiments, the relation between the nuclear ratios in conidial mixtures and in the resultant heterokaryons has now been studied in detail.

Four different stocks were used, having requirements for lysine, nicotinic, pantothenic, and *p*-aminobenzoic acids, respectively. Each experiment involved mixing conidia of two stocks in a number of different ratios so that the proportion of a given nuclear type ranged from 0.10 to 0.90. The mixtures were incubated overnight at 3°C in complete liquid medium, then placed on complete sorbose agar for 3 days. The coalesced pellets were then transferred to minimal agar, where growth and conidiation ensued. Finally, the nuclear proportions in the resultant heterokaryons were compared with those in the corresponding inocula. Five different combinations were studied and a total of 27 mixing ratios of three samples each analyzed. Every case showed a systematic deviation from the input ratios of nuclei, generally in the same direction for different input ratios of the same combination.

The nature of the deviations may be understood by considering the events taking place between the mixing of homokaryotic conidia and sampling of conidia from the resultant heterokaryons. Nuclear selection during growth of the heterokaryons cannot account for the results, because the nuclear ratios did not change significantly between the beginning and the end of long growth tubes. It seems likely, then, that deviations from the input ratios originate before the mycelium has grown away from the mixed inoculum. Microscopic examination of the conidial pellets reveal no fusions until germination has begun; and by the time sufficient fusions occur to allow thorough mixing of nuclei, many hyphae of considerable length are present. Under these conditions differential rates of germination and nuclear division prior to establishment of the final ratio are to be expected, particularly since a large proportion of the growth is homokaryotic. Changes in nuclear proportions brought about in this way may be regarded, as a first approximation, as independent of the initial proportions. However, as the input ratio becomes more extreme, it might be expected that an increasing proportion of conidia of the majority type will fail to contribute their nuclei to the general pool because they are remote from contact with hyphae which contain any nuclei of the minority type. This cause of deviation from the input ratio may be distinguished from the previous one by its strong dependence on the initial conidial proportions.

The hypothesis of unequal division rates, or equivalent phenomena, lends itself readily to mathematical evaluation. Let x_a and x_b be the initial numbers of nuclei of the two types, A and B. Although these numbers are not known, the proportion $x_a/(x_a + x_b)$ is known. Now let the division rates be such that when the final ratio is established there are $\lambda_a x_a$ and $\lambda_b x_b$ nuclei present in a given mixture. After this time the conidia of the resultant heterokaryons are sampled and the proportion, $\lambda_a x_a/(\lambda_a x_a + \lambda_b x_b)$, of type A nuclei estimated from plate counts by the approximate formula. If we let $X = x_b/x_a$ and $Y = \lambda_b x_b/\lambda_a x_a$, then $Y = (\lambda_b/\lambda_a)X$. In other words, if the explanation based on unequal division rates is correct, the quantity Y should plot linearly against the quantity X as the proportion of type A nuclei in the mixture is varied. This line should go through the origin and have a slope of λ_b/λ_a. If there is no difference in division rates the slope should be unity.

When this method of analysis was applied to the experiments, slopes differing significantly from unity were found in every case, indicating that the differential division hypothesis might be acceptable. The plots of Y against X, however, were not quite linear, and suggested that a log-log representation might provide a better fit to the data. Accordingly, the X and Y values were replotted on logarithmic paper, and in no case were any departures from linearity apparent. The equation,

$$\log Y = \log (\lambda_b/\lambda_a) + m \log X \qquad (13)$$

was fitted to each set of data by the method of least squares. The fourteen fitted curves, transformed back to original units, were essentially the same for all the heterokaryons and differed only in the parameters λ_b/λ_a and m. A typical curve is shown in Fig. 11. If the data could be explained entirely on the basis of differential division rates, only random departures from $m = 1$ should be observed. Instead, the estimates of m are all less than one and tend to cluster between 0.6 and 0.9. Clearly, an additional explanation is necessary.

Eq. 13 may be written in the form,

$$Y = \frac{\lambda_b}{\lambda_a} (x_b/x_a)^m , \qquad (14)$$

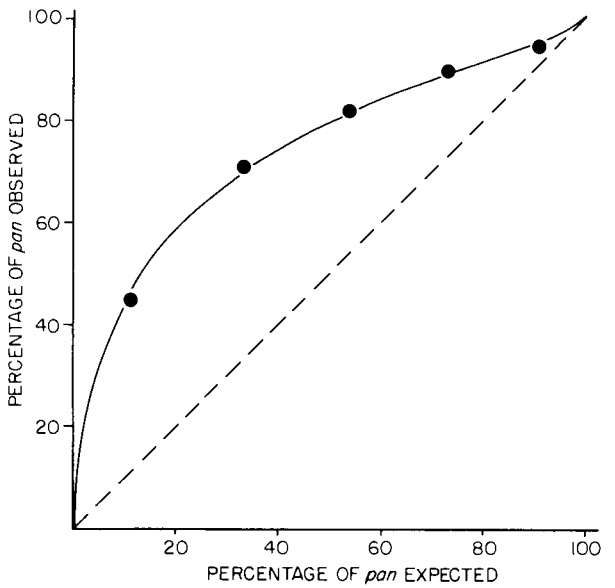

Fig. 11. Relation between final (observed) proportion of *pan* nuclei and the input (expected) proportions in *pan* + *nic* mixtures. The points are experimental and the curve theoretical, plotted according to Eq. 13 with $\lambda_b/\lambda_a = 0.254$ and $m = 0.727$.

where Y is the observed ratio of nuclear types and x_b/x_a is obviously the initial ratio at the time of mixing. If x_b, for instance, is very large compared to x_a, many of the type B nuclei may be superfluous because some type B conidia may have no opportunity to contribute their nuclei to the heterokaryon. This means that the mixture would behave as though the ratio of majority to minority types present were smaller than that estimated from the initial conidial and nuclear counts. When the input ratio is raised to a power less than 1 the effect is a reduction in the majority to minority ratio by the same amount whether type A or type B is in the majority, and for any given m this reduction becomes more pronounced as the majority to minority ratio increases. These properties of Eq. 13 and hence of Eq. 14 would be expected on the basis of a progressive failure of uniform mixing as one type of nucleus becomes more preponderant.

This relation has been found to hold for very extreme proportions. For instance, whereas a 9:1 ratio of *pan* to *nic* yields a final proportion of about 93% *pan* nuclei, a 500:1 initial ratio gives only about 99% *pan* in the resultant heterokaryon. The range over which control can be achieved by compensating for the expected deviations is more than sufficient for the anticipated studies on gene dosage.

The Effect of Cysteamine on Survival and Recessive Mutations in X-Irradiated *Neurospora*

K. C. Atwood T. H. Pittenger

It has previously been shown that cysteamine and related compounds have a marked effect in modifying the X-ray sensitivity of *Escherichia coli* B/r.[21] Similar experiments were carried out with *Neurospora*. The effect on survival and recessive mutations were studied with the heterokaryon technique previously described.[22] Conidia of the heterokaryon (*arg* + *amy,meth*) were suspended in a 0.008 M solution of cysteamine at room temperature for 1 hour. These and the controls without cysteamine received a dose of 50,000 r of 250-kvp X rays with 3 mm of Al filtration, delivered at 1000 r/minute.

The survival of heterokaryotic cells, determined by plating the conidia on minimal medium, was 21% with cysteamine and 7.6% in the control. The heterokaryotic colonies from the minimal plates were then isolated and the percentage of colonies in which the *amy,meth* component carried a recessive mutation was determined. About 300 isolates were made for each determination. In the control material, 25% of the *amy,meth* nuclei in the heterokaryotic cells carried a recessive mutation, whereas only 14.4% were found in the material treated with cysteamine. This represents a dose-reduction factor (DRF) of 1.87 for recessive mutations and 1.6 for survival. The DRF for *E. coli* B/r treated with cysteamine is about 12 in a comparable region of the survival curve.

On the Relation Between Nucleic Acid and Protein Specificity

D. Schwartz A. G. Phelps

The sequence of amino acids in the A and B chains of insulin and in β-corticotropin (Table 11) have been investigated, with the result that an

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

[22] K. C. Atwood and F. Mukai, *Biol. Quar. Prog. Rep. Nov. 10, 1951*, ORNL-1167, pp. 29-33.

TABLE 11. SEQUENCES OF AMINO ACIDS IN PROTEINS

Insulin, fraction A

Gly – Ileu – Val – Glu – Glu – CyS – CyS – Ala – Ser – Val – CyS – Ser – Leu – Tyr – Glu – Leu – Glu – Asp – Tyr – CyS – Asp

Insulin, fraction B

Phe – Val – Asp – Glu – His – Leu – CyS – Gly – Ser – His – Leu – Val – Glu – Ala – Leu – Tyr – Leu – Val – CyS – Gly – Glu – Arg – Gly – Phe – Phe – Tyr – Thr – Pro – Lys – Ala

β-Corticotropin

Ser – Tyr – Ser – Met – Glu – His – Phe – Arg – Try – Gly – Lys – Pro – Val – Gly – Lys – Lys – Arg – Arg – Pro – Val – Lys – Val – Tyr – Pro – Ala – (Gly – Glu – Asp)[a] – Asp – Glu – Leu – Ala – Glu – Ala – Phe – Pro – Leu – Glu – Phe

[a]The exact sequence of amino acids listed in parentheses is in doubt.

hypothesis has been proposed on the relation between nucleic acid structure and protein specificity. According to this hypothesis, nucleic acid confers its specificity to a protein by designating the positions occupied by the aromatic amino acids (including histidine) in the protein molecule. It is postulated that the planar rings of the aromatic amino acids would be differentially bound in cavities formed by two adjacent bases in the nucleic acid chain. Specifically, phenylalanine and tyrosine, the aromatics with only six-membered rings, would fit between two pyrimidine bases. Tryptophane and histidine which contain five-membered rings would fit between two purines. Since the distance between the bases along the sugar-phosphorus backbone on the Watson-Crick model is approximately 7 A, the interatomic spacing is of the correct order, 3.5 A, for resonance binding between the aromatic amino acids and the bases which delimit the cavities. The sites of cavities composed of a purine and a pyrimidine would correspond to the nonaromatic amino acids in the protein molecule.

The protein is pictured as being synthesized on a template formed by the doubled helix on the nucleic acid molecule, partly on one strand and partly on the other. This hypothesis imposes certain restrictions on the arrangement of the aromatic amino acids. An even number of zero aliphatics must occur between aromatics when both are of the phenylalanine-tyrosine group, and similarly when both are of the tryptophane-histidine group. An odd number of aliphatics must separate the aromatics when both are from different groups. The positions occupied by the aromatic amino acids in the proteins with known amino acid sequences support the hypothesis if the assumption is made that phenylalanine represents the position at which the switch is made from one strand on the doubled helix onto the second. The specificity governing the order of the aliphatic amino acids could be conferred partly by the arrangement of the aromatics on the nucleic acid template and partly by the cytoplasm.

The aromatic amino acids were singled out for a number of reasons: (1) structural similarities with the bases; (2) the planar rings have double bonds and are thus capable of resonance bonding; and (3) the amino acids with cyclic side chains (including proline) seem to fall into a definite sequential pattern in the insulin B molecule. Starting at the pair of phenylalanine residues and considering the numbers of aliphatics which separate the cyclic amino acids, one finds the sequence 0,0,1,2 proceeding to the C terminal end. Resuming at the N terminal end the sequence continues as 3,4,5,7. It is also of some interest to fold the insulin B chain so that the cyclic amino acids are always located on the edge of the folds as is shown in Fig. 12. In such a folded configuration the aliphatic amino acids which are located under the outer shell formed by the cyclics (inside the dotted lines), appear to be placed in quite an orderly fashion.

Experimental work is in progress to test for resonance bonding between nucleic acids and the aromatic amino acids.

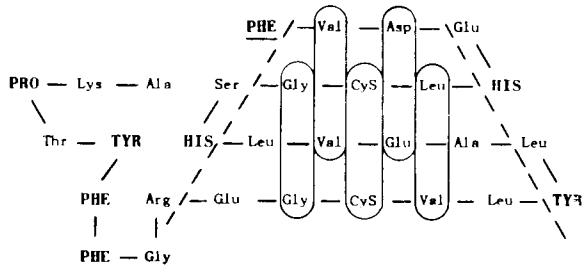

Fig. 12. Folding and arrangement of fraction B of insulin so that the amino acids with cyclic side chains (in caps) occur at the edge of the folds. The terminal phenylalanine is underlined. Notice the orderly arrangement of the aliphatic amino acids.

INSECT CYTOLOGY AND GENETICS

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R. C. von Borstel J. G. Carlson[23]
G. S. St. Amand

Feulgen-Negative Nuclear Division in *Habrobracon* Eggs After Lethal Exposure to X Rays or Nitrogen Mustard

R. C. von Borstel

The females of the parasitic wasp *Habrobracon juglandis* (Ashmead) when well fed will store eggs in the first meiotic metaphase (metaphase I) and retain them until the female is placed with the host. The unfertilized eggs develop into viable haploid males. When the untreated egg is oviposited, meiosis proceeds and the pronucleus is formed 30 min after oviposition at 30°C. The nuclei divide synchronously at the rate of approximately one division every 15 minutes. At the ninth or tenth cleavage stage (2½ to 3 hr after oviposition), most of the nuclei migrate to the egg periphery. After two or three more divisions, cell membranes slowly form between the nuclei and tissue differentiation begins. The embryo hatches about 30 hr after oviposition and feeds on the paralyzed host.

In the present experiments, well-fed females were treated with X-ray exposures of 10 kr (1.5 kr/min) or an equivalent dose of nitrogen mustard (prophase I hatchability was criterion).[24] This exposure is approximately 5 times the lethal dose for metaphase I oocytes. Even at this dose *Habrobracon* egg lethality is entirely nuclear in origin.[25] The X rays were delivered by a G. E. Maxitron 250 unit operating at 250 kvp, with a tungsten target and 1 mm of Al inherent and 3 mm of Al added filtration (hvl, 0.55 mm of Cu); the nitrogen mustard was delivered in the form of an aerosol. The subsequent development of eggs which were in metaphase I during the time of treatment was studied; the eggs were fixed in Kahle's fluid at various times after oviposition and were stained according to the Feulgen procedure after optimum hydrolysis in 1 N HCl.

Over 95% of the eggs treated with X rays and all eggs treated with nitrogen mustard were identical in their developmental pattern. Meiosis proceeds at a normal rate even when chromosome bridges are present. The first cleavage is partially inhibited, particularly but not specifically at metaphase, for 1-2 hr after oviposition; nuclear division then slowly proceeds until two to eight Feulgen-positive interphase or prophase nuclei are present, usually near the anterior end of the egg. At approximately the eighth hour-after oviposition ooplasmic areas can be seen within the yolky egg interior. These areas are ordinarily diagnostic for cleavage nuclei but nothing definite can be recognized within them except for occasional spindle-like structures. By the ninth or tenth hour, Feulgen-negative bodies which are otherwise identical to interphase nuclei begin to appear in the ooplasmic areas. These become distributed throughout the yolky interior. At the tenth or twelfth hour, 50 to 150 of the Feulgen-negative nuclei have appeared and they begin to enlarge gradually. The two to eight Feulgen-positive nuclei can occasionally be detected at this stage, and they also usually enlarge. When nuclear enlargement begins, apparently no more Feulgen-negative nuclei are formed. At 20 hr after oviposition, enlargement of the Feulgen-negative nuclei is nearly maximal (up to 200 times the normal volume) and the Feulgen-positive nuclei are no longer detectable.

It has been known for several years that, at lethal exposures, both X rays and nitrogen mustard more or less immediately and specifically interfere with deoxyribonucleic acid (DNA) synthesis whereas other intracellular syntheses are not directly disrupted. It is probable, therefore, that the strange lethal action of these mutagens on the *Habrobracon*

[23] Consultant.

[24] R. C. von Borstel, *Genetics*, in press.

[25] A. R. Whiting, *Biol. Bull.* 97, 210-220 (1949).

egg is intimately associated with a failure in DNA synthesis. The presence of a few Feulgen-positive nuclei militates against the possibilities that DNA is too diffuse to be detected or that the original pronuclear chromosomes undergo segregation and nuclear division ends with equal and complete distribution of the deoxyribonucleoprotein strands. It may be that the characteristics of DNA are changed by mutagens so that HCl hydrolysis, necessary for purine removal prior to aldehyde-fuchsin coupling, removes most or all of the DNA; this hypothesis appears to be contrary to available information.[26] Perhaps DNA synthesis has been blocked by nitrogen mustard or X rays and the new nuclei are products of other reduplicating systems which synthesize the replicate until DNA is required for further development. This proposal must be regarded with considerable caution since the participation of DNA in genetic continuity is a well-established phenomenon.

The Nuclear Localization of the Photoreactivation Phenomenon

R. C. von Borstel S. Wolff

The counteraction of ultraviolet radiation (ca. 2600 A) damage by radiation of a longer wave length (ca. 3600 A) is called photoreactivation. The phenomenon is widespread in nature (inactivation of fungi, viruses, bacteria, protozoa, higher plant cells, salamander larvae), and affects known nuclear events (mitotic rate, mutation induction, DNA synthesis). The characteristics of the *Habrobracon* egg are such that it is well suited to investigation of photoreactivation phenomena when the nucleus or the cytoplasm are separately exposed to ultraviolet radiation.

Fertilization of the *Habrobracon* egg is not required for normal development – unfertilized eggs become haploid males. The nucleus of the newly laid egg is in the first meiotic metaphase and is located at the anterior end on the convex surface (Fig. 13). The nucleus remains in this position for 30 min while meiosis is completed. The nucleus then migrates to the center of the egg and development begins.

Well-fed virgin females of stock #33 are removed from their host (the larvae of the cornmeal moth *Ephestia*) 4-12 hr before the experiments are begun

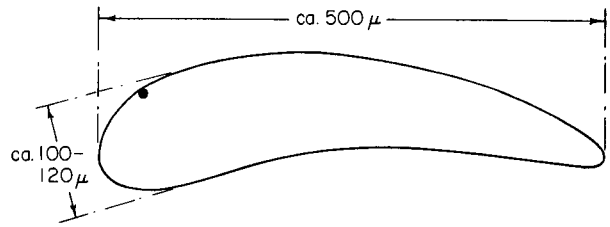

Fig. 13. Lateral view of newly laid *Habrobracon* egg. The nucleus is located at the anterior (wide) end near the convex surface.

in order that they may store mature eggs. Six females are placed in a small Stender dish with three *Ephestia* caterpillars and allowed to lay eggs; twelve to eighteen dishes may be conveniently handled. The dishes are cyclically examined for eggs; the eggs are removed with a dissecting needle and positioned on glass slides for irradiation. In this way the eggs are 0-15 min old when irradiated and have proceeded no further than the second meiotic metaphase. Under these conditions, up to 200 eggs per hour have been irradiated. Hatchabilities are recorded 2 days later.

The ultraviolet radiation source was a GE germicidal lamp at 60 cm which produced a flux of 1200 ergs/cm^2/sec; 85% of the energy produced by the lamp is reported to be at wave length 2537 A. The photoreactivating source was a GE 360 BL lamp at 4.8 cm, which produces most of its energy at wave length 3600 A. The eggs were kept at a constant temperature of 24-25°C during the exposure periods.

Figure 14 indicates that dose-hatchability following irradiation of the convex (nuclear) side of the egg is exponential. The complete data are listed in Table 12. Approximately 25% of the eggs appear to be especially resistant to the radiation; this is evinced by the concave character of the curve, and extrapolation to zero-dose of the second exponential component. This resistance is probably caused by either meiotic stage sensitivity differences or differences in location of the nucleus within the egg. Figure 14 shows also a maximum photorecovery curve from the ultraviolet damage induced by irradiation of the convex side of the egg. The dose-reduction is approximately 2.6.

A dose-effect curve for photoreactivation at a constant ultraviolet radiation exposure of 180 ergs/mm^2 is shown in Fig. 15. Photorecovery

[26] M. J. Moses, R. DuBow, and A. H. Sparrow, *J. Natl. Cancer Inst.* 12, 232-233 (1951).

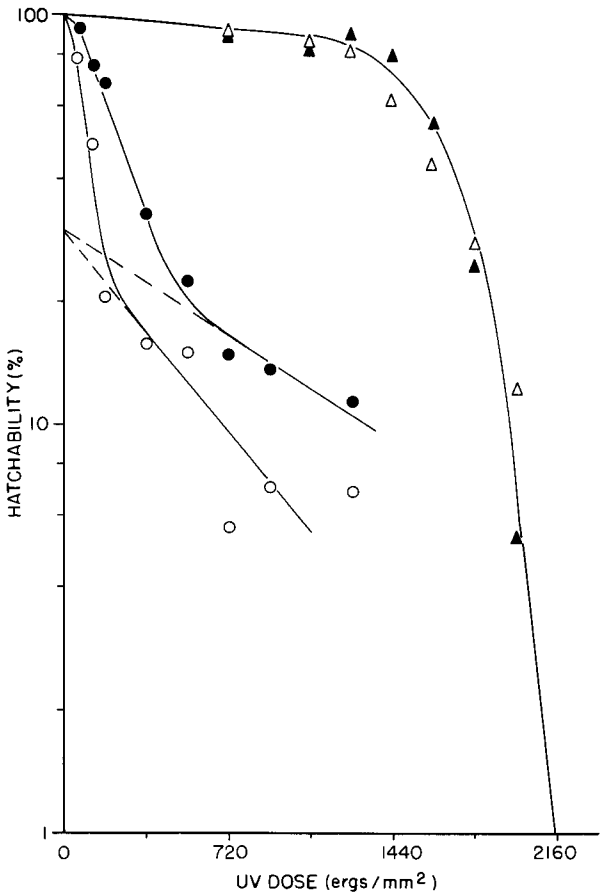

Fig. 14. Dose hatchability curves for *Habrobracon* eggs irradiated on their convex (O, ultraviolet; ●, ultraviolet plus reactivating light) or concave (Δ, ultraviolet; ▲, ultraviolet plus reactivating light) sides.

begins with very short exposures of reactivating light. Since the nucleus is in the process of meiosis at this time and probably not undergoing active synthesis, this provides evidence that the nucleus does not have to be undergoing active metabolism in order that photorecovery may occur. Controls placed under the reactivating light without prior ultraviolet irradiation showed normal hatchability even after 2 hr of constant exposure.

When the concave side of the egg is irradiated (Fig. 14 and Table 12), the hatchability curve is sigmoid and may be interpreted as being of multihit nature of high order. On the theory of Atwood and Norman,[27] the number of units which must be in-

[27]K. C. Atwood and A. Norman, *Proc. Natl. Acad. Sci. U. S.* 12, 696-709 (1949).

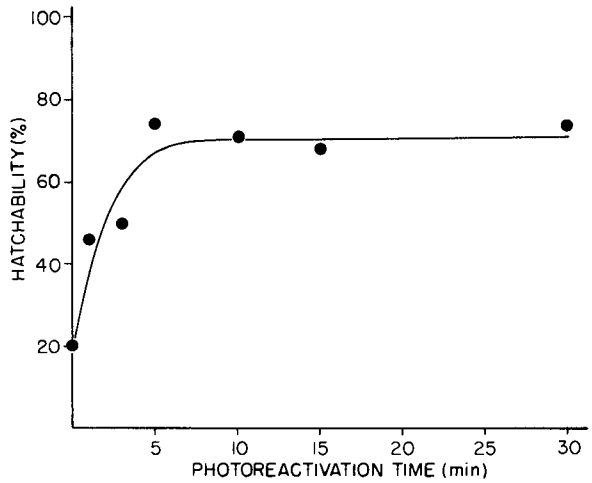

Fig. 15. Dose-recovery curve for eggs exposed to 180 ergs/mm^2 of ultraviolet radiation and to reactivating light of different duration.

activated in order to kill the egg is certainly in excess of 100 and probably in excess of 1000. It seems reasonable to assume that these sensitive units are cytoplasmic particles. Photoreactivation does not appear to take place when the egg is injured by exposing the concave side of the radiation. This indicates that the phenomenon of photoreactivation is related to events within, limited to, or governed by the egg nucleus.

Conditionally Delayed Dominant Lethal Mutations in *Habrobracon*

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

A paradoxical inequality in recessive lethal frequency as determined by two different genetic methods was reported previously.[28] The first genetic method is an F_1 generation test which discloses the apparent frequency of recessive lethal mutations induced by X rays in haploid eggs of the wasp *Habrobracon*. The second method is a second generation (F_2) analysis which reveals the frequency of recessive lethal mutations in eggs which were fertilized subsequent to irradiation. Approximately 2 times as many recessive lethal mutations were scored by the F_1 method as by the

[28]R. C. von Borstel, K. C. Atwood, and A. R. Whiting, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, pp. 28-29.

TABLE 12. HATCHABILITIES OF IRRADIATED EGGS

Dose (ergs/mm^2)	Hatchability after treatment with: Ultraviolet		UV + photoreactivating light for 15 min	
	Larvae/eggs	%	Larvae/eggs	%
Convex surface				
60	77/99	= 0.778	82/88	= 0.932
120	51/106	= 0.481	76/102	= 0.745
180	54/266	= 0.203	68/100	= 0.680
360	15/95	= 0.158	33/102	= 0.323
540	55/367	= 0.150	20/90	= 0.222
720	12/214	= 0.056	16/109	= 0.147
900	21/300	= 0.070	16/116	= 0.138
1260	7/103	= 0.068	16/140	= 0.114
Concave surface				
720	95/106	= 0.896	96/108	= 0.889
1080	87/102	= 0.852	81/100	= 0.810
1260	80/100	= 0.800	91/102	= 0.892
1440	128/209	= 0.613	158/201	= 0.785
1620	43/101	= 0.426	61/114	= 0.535
1800	28/103	= 0.272	23/97	= 0.237
1980	12/101	= 0.119	7/136	= 0.052
2160	0/103	= 0.000	0/105	= 0.000

F_2 method. Three hypotheses were proposed to account for the inequality: (1) The presence of multifactor (complementary) lethals which would appear as lethals in the F_1 but would segregate in the F_2 would account for the results; these were shown to occur but the frequency was not high enough to explain the discrepancy. (2) It was suggested that processes leading to a gene mutation could be reversed if a normal nucleus was combined with the irradiated nucleus; this hypothesis could be considered only if all others were eliminated. (3) Postembryo or conditionally delayed dominant lethal mutations could also account for the discrepancy; these are a certain class of dominant lethal mutations which are expressed by death of the haploid embryo and by death of the diploid larva or pupa. The last hypothesis is rigorously tested here and is shown to be the correct one.

In this experiment approximately 59% of the adult survivors were from fertilized eggs of mated females (Table 13); for convenience this will be assumed to be the proportion of fertilized eggs, f. The unfertilized eggs develop into haploid males and the fertilized eggs become diploid females. After X irradiation of females subsequently fertilized, the viable proportion of fertilized eggs, V_f, may be expressed as

$$V_f = \frac{m - V_u(1 - f)}{f} \qquad (15)$$

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. Among the unfertilized eggs, those which hatch carry neither a dominant nor a recessive lethal mutation;

$$V_u = (1 - D)(1 - r) \qquad (16)$$

where D is the frequency of chromosome sets having at least one dominant lethal and r the frequency of those having at least one recessive lethal mutation. The fertilized eggs which hatch are those without a dominant lethal;

$$V_f = 1 - D \qquad (17)$$

thus

$$r = 1 - (V_u/V_f) \ . \qquad (18)$$

The same method can be used to express r', the frequency of genomes bearing at least one recessive lethal mutation expressed at any stage of development,

$$r' = 1 - (V_u'/V_f') \qquad (19)$$

where

$$V_f' = \frac{m' - V_u'(1 - f)}{f} \qquad (20)$$

is the proportion of fertilized eggs reaching the adult stage.

The second method (F_2 analysis) is to isolate unmated females and determine with what frequency these are heterozygous for recessive lethals.

Table 13 contains data from a sample experiment from which the recessive lethal frequencies are calculated by the F_1 method. In the experiment considered, eggs were irradiated with 875 r in the first meiotic metaphase stage of oogenesis. The values of r and r' as calculated by the F_1 and F_2 method are compared in Table 14. The value of r as determined by F_1 analysis is much higher than that from F_2 analysis, whereas the values of r' are not significantly different. In this experiment the females used for F_2 analysis were survivors represented by V_f' in the corresponding F_1.

The similarity in r' values implies that the difference in hatchability between fertilized and unfertilized eggs is partly offset by increased mortality of the fertilized eggs during postembryonic stages. In Table 13 the mortality of the larval stages for eggs from mated and unmated females is shown, and it can be seen that larvae from eggs from mated mothers have a higher percentage mortality than larvae from eggs from virgin females.

It appears that there is a class of induced dominant lethal mutations whose time of expression depends on whether the genome is haploid or diploid. The haploids which carry such mutations die before hatching; the diploids die after hatching. These mutations will be referred to as conditionally delayed dominant lethals, and in order to compute their frequency, Eq. 16 is revised:

$$V_u = (1 - D)(1 - D')(1 - r) \qquad (21)$$

where D' is the frequency of chromosome sets having one conditionally delayed dominant lethal mutation. Then from Eq. 17 and 21

$$D' = 1 - \frac{V_u}{V_f(1 - r)} \qquad (22)$$

TABLE 13. SURVIVAL DATA AND F_1 ANALYSIS OF EGGS FROM X-IRRADIATED *HABROBRACON* FEMALES

	Offspring from mated females	Offspring from unmated females
Hatchability (larvae/eggs)	357/1100 = 0.325 (m)	236/933 = 0.253 (V_u)
Hatchability (corrected)	0.376 (V_f)	
Adults/eggs	242/1100 = 0.220 (m')	172/931 = 0.185 (V_u')
Adults/eggs (corrected)	0.278 (V_f')	
Larval lethals/larvae	84/356 = 0.236	36/236 = 0.153
Males/adults	100/242 = 0.413 ($1 - f$)	172/172 = 1.000

TABLE 14. APPARENT AND ACTUAL RECESSIVE LETHAL MUTATION FREQUENCY DETERMINED BY F_1 AND F_2 ANALYSIS

	F_1 (Computed from Table 13)	F_2 $\frac{\text{Females with lethals}}{\text{Females tested}}$
Embryo (r)	0.327 (apparent)	25/127 = 0.197
Total (r')	0.278	32/127 = 0.252

where the value of r is computed by the F_2 method. In this experiment, D' is 0.162, about one-fourth as large as D, and nearly the same as r. Values of D' as high as ½ that of D and 2½ times as high as r have been observed in other experiments when eggs in the first meiotic metaphase are irradiated. Curiously enough, conditionally delayed dominant lethal mutations appear to be associated only with irradiation of eggs in the first meiotic metaphase; in all experiments in which eggs in the first meiotic prophase have been irradiated, $D' = 0$. This phenomenon is being more fully investigated at the present time.

DROSOPHILA GENETICS

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P. Ballard

The Influence of Free Heterochromatic Duplications on the Meiotic Behavior of Univalent Chromosomes

D. L. Lindsley

In spermatogenesis in *Drosophila melanogaster* it is known that the X and Y chromosomes regularly pair and proceed to opposite poles at the first division. If the X and Y are attached (Fig. 16) a stock can be maintained which lacks a free Y chromosome; in this case the sex chromosome complement is univalent. It is known that such an attached-X-Y chromosome ($Y^SX{\cdot}Y^L$ – the center point indicates centromere position) is recovered about 85% as frequently from males of the constitution $Y^SX{\cdot}Y^L/0$ as from males of the constitution $Y^SX{\cdot}Y^L/Y$; in the latter case 50% of the progeny carry $Y^SX{\cdot}Y^L$ as expected from Mendelian segregation.[31] The deficiency of $Y^SX{\cdot}Y^L$ bearing gametes recovered from $Y^SX{\cdot}Y^L/0$ males has been interpreted as meiotic loss of $Y^SX{\cdot}Y^L$ correlated with its univalent condition. It was considered that this system offered a good opportunity to study the disjunctive and, inferentially, the pairing properties of small heterochromatic elements derived from the X chromosome; such elements marked with y^+ were derived by irradiation of Canton-S males. These duplications were then characterized with respect to whether they carried normal alleles of *ac*, *sc*, *su-w*^{a}, and *pn* distally and *car*, *su*^{W}-*f*, and *bb* proximally. The duplications actually arose through deletion of a large segment of the euchromatin of the X, and each can be described by designating which of the above markers are at the limits of the deleted segment.

With the above duplications, males of the constitution $Y^SX{\cdot}Y^L$/Dp were crossed to $\underline{y\ w}/0$ females; the progenies were examined for sex ratio and for the distribution of the duplication. These results are summarized in the first two columns of Table 15; it can be seen that the effect of two of the duplications (118 and 122) is similar to that of no duplication, and in no case does a duplication actually increase the recoverability of the $Y^SX{\cdot}Y^L$ chromosome as does the Y. If these results are to be interpreted in terms of chromosome loss, three points must be recognized: (1) Appreciable rates of loss are observed together with normal disjunction as shown by the low incidence of exceptions in Table 15; (2) any hypothesized loss cannot be merely loss of a chromosome from the meiotic spindle, but must be loss of an entire gamete as also shown by the low incidence of exceptions (i.e., nullo-XY, nullo-Dp gametes); and (3) the presence of a pairing partner may actually increase the incidence of loss, sometimes drastically. An attempt to explain these findings in terms of meiotic loss of univalents led to the hypothesis that the behavior of the $Y^SX{\cdot}Y^L$ chromosome is in some way a function of the distribution of its heterochromatin. In order to examine this

[29] Research Associate.
[30] ORINS Fellow.

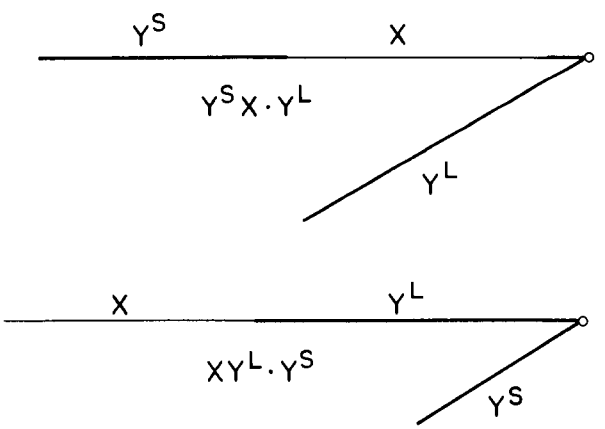

Fig. 16. Diagrammatic representation of the attached-X-Y chromosomes used in these experiments. Heavy lines represent heterochromatin.

[31] L. Sandler and G. Braver, *Genetics* 39, 365-377 (1954).

TABLE 15. RECOVERY OF THE ATTACHED X-Y CHROMOSOME EXPRESSED AS PERCENTAGE OF EXPECTATION, AND OF EXCEPTIONAL PROGENY FROM CROSSES OF X-Y/Dp MALES BY *y w*/0 FEMALES. EXPECTATION OF X-Y RECOVERY BASED ON EXPECTED EQUAL RECOVERY OF X-Y AND Dp AND ASSUMED COMPLETE RECOVERY OF Dp

Duplication	Limits of deletion	$Y^SX.Y^L$ [a]		$XY^L.Y^S$ 13		$XY^L.Y^S$ 15		$XY^L.Y^S$ 24	
		Percentage		Percentage		Percentage		Percentage	
		Recovery	Exceptions	Recovery	Exceptions	Recovery	Exceptions	Recovery	Exceptions
Y		103.9	0.1	79.4	0.8			18.2	0.4
O		88.5		85.4		34.6		3.9	
42	*pn* to su^W-*f*	55.5	3.7	39.0	8.4				
50-1	*pn* to su^W-*f*	50.4	0.2	50.4	0.8				
118	*pn* to su^W-*f*	83.0	0.1	64.6	0.4				
122	su-w^a to su^W-*f*	90.7	0.1	64.9	0.2	54.4	0.1	6.5	0
142	*pn* to *car*	18.9	4.0	18.9	5.7				
179	*pn* to *bb*	32.6	0.1	51.8	0.8				

[a]Data of Lindsley and Sandler (unpublished).

hypothesis it seemed desirable similarly to test three attached X-Y chromosomes of different constitution ($XY^L.Y^S$) derived by Parker.[32] The results of these tests are given in the remaining columns of Table 15. It is evident that $XY^L.Y^S$ 13 behaves very much like $Y^SX.Y^L$, whereas the few crosses which have been made with $XY^L.Y^S$ 15 and $XY^L.Y^S$ 24 exhibit entirely different behaviors, both from $Y^SX.Y^L$ and from each other. The remarkably low recovery of $XY^L.Y^S$ 24 from males carrying a free Y lead to the suspicion that viability difficulties might be a factor in this case. The cross $XY^L.Y^S/sc^8.Y$ ♂ × *y/y* ♀ was made to check on this point; the recovery of the $XY^L.Y^S$ chromosome from such a cross in $XY^L.Y^S/y$ females (19.1%) was no different from recovery of the same chromosome in $XY^L.Y^S/0$ males from attached X crosses (see Table 15). Since these three chromosomes can differ only in kind and amount of heterochromatin, the hypothesis that their behavior is a function of heterochromatic constitution is considered confirmed. It is too early to define the relation between heterochromatic content and disjunction.

Crossing Over in Attached-X Chromosomes

W. J. Welshons

There are indications that, under the stimulus of an autosomal inversion, the increased frequency of crossing over in attached-X chromosomes of *Drosophila melanogaster* is confined to very short localized regions of the chromosomes. Simultaneously, exchanges in very short adjacent regions have been greatly inhibited by the presence of the inversion. The inhibition of crossing over under such circumstances was completely unexpected. It should be noted that when short adjacent regions of the chromosome are so affected by an inversion that one region is stimulated to cross over while the remaining region is inhibited, the net effect for both regions considered simultaneously is to show little or no effect, since the two processes cancel each other. Only when genetic markers are close together, as they were in this experiment, can the effect be detected.

It is fortunate that further study of this effect of an inversion need not be confined to attached-X chromosomes. The obvious experiment was to perform a similar study with the same genetic markers but in this case to let the X chromosomes be unattached. The data from this experiment are presented in Table 16 along with similar data obtained from the attached-X study.

Table 16 lists the total number of exchanges which occurred in the various regions of the chromosomes defined by the genetic markers. The χ^2

[32]D. R. Parker, *Proc. Natl. Acad. Sci. U. S.* 40, 795-800 (1954).

TABLE 16. THE REGIONAL DISTRIBUTION OF CROSSING OVER ALONG THE MARKED REGIONS OF THE X CHROMOSOME

	Crossover regions					
	ptg-v	*v-m*	*m-g*	*g-sd*	*sd-f*	Total
Attached-X						
Noninversion	190	80	125	113	96	604
Inversion	103	27	108	73	49	360
χ^2	0.60	6.75	8.09	0.27	0.77	16.48
Free-X						
Noninversion	180	68	158	116	121	643
Inversion	217	85	201	154	107	764
χ^2	0.02	0.09	0.42	0.97	4.99	6.31

See text for explanation of this table.

values result from a homogeneity test which tests the hypothesis that the distribution of exchanges over the various regions is the same whether or not an autosomal inversion is present. In the case of attached-X chromosomes, a total χ^2 of 16.48 for 4 degrees of freedom is quite significant. Most of the χ^2 is contributed by discrepancies in the regions *vermilion* to *miniature* (*v-m*) and *miniature* to *garnet* (*m-g*). Furthermore, these differences are in opposite directions. When an autosomal inversion is present, there are too few exchanges in the *v-m* region and too many in the *m-g* region. When free-X chromosomes are considered, the χ^2 value equals 6.31 for 4 degrees of freedom and corresponds to a p value between 0.20 and 0.10 – not considered significant – the expected result was not obtained.

It should be noted that in the attached-X experiment, there appeared to be an interesting correlation. The region *v-m* is thought to correspond to a euchromatic section of the chromosome, and the region *m-g* is thought to contain intercalary heterochromatin.[33] It thus appeared that this inversion inhibited crossing over in a euchromatic region and stimulated exchanges in a heterochromatic one.

In spite of the failure of the free-X chromosome data to show discrepancies in these two locations, it is still possible that the generality is true. In free-X chromosomes, the χ^2 value is 6.31, and the greatest portion of this χ^2 is contributed by the region *scalloped* to *forked* (*sd-f*). This section of the chromosome appears to be euchromatic. Therefore, it would be expected, according to this generality, that when the inversion is present, crossing over in this locale would be decreased. This expectation is confirmed. The χ^2 portion of 4.99 represents a deviation in the expected direction. It is quite possible that the adjacent heterochromatic region to the right, *forked* to *carnation* (*f-car* – not marked in this experiment) has experienced and increased rate of crossing over when the inversion is present. It is interesting to note that other investigators have already reported a large increase in this *f-car* region under similar circumstances.[34] A new stock bearing markers in this region is now being synthesized in order to test this point.

It is suggested that the attachment of X chromosomes has somehow prevented the attached-X complex from responding to the influence of an autosomal inversion in precisely the same way as free-X chromosomes respond. Further study will clarify the situation.

[33] A. M. Hannah, *Advances Genet.* 4, 87-125 (1951).

[34] J. Schultz and H. Redfield, *Cold Spring Harbor Symposia Quant. Biol.* 16, 175-197 (1951).

The Somatic Behavior of an Unstable Ring Chromosome in *Drosophila melanogaster*

C. W. Hinton

Typically, ring chromosomes in *Drosophila melanogaster* experience no difficulty in mitotic divisions. This investigation concerns a unique

ring-X chromosome (called w^{vc}) which is unstable as shown by its frequent elimination from w^{vc}/rod-X zygotes to produce gynandromorphs and X0 males. Previous results have shown that w^{vc} instability is not controlled by the homologous X chromosome, the Y chromosome, or the autosomes. One dominant autosomal modifier and certain physiological factors do alter the degree of w^{vc} instability, which is highly variable from family to family. The behavior of small, spontaneously occurring, unstable ring duplications derived from w^{vc} demonstrated that w^{vc} instability is a property of the centromere region of the chromosome. These results have been supplemented and confirmed by experimental data obtained since July 1954.

In order to test the proposition that w^{vc} instability is dependent on ring structure, a special genetic situation was devised whereby the unstable ring could be opened out to form a V-shaped chromosome. Five such chromosomes have been obtained and all are completely stable. Rings reconstructed from one of the V-shaped chromosomes are again unstable, proving that ring structure is necessary for instability expression.

Earlier results had suggested that high w^{vc} instability as measured by elimination is accompanied by high mortality of w^{vc} zygotes. Eggs from the cross y/y females by w^{vc}/sc^8:Y males were tested for dominant lethality and were then raised to adulthood where the deficiency of w^{vc} adults was calculated with y/sc^8:Y males as the basis of expectation. If the w^{vc} deficiency class arises solely through dominant lethality rather than indiscriminate mortality during the entire developmental period, one should expect a straight-line relation with a slope of ½ which appears to hold at all but the highest levels of w^{vc} deficiency (Fig. 17). These results suggest that w^{vc} lethality should be used in addition to w^{vc} elimination as a combined measure of w^{vc} instability.

The following model is proposed to represent the mechanism responsible for w^{vc} instability. In the early cleavage divisions of a w^{vc} zygote, the ring chromosome may form a double dicentric chromosome at anaphase. There are two possible fates of such anaphase bridges – breakage or exclusion from daughter nuclei (Fig. 18). Qualitatively, this model accounts for (1) the finding that the ring structure is necessary for the expression of w^{vc} instability, (2) the occurrence of small ring duplications and dominant lethality as the consequences

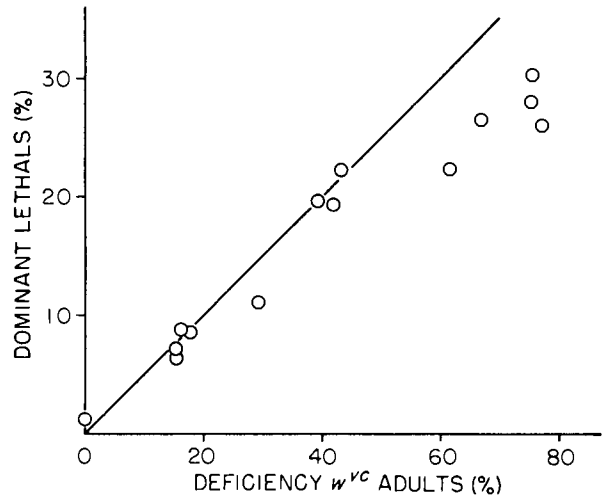

Fig. 17. The relation of dominant lethality and deficiency of w^{vc} adults. The straight line is that expected if all the w^{vc} adult deficiency arose through dominant lethals.

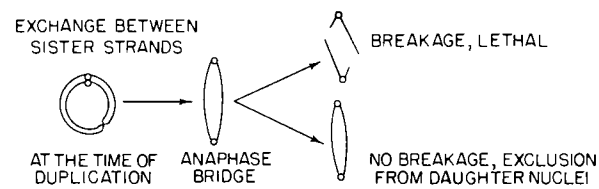

Fig. 18. Proposed model of cytological events occurring in the early cleavage divisions accounting for w^{vc} behavior.

of anaphase bridge breakage, and (3) gynandromorphs and X0 males as the result of anaphase bridge elimination. Anaphase bridges are observed in w^{vc} larval brain smear mitoses, and McClintock has found that ring chromosomes exhibit this behavior in corn.

The Composition of Sex-Linked Recessive Lethal Mutations Induced By Radiations of Different Ion Densities

C. W. Edington

It is known that induced sex-linked recessive lethal mutations arise as the result of intragenic changes (point mutations), of minute and gross chromosome deletions, and in association with gross structural changes such as inversions and translocations. It has also been observed that

most lethals which are associated with structural changes are completely linked with the aberrations.

Two hypotheses have been advanced to explain the origin of these lethal mutations. The breakage hypothesis postulates that all induced lethal mutations arise as the result of breakage of the chromosome per se with few, if any, produced by intragenic changes or position effects. According to this hypothesis, any lethal which appears to be caused by a point mutation may be regarded as having occurred by chromosome breakage followed by restitution of the broken ends. The position effect hypothesis postulates that the majority of lethals are caused by intragenic changes (no breakage involved) and to position effect rather than by the breakage process itself.

In an effort to gain further information concerning this problem, males of the Oregon-R strain of *Drosophila melanogaster* were exposed either to fast neutrons in the Oak Ridge National Laboratory's 86-inch cyclotron or to Co^{60} gamma rays and the frequency of induced sex-linked recessive lethal mutations was determined by the Muller-5 technique. Since the relative biological effectiveness (RBE) of fast neutrons as compared to X rays for dominant lethals and chromosome aberrations has been shown to be high (4-7 for dominant lethals and 5-6 for translocations), it is reasonable to assume that a relatively high RBE would be obtained for recessive lethals if a substantial number of them were caused by position effect. When the slopes of the regressions of lethal frequency on dose for neutrons and gamma rays (Fig. 19) are compared to the slope of the regression for X rays, it can be seen that the RBE of neutrons as compared to X rays is ~1.25 and to gamma rays ~2. Therefore, it becomes apparent that the RBE for recessive lethals is much lower than that for genetic effects arising from multihit observations. It should also be noted that, per unit dose, X rays are about 1.6 times as effective in producing recessive lethals as gamma rays.

Furthermore, if lethals arise chiefly as the result of chromosome breakage, then the frequency of recessive lethals induced in a closed-X chromosome should be lower than the frequency in a rod-X chromosome. This is caused by torsional restitution of the broken ends and the formation of double and interlocked rings with subsequent loss of the ring. In order to make this comparison, males bearing a closed-X chromosome, X^{cl}, and

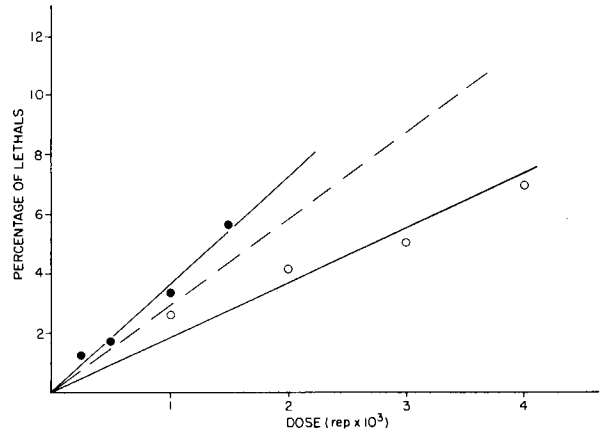

Fig. 19. The frequency of sex-linked recessive lethal mutations induced by radiations of different ion densities. ●, fast neutrons; O, gamma rays; – – –, X rays.

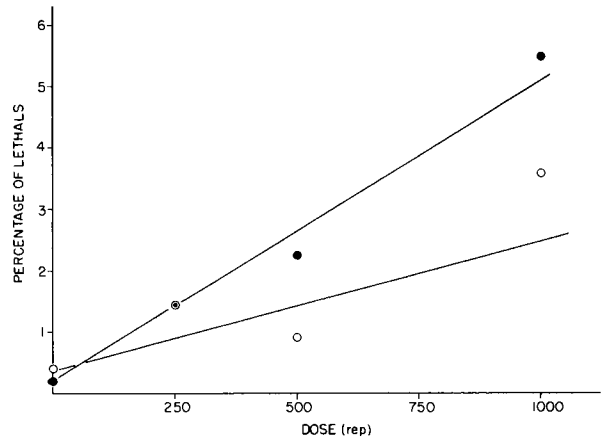

Fig. 20. The frequency of sex-linked recessive lethal mutations induced by fast neutrons in a closed-X and rod-X chromosome. ●, rod-X chromosome; O, closed-X chromosome.

males bearing a rod-X chromosome, In(1)EN (which arose from an opening-out of the X^{cl} chromosome), were exposed to fast neutrons and the frequency of recessive lethals determined. It is apparent from Fig. 20 that the frequency induced in the closed-X chromosome is lower than that in the rod-X. When the slopes of the regression lines are tested for significance by the "t" test, it is found that they are significant at the 7% level. It is believed that this difference is real since the

experimental method gives a minimum estimate of recessive lethal frequency in the rod chromosome. The data tabulated in Table 17 demonstrates a sex ratio distortion which confirms the belief that rings are lost owing to breakage and torsional restitution. The table also shows a lowered frequency of dominant *Minutes*, which are considered to be largely the result of chromosome deletion. This confirms the finding that the lowered frequency of recessive lethals in closed-X chromosomes as compared to the frequency in rod-X chromosomes can be attributed to actual chromosome breakage with loss of the ring.

From the results reported here, it seems valid to conclude that an appreciable number of recovered lethals are the result of a chromosome break and are independent of the eucentric manner in which the breaks rejoin.

TABLE 17. SEX-RATIO DISTORTION AND FREQUENCY OF *MINUTES* INDUCED BY FAST NEUTRONS IN CLOSED-X AND ROD-X CHROMOSOMES

Chromosome	Dose (rep)	Number of F_1 males	Corrected percentage of F_1 males	Number of F_1 females	Number of *Minutes*	Percentage of *Minutes*
Rod-X	0	2511	50.0	2745	0	
	250	2310	50.65	2465	5	0.203
	500	1837	50.34	1984	7	0.353
	1000	1724	49.64	1912	18	0.941
Closed-X	0	2392	50.0	2740	0	
	250	1620	54.07	1595	1	0.063
	500	1698	58.76	1403	3	0.214
	1000	1115	60.98	847	4	0.472

RADIATION PROTECTION AND RECOVERY

PROTECTION AND RECOVERY IN BACTERIA AND MICE

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G. E. Stapleton
D. Billen
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D. H. Woodbury

Effect of Growth Conditions on Bacterial Recovery

G. E. Stapleton
A. J. Sbarra
D. H. Woodbury

It had been shown that *Escherichia coli* B/r can partially overcome the damaging effects of X or gamma rays if the cells are supplied with the necessary nutritional factors after irradiation.[1] The recovery effect is more pronounced if the cells are incubated after irradiation at a temperature well below that which is optimal for growth of this organism.[1,2] In all the reported investigations, cells had been cultured prior to irradiation on a rich natural medium (nutrient broth). Nonirradiated cells grow equally well on a simple inorganic salts-glucose medium as on the richer media. Moreover, after culture on the rich media, they adapt well to growth on the simple media. Irradiated cells, on the other hand, lose much of this adaptability.

Some experiments were carried out to determine the effect of the medium (see Table 18 for composition of media) on which the cells were grown prior to irradiation on their sensitivity as well as their requirement for nutritional factors after exposure to gamma rays. Table 19 shows data on the effect of cultural conditions on the sensitivity of cells to gamma rays. Medium III, as described, constitutes the chemically defined medium found to substitute for natural tissue extracts in promoting recovery. It is clear from this table that cells cultured prior to irradiation on medium I show greater survival when plated on the simple medium after irradiation than do cells cultured on nutrient broth. Nevertheless, their viability is increased on yeast extract or medium III, indicating a stimulatory effect of these media on recovery. It is of interest that cells cultured on medium I plus added purines or pyrimidines, or amino acids prior to irradiation show requirements for these compounds in the plating medium after irradiation. In all experiments either medium III or yeast extract stimulate recovery in the cells, regardless of how they were cultured prior to irradiation.

It is of interest that cells cultured on the chemically defined recovery medium (III) are far more resistant than cells cultured on other media. The nature of this resistance is under investigation at the present time.

The results, to date, indicate that cells cultured on a rich medium, have a limited enzyme constitution since they have had supplied to them, in their culture medium, a complex of metabolic intermediates. When such cells are placed in an

TABLE 18. COMPOSITION OF MEDIA

Components	Concentration (mg/ml)
Medium I	
KH_2PO_4	1.0
$(NH_4)_2HPO_4$	4.0
$MgSO_4$	0.7
Na citrate	0.5
Glucose	10.0
pH 6.8	
Medium III	
K_2HPO_4	0.5
KH_2PO_4	0.5
$MgSO_4$	0.2
NaCl	0.01
$FeSO_4$	0.01
$MnSO_4$	0.01
Guanine	0.05
Uracil	0.05
Glutamic acid	0.15
Glucose	10.0
pH 6.8	

[1] G. E. Stapleton, A. J. Sbarra, and I. W. Bacon, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, pp. 35-36.

[2] G. E. Stapleton, A. J. Sbarra, and C. W. Edington, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, pp. 35-36.

TABLE 19. EFFECT OF CULTURE MEDIUM ON RADIATION SENSITIVITY

Surviving fractions are averages of three or more runs. Incubation temperature, 37°C

Culture medium	Surviving fraction of cells after 85 kr of γ rays plated on:				
	Medium I	Medium I + G + U	Medium I + AA	Medium III	Medium I + 20 mg of yeast extract
1. Medium I	2.2×10^{-6}	3.1×10^{-6}	4.0×10^{-6}	2.2×10^{-5}	1.3×10^{-5}
2. Medium I + G + U[a]	1.0×10^{-7}	1.0×10^{-6}	8.0×10^{-7}	3.0×10^{-6}	2.6×10^{-6}
3. Medium I + AA[b]	2.3×10^{-7}	2.9×10^{-6}	2.3×10^{-5}	5.0×10^{-5}	6.0×10^{-5}
4. Nutrient broth	3.0×10^{-8}	5.0×10^{-8}	8.0×10^{-7}	2.7×10^{-6}	3.5×10^{-6}
5. Medium III	1.0×10^{-4}			6.0×10^{-4}	3.0×10^{-4}

[a]G, guanine; U, uracil (50 μg each).
[b]AA = Difco caseamino acids.

inorganic salts-glucose medium (I) void of these compounds they not only must make these intermediates but the enzymes for synthesis of these compounds. Normal, nonirradiated cells can perform this task, whereas irradiated cells are inhibited, and partially overcome the inhibition if the proper nutritional factors are supplied to them immediately after irradiation.

Protection, Recovery, and Mutagenesis in X-Irradiated *Escherichia coli*

C. O. Doudney A. Hollaender

A study of the effects of cysteamine (β-mercaptoethylamine) and β-mercaptoethanol on the induction of revertants in several auxotrophic strains of *Escherichia coli* (P82/r purine$^-$, T83-8 tyrosine$^-$, P83-5 phenylalanine$^-$, and 113-3 methionine$^-$) has been carried out. Both these compounds have a marked ability to protect *E. coli* from the lethal effects of X and gamma irradiation.[3] Although protection by pretreatment with mercaptoethanol shows little dependence on postirradiation nutrition, a major part of the increased survival of cysteamine-treated *E. coli* is dependent on postirradiation recovery requiring nutritional supplements.[4] Figure 21 compares the results obtained with strain P83-5 following treatment with nitrogen, mercaptoethanol (0.1 M), and cysteamine (0.006 M) for 30 minutes. Mercaptoethanol markedly reduced the reversion rate at a given dose of X ray in these strains. A similar reversion rate for equivalent surviving fractions is observed when treated

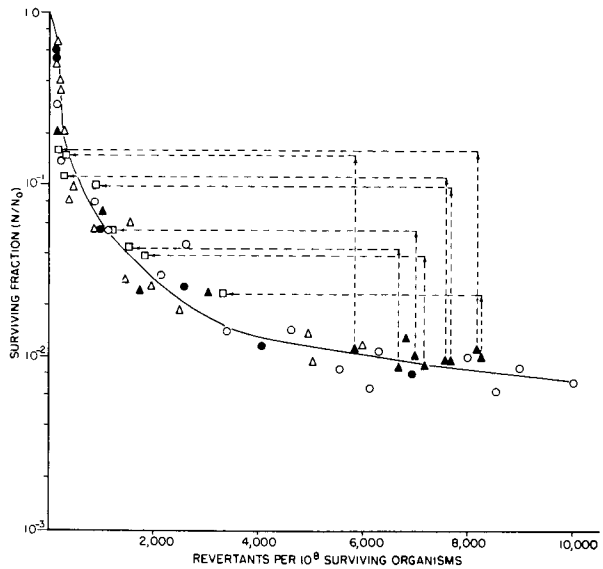

Fig. 21. The relation of survival to induced reversion rate following X irradiation of *E. coli* strain P83-5. O, air; ●, nitrogen; Δ, β-mercaptoethanol; ▲, cysteamine (unrecovered); □, cysteamine (recovered).

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

[4]C. O. Doudney and A. Hollaender, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 36.

and untreated cells are compared at varying dose levels. The cysteamine-treated bacteria, which are prevented from recovering by withholding a yeast extract supplement following irradiation, show a comparable relation of survival to reversion rate. In our studies of the effect of postirradiation recovery following treatment with cysteamine prior to irradiation in strain P83-5 (Fig. 21), results demonstrate that the postirradiation recovery process reduces the reversion rate to a degree corresponding to the increase in survival produced. These data thus extend the observation of a relation of survival to reversion rate following X irradiation in oxygen and nitrogen[5,6] to chemical protection in several auxotrophic strains and to postirradiation recovery in one. The results with strain P83-5 suggest that the mechanism involved in postirradiation recovery is also effective in reducing the reversion rate following irradiation.

Effects of X Irradiation on the Synthesis of Cellular Constituents

D. Billen

In studies of the effects of X rays on the macromolecular organization of *Escherichia coli* strain B/r it was found that when the exposed cells were resuspended in a growth medium such as nutrient broth and incubated at 37°C, an enzymically active, ribonucleic acid-rich particulate component was observed to breakdown as was also a deoxyribonucleic acid (DNA) component.[7] Earlier investigations had shown that growth, as measured by turbimetric increase, occurred to a limited degree when X-irradiated cells were incubated under similar conditions.[8] With these observations in mind and the reports of other investigators showing complete inhibition of DNA synthesis and a modified ribonucleic acid (RNA) and protein synthesis in ultraviolet-exposed cells,[9,10] a study of nucleic acid metabolism was undertaken to determine the relation between this altered nucleic acid metabolism and the cellular component changes observed.

As in the ultraviolet studies of the investigators mentioned, it was found that synthesis of DNA (net increase) was completely inhibited in X-irradiated (60 kr) *E. coli* strain B/r reincubated in a minimal salts-glucose medium, although some incorporation of C^{14} from labeled glycine occurred. RNA and protein synthesis took place but at a reduced rate as compared to the nonirradiated controls. The degree of inhibition of RNA and protein synthesis varied from experiment to experiment. If the exposed cells are pretreated with cysteamine and incubated in a minimal salts-glucose medium supplemented with yeast extract, the inhibition of DNA synthesis is reversed after a brief lag period (Fig. 22). Such treatment has been found by other investigators in this laboratory markedly to increase the survival of X-irradiated

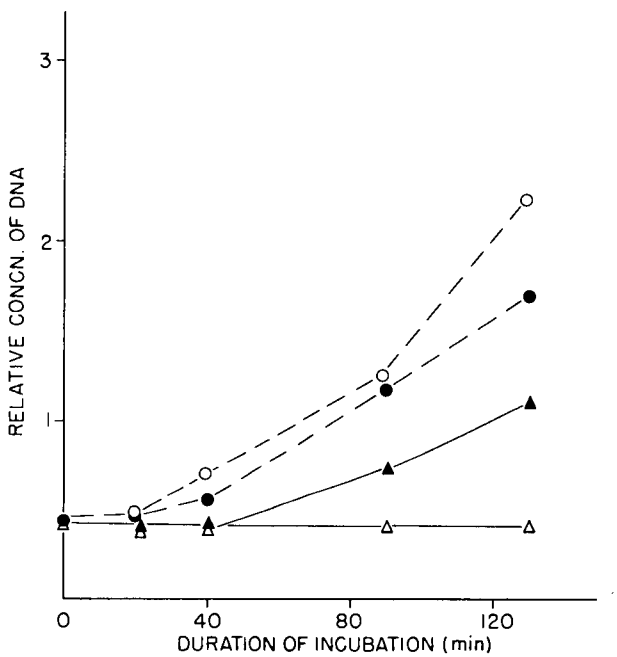

Fig. 22. The synthesis of DNA in *E. coli* strain B/r X-irradiated with and without cysteamine pretreatment, when incubated at 37°C in a minimal salts-glucose medium supplemented with yeast extract. – – – O, unirradiated cells; – – – ●, unirradiated cells, cysteamine-treated; Δ—, X-irradiated cells; ▲—, X-irradiated cells, cysteamine-treated.

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

[6] D. Billen and R. W. Whittle, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 40.

[7] D. Billen and E. Volkin, *Biol. Quar. Prog. Rep. Feb. 10, 1953*, ORNL-1497, p. 21.

[8] D. Billen, *Biol. Quar. Prog. Rep. May 10, 1951*, ORNL-1026, p. 27.

[9] A. Kelner, *J. Bacteriol.* 65, 252-262 (1953).

[10] S. S. Cohen and H. D. Barner, *Proc. Natl. Acad. Sci. U. S.* 40, 885-893 (1954).

E. coli.[4] The effect observed here is similar to that found in DNA synthesis studies on photoreactivated cells that had been exposed to ultraviolet light.[9] If the yeast extract is not added, DNA synthesis remains blocked. When non-cysteamine-treated cells are incubated in the minimal salts-glucose medium supplemented with yeast extract, no net increase in DNA is found (Fig. 22). Thus, as an extension of the findings in the survival studies,[4] it has been found that the addition of cysteamine prior to irradiation alters the effects of exposure to the extent that a compound or compounds present in yeast extract enables the exposed cells to overcome some block in the events leading to DNA synthesis. It is hoped that the responsible compound(s) will soon be identified since such information will help unravel the mechanism of the radiation-induced block in DNA synthesis. It also remains to determine the effects of such treatments on the particulate components in irradiated cells pretreated with cysteamine.

Effect of Certain Nitrogen- and Sulfur-Containing Compounds on the Survival of X-Irradiated Mice

W. T. Burnett, Jr. D. G. Doherty
A. W. Kimball[11]

Protection against ionizing radiations has been afforded to mice, in this and other laboratories,[12-16] by certain chemical compounds. Several of these contain a sulfhydryl group or groups. However, from the standpoint of toxicity and of limited ability to enhance the survival of irradiated mice, cysteine, glutathione, BAL, and β-mercaptoethylamine (MEA) definitely are not ideal protective agents in spite of their unique protective properties in mice and in bacteria.[17,18] Heretofore, the best protective agent reported (Bacq) appeared to be MEA. The search for a suitable protective agent led to a systematic modification of the chemical structure of this compound, the primary objective being to elucidate the mechanism of protection of sulfhydryl compounds in general.[14]

Special emphasis in this report will be given to the protective properties of S,β-aminoethylisothiouronium·Br·HBr (AET bromide). It will be shown that this protective agent is less toxic than the parent compound. Moreover, on the basis of as yet circumstantial evidence, the protective action is probably sustained over a much longer span of time. The results of experiments now in progress show that mice can survive higher doses of total-body radiation following treatment with AET bromide than hitherto realized with other protective agents. Further advances are being made by use of the principle of "multiple therapy" following exposure to ionizing radiations. Comparative data are given for X and gamma radiations.

Sequential Screening Tests. The scope of the program called for an economical and time-saving screening test. A sequential design of comparative experiments[19,20] was adopted. The test is objective, and it minimizes the chances of errors of both the first and second kinds, namely, the error of falsely stating that a compound is protective and the error of failing to detect real protection. In this type of experiment, the sample size is not fixed but instead depends on the outcome of the experiment. The savings can be as much as 50%.

Briefly, the strategy was to expose five mice to an LD_{100} dose of total-body X radiation (800 r measured in air) for 10 min following the intraperitoneal injection of 0.2 ml of saline. Simultaneously, an additional five mice were given 800 r of X rays following the intraperitoneal injection of a solution of the test compound. An examination of the difference in the number (E) of surviving mice treated with the test compound

[11] Mathematics Panel.

[12] H. M. Patt, E. B. Tyree, R. L. Straube, and D. E. Smith, *Science* 110, 213-214 (1949).

[13] E. P. Cronkite, J. Brecher, and W. H. Chapman, *Proc. Soc. Exptl. Biol. Med.* 76, 396-398 (1951).

[14] D. G. Doherty and W. T. Burnett, Jr., *Abstr. of Papers, 126 Natl. Meet. Am. Chem. Soc.*, p. 9C (1954).

[15] H. M. Patt, D. E. Smith, E. B. Tyree, and R. L. Straube, *Proc. Soc. Exptl. Biol. Med.* 73, 18-21 (1950).

[16] Z. M. Bacq, G. Dechamps, P. Fischer, A. Herve, H. Lebihan, J. Leconte, M. Pirotte, and P. Rayet, *Science* 117, 633-636 (1953).

[17] W. T. Burnett, Jr., G. E. Stapleton, M. L. Morse, and A. Hollaender, *Proc. Soc. Exptl. Biol. Med.* 77, 636-638 (1951).

[18] C. O. Doudney, D. G. Doherty, and A. Hollaender, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, pp. 38-39.

[19] A. Wald, *Sequential Analysis.* Wiley, New York, 1946.

[20] O. L. Davies, *Design and Analysis of Industrial Experiments.* Hafner, New York, 1954.

and in the number (S) of mice in the control group was made on the day on which the third mouse in the control group died (ith day). This was called trial 1. A compound was considered protective when, on repeated trials, $\Sigma E_i - \Sigma S_i \geqq 6$, and nonprotective when, after several trials, $\Sigma E_i - \Sigma E_i \leqq 6$, where the subscript i denotes the number of trials. Usually, the tests were truncated following the third trial, but in some cases, following the second trial, when the compound did not appear promising as a protective agent. The test was designed to detect a 20% difference in survival time, with $\alpha = \beta = 0.10$ (α = maximum risk of reporting a compound protective when an equivalent or greater survival could actually be obtained with saline. β = maximum risk of reporting that the saline controls would survive longer than mice given the test compound when this is actually not the case). The applications of the sequential tests for representative nitrogen- and sulfur-containing compounds at one or two dose levels are summarized in Tables 20 and 21. The results for protective compounds are given in Table 20 and for nonprotective compounds in Table 21.

C_3H male mice 10-14 weeks old and weighing, on the average, ~24 g were used in the sequential tests. The mice were randomized in each test, and were housed in lots of ten each in porcelain pans. The effect of housing in groups and of housing conditions on radiation-induced mortality will be discussed later in this report. Water and Purina Laboratory Chow were given *ad libitum*. The source of X rays was a General Electric Maxitron 250 operated at 30 ma, 250 kvp, beryllium window, 2 mm of Cu filtration added, hvl 3.8 mm of Cu and TSD 46 cm (center of animal in a horizontal or crouched position). Further details have been given by Burnett *et al.*[21] The mice were segregated for at least 2 weeks before use, following receipt of shipments from the Jackson Memorial Laboratory, Bar Harbor, Maine. With some exceptions, where the tests indicated that a compound was protective against X irradiation, the protected mice survived for at least 28 days.

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

TABLE 20. PROTECTIVE NITROGEN- AND SULFUR-CONTAINING COMPOUNDS. SEQUENTIAL ANALYSES, TOXICITY TESTS, AND 28-DAY SURVIVORS. C_3H MALE MICE GIVEN AN LD_{100} DOSE OF X RADIATION (800 r)

Compound	Toxicity LD_{50} (mg/mouse)	Dose (mg/mouse)	$\Sigma E_i - \Sigma S_i$[a]	28-Day survival (%)
β-Mercaptoethylamine·HCl	5.4	1.8	4^3	0
		3.6	8^3	7
		3.6	8^3	13
		3.6	8^2	10
S,β-Aminoethylisothiouronium·diHBr	13.9	4.4	8^3	40
		8.8	6^2	80
		8.8	7^3	20
		8.8	8^2	60
		8.8	6^2	70
		8.8	7^2	90
		8.8	6^2	30
α-Aminopropylisothiouronium·diHBr	6.9	2.5	9^3	13
		5.0	7^3	60
β-Aminoethylisothiouronium·diHCl	>4.5	2.3	7^3	0
		4.5	8^3	0

[a] Survivors in treated group minus survivors in saline group. Superscripts show number of trials made in sequential test before a compound was considered protective.

TABLE 21. NONPROTECTIVE NITROGEN- AND SULFUR-CONTAINING COMPOUNDS. SEQUENTIAL ANALYSES AND TOXICITY TESTS. C_3H MALE MICE GIVEN AN LD_{100} DOSE OF X RADIATION (800 r).[a]

Compound	Toxicity LD_{50} (mg/mouse)	Dose (mg/mouse)	$\Sigma E_i - \Sigma S_i$[b]
γ-Hydroxypropylisothiouronium·Cl	5.4	2.7	-3^3
		5.4	-3^3
β-Cyanoethylisothiouronium·Cl	>5.2	2.6	0^3
		5.2	4^3
β-Thiouronium acetic acid·HBr, pH2	~2.0	0.5	3^1
		1.0	-3^2
β-Thiouronium ocetamide	>3.0	1.5	4^3
		3.0	0^3
BAL-diisothiouronium·diCl	>11.2	5.6	3^3
		11.2	4^3
Di-(S,β-isothiouroniumethyl ether)·diCl	81	16	2^3
		32	2^3
Methylaminoethylisothiouronium·2HCl	2.6	0.9	-1^3
		1.7	2^3
Dimethylaminoisopropylthiouronium·2Cl	>3.7	1.9	1^3
	<7.4	3.7	-4^3
Cholineisothiouronium·2HBr	0.86	0.17	-1^3
		0.33	0^3
N-Acetylaminoethylisothiouronium·Br	>7.6	3.8	-1^2
		7.6	-1^2
Methyl bis(isothiouronium methyl)amine·3HCl	~1.5	0.5	2^1
		1.0	2^1
Bis(isothiouronium ethyl)amine·3HCl	~1.5	0.5	2^1
		1.0	4^3
Pyridine-2-isothiouronium·Br·HBr, pH 3	~9.0	5.0	1^1
		10.0	-1^2
β(1-Piperdyl)ethylisothiouronium·2HCl	2.4	1.5	0^3
γ(1-Piperdyl)propylisothiouronium·2HCl	4.8	1.4	0^3
		2.8	-1^3
γ(1-Piperdyl)butylisothiouronium·2HCl	3.3	2.5	0^2
β(1-Pyrolidyl)ethylisothiouronium·2HCl	4.0	1.4	-3^2
		2.8	-3^2
γ(1-Pyrolidyl)propylisothiouronium·2HCl[c]	5.5	2.0	4^3
		4.0	6^3
δ(1-Pyrolidyl)butylisothiouronium·2HCl[c]	4.0	1.6	8^3
β(4-Morpholyl)ethylisothiouronium·2HCl	~3.0	1.5	3^3
		3.0	0^3

TABLE 21 (continued)

Compound	Toxicity LD_{50} (mg/mouse)	Dose (mg/mouse)	$\Sigma E_i - \Sigma S_i$[b]
γ(4-Morpholyl)propylisothiouronium·2HCl[c]	>6.0	3.0	0^3
		6.0	5^3
δ(4-Morpholyl)butylisothiouronium·2HCl	9.0	4.5	0^3
		9.0	-1^3
β-Alanylmercaptoethylamine	>10.0	5.0	3^2
		10.0	3^2
Pantoylaminoethylthiol	>6.6	3.3	1^3
		6.6	0^3
Pantetheine	>8.8	4.4	-2^2
		6.6	0^3
2-Butylaminopropylthiol	>2.4	1.2	2^3
	<4.8	2.4	-3^3
2-Dibutylaminopropylthiol	>6.6	3.3	0^3
		6.6	-1^3
o-Aminobenzenethiol (sodium salt?)	>2.0	1.0	2^3
		2.0	0^3
2,2′-Dithioethanol[c]	>4.8	2.4	6^3
		4.8	3^3
Diethylaminoethyl sulfide	>5.6	2.8	4^3
		5.6	-2^3
Diisopropylaminoethyl sulfide	>3.2	3.2	4^3
Di(isothiouronium-3-diethyl)formal·2HCl	>13.1	6.6	0^1
		13.1	0^2
4,6-Diamine-2S-thiazinethiol	>2.4	1.2	2^1
		2.4	1^2
2,5-Dimercapto-3,4-thiodiazol	>18.0	9.0	1^2
		18.0	1^2
2-(Dibutylaminoethyl mercapto)-2-thiazoline	~3.0	1.0	0^1
		2.0	4^3
Thialdine	>5.2	2.6	1^3
		5.2	1^3
Diisothiouronium pyrimidine·Cl (suspension)	>8.0	8.0	-3^2
Cyclohexylethylisothiouronium·Br	>1.0	0.5	5^3
		1.0	2^3
Phenylethylisothiouronium·Br	>1.6	0.8	2^3
		1.6	4^3
p-Nitrobenzylisothiouronium·Br	>4.0	2.0	-1^2
		4.0	-3^2

[a]No 28-day survivors.

[b]Survivors in treated group minus survivors in saline group. Superscripts show number of trials made in sequential test before a compound was considered protective.

[c]To be examined further for protective activity.

"Up-and-Down" Toxicity Tests. Before testing for protective ability, the toxicity of a given compound was estimated usually by an "up-and-down" method.[22] A set of doses, usually equally spaced on a log scale, was chosen *a priori*. One animal was injected at a dose believed to be near the LD_{50}. If this animal died, a second animal was injected with the next lower dose; if the first animal lived, the second animal was injected with the next higher dose. The procedure was continued until a predetermined number of animals had been tested. A toxic dose (LD_{50}) was considered to be one that produced moderate or marked convulsions (clonic spasms) and/or death within 30 min following the intraperitoneal injection of a solution (occasionally, a suspension) of the compound. Actually, the LD_{50} could be estimated within 5% with only ten animals tested. Other methods, such as probit analysis, of estimating the LD_{50} could easily require fifty or more animals to achieve standard errors of 5% or less. C_3H female mice 10-14 weeks old were used in the toxicity tests.

Protection by MEA and AET Bromide. Toxicity data for the parent compound, MEA, and its derivative, AET bromide are given in Table 22. It will be noted that there is only a small difference between the LD_5 and LD_{95} in each case. After Bacq, we selected a dose of 3.6 mg of MEA for our standard in the sequential tests. This dose is, of course, approaching the toxic range. In our hands, a dose of 1.8 mg of MEA does not provide significant protection to X-irradiated mice. The dose levels for other test compounds were selected so as to be equimolar to 1.8 and 3.6 mg, respectively, of the parent compound unless the toxicity of the compound precluded the calculated doses. In the latter case, doses were usually selected so as to be equimolar to 0.9 and 1.8 mg of MEA.

The summary given in Table 20 suggests that AET bromide is a better protective agent for mice than the parent compound, MEA; it has therefore been adopted as the standard in recent screening tests. In Table 20, it should be noted that 4.4 mg of AET bromide affords almost as much protection as 8.8 mg per mouse, in contrast to the failure of 1.8 mg of MEA (equimolar to 4.4 mg of AET bromide) to protect.

[22] K. A. Brownlee, J. L. Hodges, Jr., and M. Rosenblatt, *J. Am. Stat. Assoc.* 48, 262-277 (1953).

TABLE 22. TOXICITY OF β-MERCAPTOETHYLAMINE AND S,β-AMINOETHYLISOTHIOURONIUM·Br·HBr GIVEN BY INTRAPERITONEAL INJECTION TO C_3H FEMALE MICE. PRODUCTION OF CONVULSIONS AND DEATH

	Mercaptoethylamine (mg/mouse)	Aminothiouronium (mg/mouse)
LD_{95}	6.38 (5.57 – 7.19)*	14.77 (13.52 – 16.02)*
LD_{50}	5.40 (5.12 – 5.68)*	13.57 (13.22 – 13.92)*
LD_5	4.42 (3.61 – 5.23)*	12.37 (11.12 – 13.62)*

*95% Confidence interval.

The Additive Effect of S,β-Aminoethylisothiouronium·Br·HBr, Bone Marrow, and Streptomycin on the Survival of Irradiated Mice

W. T. Burnett, Jr. D. G. Doherty
Alexander Hollaender

Little is known about the mechanisms involved in the death processes induced by ionizing radiations. In this connection, it is of interest to note that the first wave of deaths usually occurs in irradiated mice in the period 4-7 days following exposure to LD_{100} doses. The peak of deaths narrows down to 5-5½ days with doses in the range of 1000-4000 r. However, the effect of protective agents in mice given doses within this range has received little attention. In view of the sharpness of the mortality curves, it is evident that a gain of a few days in survival time would represent significant protection, although the animals were destined to die before the 28-day period conventionally used in most protection studies. A shift in the mortality peak should indicate protection with respect to a relatively few of the death processes induced by ionizing radiations. Protection achieved in this region could well be proportional to the dose in contrast to lower doses where no simple mathematical relation is usually evident.

Protection by AET Bromide at High Doses of Radiation. Exploratory experiments with doses of X and gamma rays in the range 572-2400 r were made, AET bromide being used as the protective agent. C_3H female mice 10-14 weeks in age were used in these experiments. The X-ray exposures were made with the 250 Maxitron. The gamma-ray

exposures were made with a recently recalibrated cobalt source delivering about 57 r/min to the center of the animal in an upright position. Details of the exposure box have recently been described by Upton *et al.*[23,24]

A dose of 8.8 mg of AET bromide given by intraperitoneal injection was adopted as a standard. Survival times following irradiation were estimated to the nearest 6 hours. Two trials were made with X rays and two with gamma rays. Five mice irradiated simultaneously were used for each dose in each trial.

The five saline controls and five AET bromide-treated mice for each dose level were housed together in porcelain pans. The results of several experiments are presented in Table 23 and Fig. 23. The appearance of the treated mice that died was good at the time of death; in some cases, they were apparently on the road to recovery, as judged by weight curves. The point to be emphasized here is that many animals, after surviving for approximately 7-11 days, rapidly declined and died. Since it is well established that a massive bacterial invasion of the blood stream occurs in this period and that a few antibiotics, particularly streptomycin, are effective in reducing mortality

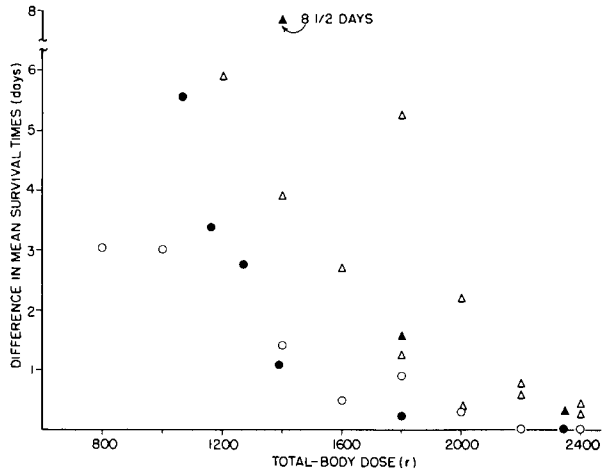

Fig. 23. Enhancement of mean survival times in C_3H female mice by treatment with aminoethylthiouronium prior to irradiation with X and gamma rays. X-ray experiments: ●, first; O, second. Gamma-ray experiments: ▲, first; Δ, second.

[23] A. C. Upton, F. P. Conte, G. S. Hurst, and W. A. Mills, submitted for publication 1955.

[24] J. S. Kirby-Smith and D. S. Doniels, *Genetics* 38, 375 (1953).

TABLE 23. EFFECT OF S,β-AMINOETHYLISOTHIOURONIUM·Br·HBr ON THE SURVIVAL OF C_3H FEMALE MICE EXPOSED TO TOTAL-BODY X AND GAMMA RADIATIONS

Dose (r)	X rays				Gamma rays			
	Saline		Thiouronium		Saline		Thiouronium	
	*	**	*	**	*	**	*	**
572	0/5	7½	5/5		2/5	16	5/5	
648	0/5	10½	4/4		2/5	14	5/5	
762	0/5	9	4/4		0/5	11	5/5	
800	0/5	9	2/4	15½	1/5	10	4/5	12
838	0/4	8	5/5		0/5	8½	5/5	
914	0/5	8	3/5	10	0/5	8	4/5	18
991	0/5		3/4		0/5		3/5	
1067	0/5	6	1/5	12	0/5	9	5/5	

*No. 28-day survivors/no. irradiated.

**Mean death time (days) for nonsurvivors.

in the range of radiation doses around the LD_{50},[25,26] it was felt that the mice which had survived for the 7 to 11 days might have been saved by suitable antibiotic treatment. Similar reasoning was applied to the possible use of bone marrow[27,28] as a recovery agent in conjunction with the protective agent.

Protection by Combined Treatment. Male mice of the 101 × C_3H strain approximately 6 months old have been used in an examination of the protective effect of AET bromide followed by a treatment with bone marrow and streptomycin.[29] Five trials have been made with graded doses of gamma rays in the range 1600 to 2200 r. The design of the experiment was a 2^3 factorial, repeated at each of eight dose levels (see Tables 24 and 25). In each experiment, one mouse was exposed to each dose of radiation indicated for each treatment. In Tables 24 and 25 the results of five experiments have been pooled. These tables give the number of surviving mice under each condition. The mean survival time of the animals that died is also

[25] C. P. Miller, C. W. Hammond, M. Tompkins, and G. Shorter, *J. Lab. & Clin. Med.* 39, 462-479 (1952).

[26] H. Y. Canter, and M. M. Grenan, *Am. J. Physiol.* 172, 351-358 (1953).

[27] L. O. Jacobson, E. L. Simmons, E. K. Marks, E. O. Gaston, M. J. Robson, and J. H. Eldredge, *J. Lab. & Clin. Med.* 37, 683-697 (1951).

[28] E. Lorenz, C. Congdon, and D. Uphoff, *Radiology* 58, 863-877 (1952).

[29] R. Q. Marston, H. J. Ruth, and W. W. Smith, *Proc. Soc. Exptl. Biol. Med.* 83, 289-291 (1953).

TABLE 24. EFFECT OF S,β-AMINOETHYLISOTHIOURONIUM·Br·HBr, BONE MARROW SUSPENSIONS, AND STREPTOMYCIN ON THE SURVIVAL OF 101 × C_3H MALE MICE EXPOSED TO TOTAL-BODY GAMMA RADIATION

Dose (r)	Thiouronium		Thiouronium Streptomycin		Thiouronium Marrow		Thiouronium Marrow Streptomycin	
	*	**	*	**	*	**	*	**
1600	5/5		4/5	20	5/5		4/5	9½
1800	2/5	10	2/5	9	4/5	8½	4/5	8
2000	1/5	7½	4/5	6½	4/5	19	5/5	
2200	0/4	7½	2/5	11	2/4	6	3/4	15½

*No. 28-doy survivors/no. irradiated.

**Mean death time (days) for nonsurvivors.

TABLE 25. EFFECT OF BONE MARROW SUSPENSIONS AND STREPTOMYCIN ON THE SURVIVAL OF 101 × C_3H MALE MICE EXPOSED TO TOTAL-BODY GAMMA RADIATION

Dose (r)	Saline		Streptomycin		Marrow		Streptomycin Marrow	
	*	**	*	**	*	**	*	**
1600	0/4	5	0/4	10	2/4	5½	1/4	5½
1800	0/4	5½	0/4	6½	1/4	5	0/4	6½
2000	0/4	5½	0/4	6½	0/4	5	1/4	5
2200	0/4	5½	0/4	5½	0/4	5½	0/4	6½

*No. 28-day survivors/no. irradiated.

**Mean death time (days) for nonsurvivors.

given. At each dose level, the mean survival time of the saline controls was approximately 5½ days. A moderate gain in survival time was achieved with streptomycin alone and in combination with bone marrow. With bone marrow alone and in combination with streptomycin, a few animals survived at the 1600-, 1800-, and 2000-r levels of gamma radiation. Within 1 hr following irradiation, bone marrow was given by intravenous injection of a saline suspension (approximately one-third of the homologous bone marrow from one femur per mouse). Streptomycin (5 mg) was given, as indicated, by daily subcutaneous injection to each mouse for a period of 10 days, beginning on the third day following irradiation. The results gained with bone marrow are about those to be expected on the basis of the experience of other investigators. For a rough estimate one might deduce from the data in Table 25 that the LD_{50} of the AET bromide-treated hybrid mice was about 1800 r. It would appear that the additional streptomycin treatment, bone marrow treatment, and both treatments together produced an additional increase in survival over that given by AET bromide. Although some of the animals died in less than 28 days, the extension of their life span was sufficient to indicate some protection. All of the mice listed as surviving are still alive 40 days after irradiation. At the end of the initial 28-day period, the first signs of graying were noted in mice having thin spots on their hair coat; otherwise, the mice appeared to be in good condition, as judged by visual inspection and complete recovery of the weight loss in the second week following irradiation. Another group of five C_3H male mice treated with AET bromide alone, kept for 70 days, now show signs of striking patterns of depigmentation.

Effect of Housing Conditions on Survival. It is well known that animals housed in colonies behave differently to those housed in individual cages. Casual observations by Furth, Upton, Conte, and Burnett in this Laboratory have been made which indicated that the mortality pattern of irradiated animals is influenced by the housing conditions. Recently, W. L. Russell (private communication, 1955) has found that the LD_{50} of irradiated mice is enhanced by housing in individual cages. We have adopted a simple wire box-type of cage having the dimensions 4 × 4 × 6 inches. Four of the cages are placed on an open metal rack which permits the excreta to fall on thick pulp paper. The cages are replaced with clean, sterilized cages and the pulp paper replaced at least at intervals of 3 days. Any animal having diarrhea is segregated. As mentioned earlier in this report, treatment with an antibiotic daily, beginning on the third day and continuing until the thirteenth day following irradiation, produces a further reduction in mortality. This schedule of streptomycin treatments was adopted on the basis of experience of other workers.

Another important phase of the program is the determination of the best routes and times for administration of the protective agents, recovery agents, and antibiotics, as well as of the best doses and combinations. It is now anticipated that, with only a few modifications of the experiments reported here, mice could be protected against >2400 r of total-body gamma radiation.

ENZYMOLOGY AND PHOTOSYNTHESIS

ENZYMOLOGY

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Metabolism of Formate-C^{14}, Glycine-2-C^{14}, and Adenine-8-C^{14} by Chick Embryos

J. V. Passonneau J. R. Totter

Experiments previously described[3] have been extended to include use of the glycine-2-C^{14} as a labeling agent. Ribonucleic (RNA) and deoxyribonucleic (DNA) acids from chick embryos that had been incubated with formate-C^{14}, glycine-2-C^{14}, or adenine-8-C^{14} were extracted with cold 1 N $HClO_4$ and hot 0.5 N $HClO_4$ respectively, and degraded with hot 70% $HClO_4$. The bases resulting from the hydrolyses were chromatographed on paper with 60% isopropanol-2 N HCl, and the specific activities of the eluted bases determined. All other experimental conditions were the same as those described in the previous report. The activities found for the RNA and DNA bases in typical experiments are recorded in Table 26. It may be seen that gamma irradiation failed to reduce the DNA adenine activity, with any of the three precursors tested. On the other hand the activities of both guanine and thymine (when the precursor was glycine-2-C^{14} or formate-C^{14}) were depressed by gamma irradiation. These results confirm the previous finding and lend weight to the interpretation offered earlier. It seems most probable that adenine is incorporated by some route which does not involve new synthesis of DNA. This has an important bearing on interpretation of the results of many experiments related to the biosynthesis of nucleic acid.

[1]Consultant.

[2]Research Participant.

[3]J. V. Passonneau and J. R. Totter, *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, p. 37.

PHOTOSYNTHESIS

W. A. Arnold E. S. Meek

Possible Mutation in *Chlorella*

W. A. Arnold E. S. Meek

An attempt has been made to induce mutations in *Chlorella* cells by ultraviolet irradiation. It is well known that, under ordinary circumstances *Chlorella* will grow in a completely inorganic medium. A requirement for the presence of trace elements, vitamins, or any specific growth factor in the medium has not been shown.

A suspension of *Chlorella pyrenoidosa* (Emerson) was irradiated with wave length 2537 A for various times. The suspension was such that 0.1 ml contained approximately 200 *Chlorella* cells. At certain intervals, 0.1 ml was plated out onto a complete medium. The complete medium contained the required inorganic salts or minimal medium, a trace element mixture, a vitamin mixture, glucose, tryptone, and yeast extract.

After 2 weeks' incubation at 23°C in the presence of neon lights, 69 colonies were obtained.

TABLE 26. THE EFFECT OF GAMMA IRRADIATION ON THE SPECIFIC ACTIVITIES OF THE NUCLEIC ACID BASES WITH FORMATE-C^{14}, GLYCINE-2-C^{14}, AND ADENINE-8-C^{14} PRECURSORS[a]

Dose (r)	Base →	DNA Fraction								RNA Fraction					
		Adenine, cts/sec/μmole			Guanine, cts/sec/μmole			Thymine, cts/sec/μmole		Adenine, cts/sec/μmole			Guanine, cts/sec/μmole		
	Precursor →	For-C^{14}	Gly-2-C^{14}	Ad-8-C^{14}	For-C^{14}	Gly-2-C^{14}	Ad-8-C^{14}	For-C^{14}	Gly-2-C^{14}	For-C^{14}	Gly-2-C^{14}	Ad-8-C^{14}	For-C^{14}	Gly-2-C^{14}	Ad-8-C^{14}
Control		47.5	6.4	14.0	38.0	5.45	1.8	56.0	13.6	70	8.35	22.8	47.5	11.5	3.3
1,000		54.0	7.3	21.6	30.4	5.6	1.6	72.5	10.1	93	10.8	33.0	32.8	7.6	3.8
5,000		34.0	4.5		24.6	3.2		27.5	6.6	85	8.85		28.2	7.3	
10,000		48.0	5.2	18.6	25.6	3.0	2.3	29.5	7.2	79	7.2	31.8	36.4	6.15	3.8
20,000		43.5	5.8		24.2	3.5		34.4	5.9	76	7.0		41.5	3.34	

[a]Specific activities of precursors: Formate, 8 mc/mmole; glycine, 1.61 mc/mmole; adenine, 1.17 mc/mmole.

One colony failed to grow in minimal medium, minimal medium plus trace elements, or minimal medium plus glucose. This colony did, however, grow in the complete medium, the minimal medium plus vitamins, minimal medium plus tryptone, and the minimal medium plus yeast extract. It is hoped that added investigations will determine the specific growth requirements of the mutant obtained.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell, Section Chief

E. F. Oakberg	J. W. Bangham
L. B. Russell	R. L. DiMinno
J. S. Gower	M. K. Freeman
J. C. Kile, Jr.[1]	E. M. Kelly
L. Wickham	M. H. Major
R. Auerbach[2]	R. J. Spear

Radiation-Induced Mutations in the Mouse

W. L. Russell	M. K. Freeman
J. S. Gower	E. M. Kelly
J. W. Bangham	M. H. Major

Collection of data on presumed mutations at specific loci in the 300-r X-ray experiment is almost complete. The additional information obtained strengthens the conclusions presented earlier.[3] A more detailed account of this experiment and experiments at other doses will be presented when the breeding tests of the mutations are concluded.

Duration of Spermatogenesis, Timing of Tubule Stages, and Renewal of Spermatogonia in the Mouse

E. F. Oakberg R. L. DiMinno

It is general knowledge that in a cross section of the testis, individual tubules vary greatly in the cell associations represented. This results from two sequential processes, one a rhythmic multiplication of spermatogonia (the spermatogenic "wave"), the other the development of spermatogonia into sperm. In rodents, the rate of the respective processes is such that four cycles of spermatogonial multiplication occur at a given point during the time required for dormant type A spermatogonia to develop into spermatozoa. This results in the presence of four stages of spermatogenesis in most tubule sections. The regularity of this process gives rise to definite cell associations which can be used for systematic classification of tubule stages.[4,5] Recent interpretations of the dynamics of spermatogenesis, especially as revealed by periodic acid-Schiff staining,[5] have made possible a reinterpretation of the radiation damage to spermatogenic cells.[3]

Demonstration of the high sensitivity of intermediate and type B spermatogonia to irradiation, with expression of this damage as cell death,[3] emphasized the need for reevaluation of estimates of the duration of spermatogenesis. For, it is now apparent that irradiation exposures used in the estimates made by various investigators[6,7] have been so high that one entire cycle of developing spermatogonia must have been destroyed. As a result, the 26-27 days commonly accepted as the time required for development of sperm from spermatogonia actually represents only the completion of development of primary spermatocytes. Therefore, the 26-27 estimate should be divided by 3 to get the estimate of the length of one cycle of the seminiferous epithelium, and not by 4 as has previously been done.[5]

In order to verify this hypothesis, 101 × C_3H F_1 hybrid male mice were exposed to 100 r of X rays, and killed at the times shown in Table 27. All intermediate and type B spermatogonia are destroyed with 100 r, but only minor damage will be caused to primary spermatocytes. Therefore, subsequent development of the germinal epithelium should demonstrate a sharply defined disappearance of cell populations which, in the absence of irradiation, would have resulted from cells which were spermatogonia x hr or 1, 2, or 3 cycles + x hr earlier. Determination of the time required for the disappearance of certain post-spermatogonial cell types should allow estimation of length of the cycle of the seminiferous epithelium. Tubules in stage VII were selected for scoring because this is the only stage that can be identified accurately at all time intervals used

[1] Consultant.

[2] Research Associate.

[3] E. F. Oakberg and J. C. Furney, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 49.

[4] E. C. Roosen-Runge and L. O. Giesel, *Am. J. Anat.* 87, 1-30 (1950).

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

[6] A. B. Eschenbrenner and E. Miller, *Arch. Pathol.* 50, 736-749 (1950).

[7] S. L. Shaver, *Am. J. Anat.* 92, 391-432 (1953).

TABLE 27. ESTIMATION OF TIME REQUIRED FOR COMPLETION OF ONE CYCLE OF THE SEMINIFEROUS EPITHELIUM AND DURATION OF SPERMATOGENESIS

Time after 100 r of X rays at which observation was made[a]	Limited to stage-VII tubules			
	Characteristic scored	Proportion of tubules in specified class (%)[b] at time shown in column 4	Time at which effect was observed	Time between successive cell generations (cycle length)
24, 30, 36, 48 hr	Absence of resting primary spermatocytes	76[c]	38 hr[c]	
7, 8, 9, 10, 11 days	Absence of primary spermatocytes in pachytene	78	10 days	8 days, 10 hr
16, 17, 18, 19, 20 days	Absence of spermatids	74	19 days	9 days
25, 26, 27, 28, 29 days	Absence of spermatozoa	74	27 days-8 hr[b]	8 days, 8 hr
25, 26, 27, 28, 29 days	Reappearance of spermatids	54	25 days	
34, 35, 36, 37, 38 days	Reappearance of spermatozoa	51	34 days	9 days
Total of 4 cycles				34 days, 18 hr

[a]Four mice killed at each time interval, 20 stage-VII tubules scored for each mouse.

[b]Based on 80 tubules, 20 from each of 4 mice.

[c]Frequencies of tubule classes observed at these times not directly comparable to frequencies with which they are to be compared. Values for 24-48 hr and 25-29 days estimated from a rough plot of all data at each of these intervals.

in this experiment. During the first 48 hr, a deficiency of resting primary spermatocytes occurs. As a result of the cyclical process of development, this results in absence of pachytene stages of the primary spermatocyte one cycle length later, disappearance of spermatids two cycles later, and absence of maturing spermatozoa after three complete cycles. Reappearance of spermatids in the 25-29 and maturing spermatozoa in the 34- to 38-day group was used for the final estimate of cycle length. The estimate of about 35 days as the duration of spermatogenesis, with a cycle length of 8 days 18 hr (Table 26) is remarkably close to the suggestion that previous estimates of duration of spermatogenesis (26-27 days) should be divided by 3 to obtain the length of each cycle.

Description of the cycle of the seminiferous epithelium of the rat[5] is directly applicable to the mouse for tubule stages I-IX and the initiation of stage X. Stages XI, XII, and XIII as described for the rat are not clearly distinguishable in the mouse, and a classification using twelve stages has been adopted. The appearance of acute, sharp angles at the caudal end of the spermatid nucleus has been used to indicate the beginning of stage XI. Stage XII begins with contraction in length of the spermatid. Diakinesis and both meiotic divisions occur during this stage and afford the most easily recognizable means of classification. Thus stage XII in the mouse is comparable to stage XIV for the rat. Estimation of the duration of each tubule stage should be possible by combining the 8-day–18-hr cycle estimated from the data of Table 26 with a frequency distribution of tubule stages. For this purpose, 250 random tubule cross sections were scored from each of twelve control mice. These data are summarized in Table 28.

In order to obtain information on the process of renewal of spermatogonia in the mouse, the numbers of spermatogonia and resting primary

TABLE 28. FREQUENCY DISTRIBUTION OF 3000 RANDOMLY SELECTED TUBULE STAGES, DURATION OF EACH STAGE, AND MEAN NUMBER OF SPERMATOGONIA AND RESTING PRIMARY SPERMATOCYTES PER TUBULE CROSS SECTION IN CONTROL MICE

Stage	Number of tubules in given stage	Percentage of tubules per stage	Duration of each stage (hr)	Mean number of "normal" spermatogonia per tubule cross section[b]			Mean number resting primary spermatocytes per tubule cross section
				Type A	Intermediate	Type B	
I	322[a]	10.73	22.5	6.59			
II	262	8.73	18.3	8.62	0.53		
III	126	4.20	8.8	2.22	10.65		
IV	270	9.00	18.9	2.05	10.49	2.67	
V	164	5.47	11.5	1.97		19.45	
VI	262	8.73	18.3	2.15		17.13	7.06
VII	298	9.93	20.9	2.24			38.20
VIII	301	10.03	21.1	2.30			
IX	220	7.33	15.4	2.86			
X	164	5.47	11.5	3.46			
XI	310	10.33	21.7	4.01			
XII	301	10.03	21.1	5.13			
	3000		210.0				

[a]250 tubules scored per mouse.
[b]Sectioned at 5 μ.

spermatocytes were counted in the 3000 tubules scored. Development of primary spermatocytes from type A spermatogonia has been found to occur in the same manner as previously described for the rat.[8] In the mouse, three peaks of mitoses occur during multiplication of type A cells; at stages IX, XI, and II. After the division at stage II, most spermatogonia transform into the intermediate type, but a few type A cells "escape" this differentiation and remain dormant (as far as division is concerned) until the division peak of stage IX. Intermediate spermatogonia formed by differentiation of type A cells divide at stage IV to form type B cells. The division of type B spermatogonia at stage VI then gives rise to resting primary spermatocytes.

Definite cell associations and predictable numbers in each cell generation are correlated with the tubule stages identified by development of the acrosomic system of spermatids. The main purpose of the above work on timing of tubule stages was to make possible the elucidation of the radiation response of any given cell at any selected subsequent stage of development.

[8]Y. Clermont and C. P. LeBlond, *Am. J. Anat.* **93**, 475-501 (1953).

Changes in the Sensitivity of Oocytes to the Induction of Dominant Lethals

L. B. Russell R. J. Spear

It was shown earlier[9,10,11] that the death of embryos conceived *subsequent* to irradiation of the female was due largely or entirely to dominant

[9]L. B. Russell *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, pp. 52-54.

[10]L. B. Russell and R. J. Spear, *Rec. Genetics Soc. Amer.* **23**, 64 (1954).

[11]L. B. Russell and W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* **19**, 50-59 (1954).

lethals produced by radiation in the oocytes. Since superovulation occurs within the postirradiation interval to be considered here,[12,13] litter-size cannot be used to determine dominant lethal incidence. Dominant lethal incidence for the results described in this report is expressed as follows:

$$1 - \frac{\text{(living embryos/corpora lutea)/irradiated female}}{\text{(living embryos/corpora lutea)/control female}} .$$

Ideally, the computations should be made on fertilized eggs rather than corpora lutea. But the determination of fertilized eggs is technically difficult and tedious; and, furthermore, survival of embryos cannot be measured in the same females in which eggs are scored. That the use of corpora lutea is justified was shown in a separate experiment, involving two simultaneously treated comparable series of females, in which it was determined that, for ovulations made at the first estrus following treatment, irradiated and corresponding control females are approximately equal in their ratios of the number of fertilized eggs (scored within 16 hr after fertilization) to the number of corpora lutea (scored at about 2 wk of pregnancy) (Table 29).

Earlier results[9,11] indicated that although matings made 1½ to 4½ days after 400 r to the ovaries produced a dominant lethal incidence of 28%, matings made ½ day after 400 r resulted in a dominant lethal incidence of 69%. Since it is known[14] that the first meiotic division occurs shortly before ovulation, it became of interest to determine whether the higher dominant lethal rate induced in oocytes ½ day prior to ovulation was due to this fact, and whether even higher sensitivities might be demonstrated at preovulation intervals slightly shorter or longer than ½ day.

Females of tested fertility, and of the same strain – $(101 \times C_3H)F_1$ – and age used earlier, were irradiated with 400 r X rays at one of three times of day: 11:04-11:26 AM, 2:37-3:05 PM, and 6:44-7:13 PM. Males were added at 6:30-7:30 PM of the same day. Of 159 females entered into the experiment, 27 had vaginal plugs the morning after treatment, and 18 the following morning; the rest were not used for this series. Females were sacrificed 13½ or 14½ days after fertilization, their uterine contents examined, and corpora lutea counted. The results are shown in Table 30.

The dominant lethal incidence of 24% for females irradiated around 36.5 hr prior to fertilization

[12] L. B. Russell and M. H. Major, *Genetics* 38, 687-688 (1953).

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

[14] G. D. Snell, in *Biology of the Laboratory Mouse*, ed. G. D. Snell, pp. 59-65, 73-78, Blakiston, Philadelphia, 1941.

TABLE 29. COMPARISON OF MEAN NUMBER OF FERTILIZED EGGS AND MEAN NUMBER OF CORPORA LUTEA IN TWO STRICTLY COMPARABLE SERIES OF FEMALES UNDER VARIOUS CONDITIONS

Prefertilization day treated ⟶	0 r		400 r	
	½	1½-3½	½	1½-3½
Mean number fertilized eggs[a] (per ♀)	7.2	8.9	6.8	10.7
Mean number corpora lutea[b] (per ♀)	10.3	10.0	11.0	13.2
Fertilized eggs/corpora lutea	0.70	0.89	0.62	0.81

[a] Based on total of 351 examined within 24 hr after fertilization under dark-contrast-medium phase.

[b] Based on total of 265 counted at approximately 2 wk postfertilization.

TABLE 30. PERCENTAGE OF DOMINANT LETHALS INDUCED IN OOCYTES BY IRRADIATION AT VARIOUS INTERVALS PRIOR TO FERTILIZATION

Mean irradiation-to-fertil. interval[a] (hr)	Time treated[b]	Number females	Mean number per female		Percentage of total death occurring before implantation	Percentage dominant lethals[e]
			Corpora lutea	Living embryos		
36.5	11:19 AM- 6:52 PM[c]	10	11.0	7.6	55.9	23.9
16.1	11:11-11:26 AM	4	10.5	7.8	54.5	18.7
12.6	2:37- 3:05 PM	8	10.5	4.5	37.5	52.8
8.5	6:44- 7:13 PM	10	?[d]	0.1	93.9	98.8
Controls.		13	9.2	8.3	54.8	

[a]Mean of the extremes of the range. The range is computed by assuming fertilization to occur between 2:00 and 5:00 AM (text footnote 14).

[b]Treatment consisted of 400 r of X rays (250 kvp, 15 ma, ca. 80 r/min).

[c]Eight of the 10 females were irradiated between 2:37 and 3:05 PM.

[d]Corpora lutea retrogress rapidly after the death of the last embryo and, since in 9 of the 10 females in this group there were no living embryos, reliable corpora lutea counts could not be made. To compute percentage dominant lethals an average number of corpora lutea equal to controls was assumed. This would give an underestimate of dominant lethals should super ovulation have occurred.

[e]Computed as described in first paragraph of text.

agrees well with the 28% incidence found in an earlier experiment[9,11] for the 1½- to 4½-day prefertilization intervals. It may be seen from Table 30 that the dominant lethal incidence for females irradiated around 16.1 hr prefertilization is no higher than that for females treated 1 day earlier, i.e., around 36.5 hr prefertilization. However, a radical change in sensitivity to dominant lethal induction occurs in oocytes between ca. 11:30 AM and ca. 7:00 PM of the day preceding ovulation. The group irradiated in the afternoon (2:37-3:05 PM) is intermediate in average. It is possible that an abrupt change from "slightly sensitive" to "extremely sensitive" occurs during the afternoon, since, when results from individual females irradiated 2:37-3:05 PM are examined, this group is found to be heterogeneous, two of the females having *no* living embryos, like the 7:00-PM group, the rest of them averaging 6.0 living embryos, which is almost as high as the 11:30-AM group. A histological study of the ovary is under way to determine if the different sensitivities to dominant lethal induction are correlated with nuclear events occurring in the oocytes.

[15]L. B. Russell, *J. Exptl. Zool.* 114, 545-602 (1950).

The Development of Some X-ray-Produced Abnormalities in the Mouse

R. Auerbach R. L. DiMinno

Strain-101 female mice were mated to C_3H males and irradiated with 300 r of X rays 9½ days after the appearance of a copulation plug. This stage has been shown to be sensitive to radiation production of a number of abnormalities, including the ones whose development is now under study.[15] Radiation factors in our experiments were 250 kvp; 15 ma; 3 mm of Al; hvl 0.4 mm of Cu.

Embryos were obtained by dissection at ½-day intervals following irradiation and gross abnormalities were recorded. Among the most frequently observed gross abnormalities are blebs, coloboma, tail defects, and "spina bifida." The histological examination of these abnormal regions will be described below.

Blebs consisted of local accumulation of dead cells. They were usually restricted to the dorsal lumbosacral region and the most anterior part of the head of embryos 10-11½ days old; occasionally, blebs persisted into later stages.

Coloboma involved only a small part of the pigment ring of the eye. The histological material studied to date suggests that faulty formation as

well as abnormal reconstitution of the retina may be involved in the production of this abnormality.

Tail defects occur in many embryos $10\frac{1}{2}$-$12\frac{1}{2}$ days old but are almost always present in later fetuses. Preliminary study indicates that in the former group tail formation is initially abnormal, the degree of abnormality being related to the degree of neural malformation of the lumbosacral region; the subsequent abnormality of all tails may be indicative of secondary degenerative processes. That such processes are indeed involved is also suggested by the appearance at days $11\frac{1}{2}$-$13\frac{1}{2}$ of terminal tail hematomata coupled with local degeneration of terminal neural tissues.[16]

X-ray-induced "spina bifida" was found to be the end result of disturbed differentiation of the lumbosacral region.[17] In our material, abnormalities of the ganglionic, cartilaginous, and muscular elements bear a close relation to previous somite disorientation which, in turn, seems causally related to initial malformation and degeneration of the neural tube and its subsequent reconstitution. It is interesting to compare these results with hereditary spina bifida in the mouse in an attempt to understand more clearly the nature of this abnormality. Spina bifida can occur in response to a number of mutations with strikingly different initial developmental disturbances;[18,19] a common characteristic, though not necessarily cause, of these genetically determined types of spina bifida is the disruption, in the developing mouse embryo, of the normal formation or maintenance of the neural tissues of the lumbosacral region. The X-ray results suggest that such disruption at an appropriate time may, in itself, be sufficient to result in abnormal dependent differentiation leading to spina bifida.

[16] See alsa Hamburgh's results (*Anat. Record* 119, 409, 1954) with trypan blue; and hereditary tail defects (e.g., S. Gluecksohn-Waelsch, *Quart. Rev. Biol.* 28, 115, 1953).

[17] R. Auerbach, *Anat. Record* (1955). (Abstract, in press.)

[18] See H. Gruneberg, *The Genetics of the Mouse*, 2nd ed. Martinus Niyhaff, The Hague, 1952.

[19] See R. Auerbach, *J. Exptl. Zool.* 127, 305-329 (1954).

PATHOLOGY AND PHYSIOLOGY

PATHOLOGIC AND PHYSIOLOGIC EFFECTS OF RADIATION

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Preliminary Studies on the Role of the Thyroid-Pituitary Axis in Resistance to Lymphoid Leukemia

D. M. Morris F. F. Wolff

Previous studies suggest that the thyroid-pituitary axis significantly influences the growth of lymphoid tissues.[6,7] The following experiments were undertaken to explore the action of thyroid and thyroid-stimulating hormones on the resistance of AK/R mice to transplanted lymphosarcoma.

Young adult AK/R mice, previously thyroidectomized by subcutaneous administration of I^{131}, were injected intraperitoneally with suspensions of AK/R lymphoma cells. Inoculations were also made into euthyroid and radiothyroidectomized hosts receiving thyroid hormone and thyroid-stimulating hormone (TSH). Thyroidectomy was achieved by the injection of 200–230 μc of I^{131} intraperitoneally or subcutaneously; 14 days later subcutaneous administration of hormone (or saline placebo) was begun and repeated every other day for three injections, then continued every third day until death. Thyroxine was prepared in a concentration of 1 mg/ml, and the thyroid-stimulating hormone preparation contained 10 USP units/milliliter. All solutions were made up in physiological saline, the thyroxine solution and saline for the controls being made slightly alkaline. The lymphoid tumor suspension was administered 1 or 2 days after the third injection of saline or hormone. All mice were autopsied at death and the diagnosis of leukemia was confirmed by microscopic study when doubtful.

The mean survival time (Table 31) for the forty-nine thyroidectomized animals (13.4 days) was significantly longer ($p < 0.001$) than for the thirty-nine euthyroid controls (11.4 days). All mice died with leukemia. When supplementary thyroid was administered to the thyroidectomized mice, the mean survival time was significantly reduced (10.4 days, $p < 0.001$). Enhancement of the already high level of endogenous TSH in thyroidectomized animals by supplementary injected TSH further prolonged the survival time (15.6 days); this difference proved to be significant at the 3% level; however, the administration of thyrotrophin to euthyroid mice resulted in a slight reduction in survival (Table 31), presumably by causing hypersecretion of thyroid hormone.

[1]Consultant.

[2]Research Participant (summer 1954).

[3]U. S. Air Force.

[4]Temporary employee (summer 1954).

[5]Research Associate.

[6]T. F. Dougherty, *Phys. Rev.* 32, 379-401 (1952).

[7]H. S. Kaplan, S. Nagareda, and M. B. Brown, *Recent Prog. Hormone Res.* 10, 293-338 (1954).

TABLE 31. THE SURVIVAL OF AK/R LEUKEMIC HOSTS AS AFFECTED BY THYROID OR THYROTROPHIC HORMONE

Treatment	Number of mice	Mean survival time (days)	Range of survival times (days)
No treatment	39	11.4	9 – 23
Thyroidectomy	49	13.4	9 – 25
Thyroidectomy + thyroxine	44	10.4	9 – 22
Thyroidectomy + thyroxine + thyrotrophin	27	11.7	7 – 26
Thyroidectomy + thyrotrophin	10	15.6	12 – 18
Thyrotrophin	19	10.9	7 – 11

These observations, although indicating conclusively that thyroidectomy prolonged the survival of leukemic mice, fail to disclose the mechanism of the protective effect. The data suggest that thyroid hormone adversely affects and that thyrotrophin enhances, survival but under the conditions of these preliminary experiments the interrelation of these two hormones, as well as their effects on other endocrine glands, cannot be completely analyzed. Further investigations are in progress.

A Cytochemical Study of Human and Rat Platelets

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

In an attempt better to understand the role of the blood platelet in hemostasis, cytochemical studies of human and rat platelets have been undertaken, some preliminary results of which are presented.

Whole blood was collected in a 20-ml silicone-treated syringe, to which had been added 0.5-1.0 ml of Sequestrene (disodium ethylenediamine tetra-acetic acid, 1% in 0.7% saline), and immediately transferred to a 15-ml silicone-treated centrifuge tube in an ice bath. Rat blood was obtained from the abdominal aorta of male Sprague-Dawley rats which had been anesthetized with nembutal and injected intravenously with 0.5 ml of anticoagulant (0.5 mg of heparin). Human blood was withdrawn from the antecubital vein of one of the authors.

The blood was separated into plasma, platelets, and erythrocytes by differential centrifugation. Platelets were suspended in plasma and smears were made on clear glass slides and allowed to dry at room temperature. The slides were then placed in one of the following fixatives: absolute methanol, Carnoy's solution, Flemming's solution, formol-acetic acid, Baker's formalin-calcium chloride, mercuric chloride-acetic acid, 95% ethanol, and 9 parts saturated alcoholic solution of picric acid with 1 part formalin. A series of staining techniques was then applied to samples of each fixed smear. Air-dried unfixed smears were also stained.

The rat platelet was observed to be considerably smaller than the human platelet, which, according to Downey[8] varies in size from 2 to 4 μ in fixed smears; in a series of measurements of rat platelets fixed in methyl alcohol and stained with Wright-Giemsa the size varied from 0.30 to 1.65 μ in diameter with a mean of 0.86 μ. The individual rat platelet appeared as an irregularly spherical body and was comprised of a clear, homogeneous, peripheral area, called the hyalomere (often thin filaments were seen projecting from its edge), and coarse granules, usually centrally located, called the granulomere. In a smear preparation the Wright-Giemsa procedure differentially stained these structures, the granules appearing a brilliant, reddish-purple, and the hyalomere light blue. One of the effects of various fixatives is to emphasize or to obscure the appearance of the hyalomere with its filaments.

The cytochemical observations indicate the presence of mucopolysaccharide and phospholipid in the human and rat platelets. The absence of deoxyribonucleoprotein is confirmed; however, the presence of ribonucleoprotein could not be demonstrated conclusively with these methods. The investigations are currently being extended to include the study of platelets in animals with radiation-induced hemorrhage.

[8] H. Downey, ed., *Handbook of Hematology*, Vol. I, Sect. VII, p. 451, Hueber, New York, 1938.

Studies of the Distribution of S^{35} Sulfate in Whole-Body Irradiated Rats

L. H. Smith B. Anderson

Localization of sulfate, either free or bound, in animal tissue has been successfully studied with the use of $Na_2S^{35}O_4$. The primary object of the present experiment was to study the effects of X irradiation on the distribution of S^{35}-labeled sodium sulfate in the Sprague-Dawley rat. Rats exposed to 750 r of whole-body X radiation were injected intravenously 1, 2, 3, 4, or 5 days later with 300 μc of S^{35}-labeled Na_2SO_4. Twenty-four hr post-injection, skin and cartilage were obtained for $S^{35}O_4$ determinations; these tissues were chosen because of their relatively large content of sulfonated mucopolysaccharides.

In preliminary experiments this dose of X radiation had no conclusive effect on the $S^{35}O_4$ uptake of skin or cartilage. Similarly, the amounts of $S^{35}O_4$ excreted in the urine during the first 24 hr postinjection were not consistently different in X-irradiated and control rats; however, the plasma level of labeled sulfate was usually much lower in the exposed animals on the third day post-irradiation (Table 32).

TABLE 32. S^{35} ACTIVITY IN PLASMA OF RATS AT INTERVALS FOLLOWING WHOLE-BODY EXPOSURE TO 750 r OF X RAYS

Time after irradiation (days)	Experiment no.	Plasma activity (cts/min/100 μl)	
		Control	X-rayed
1	2	7,276[a]	9,159[a]
		9,537	6,773
2	2	5,947	8,522
		7,719	4,503
2	4	7,071	11,447
			8,061
			7,360
3	2	6,581	2,771
		6,429	3,142
3	3	6,924	3,413
		6,403	3,280
3	5	3,370	2,097
		3,017	4,319
3	4	11,805	1,917
3	5	3,676	3,886
		2,758	4,794
4	6	6,353	5,674
			11,058
			4,577
5	6	5,991	7,551
			3,609
			2,537

[a]Each value represents the average of duplicate determinations from one rat.

In view of these results, an effort was made to determine the plasma distribution of $S^{35}O_4$ in normal rats, particularly that bound to the plasma proteins. Ammonium sulfate fractionation of plasma proteins indicated that $S^{35}O_4$ was distributed as follows: fibrinogen, 14%; euglobulin, 29%; pseudoglobulin, 23%; and albumin plus undetermined residue, 34%. The crude plasma mucoprotein fraction, obtained according to the method of Winzler *et al.*,[9] contained less than 1% of the total plasma $S^{35}O_4$ activity. In view of technical limitations, these values were not considered conclusive.

[9]R. J. Winzler, A. W. Devor, J. W. Mehl, and I. M. Smyth, *J. Clin. Invest.* 27, 609-616 (1948).

More precise identification of labeled sulfate with specific plasma or serum proteins was attempted with the use of a modification of the filter paper electrophoresis method of Durrum.[10] Samples (0.025 ml) of plasma or serum[11] obtained from rats injected intravenously 24 hr earlier with 300-600 μc of $Na_2S^{35}O_4$ were placed on strips of Whatman No. 1 filter paper and subjected to electrophoresis for 10 hr at 1.0 ma and 160-250 volts. The paper strips were dried, developed in tetrabromphenolsulfonphthalein, dried again, and scanned with a Photovolt photometer. The resulting optical densities, recorded at 3-mm intervals, produced a plasma or serum protein distribution curve (Fig. 24). The strips were then cut into 3-mm widths, placed on planchets, and the $S^{35}O_4$ activity of each section determined with a gas flow proportional counter (Fig. 24). The filter paper sections possessing the highest activity correspond to the area between the α_1 globulin and the albumin peak. In spite of the low counts obtained, these results have been duplicated in four separate determinations.

[10]E. L. Durrum, *J. Am. Chem. Soc.* **72**, 2943-2948 (1950).

[11]Either plasma or serum samples were used on the assumption that the presence or absence of fibrinogen would not appreciably affect the results.

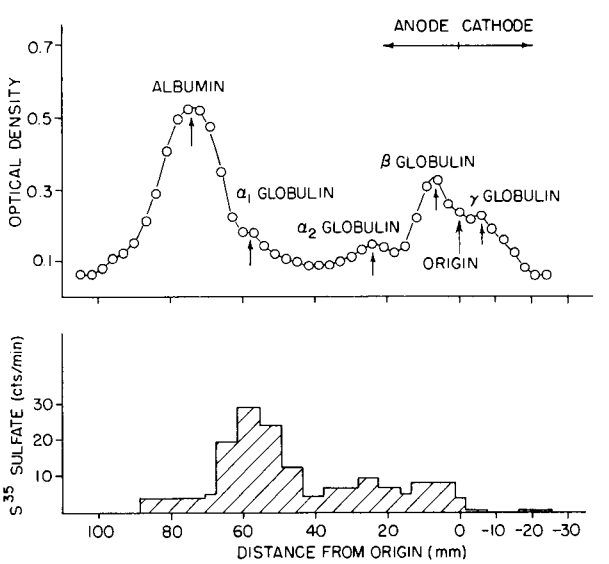

Fig. 24. Filter paper electrophoretic pattern of proteins and associated S^{35} sulfate activity of blood plasma from normal rats.

To eliminate the possibility that the S^{35} distribution on the paper strips resulted from independent migration of free sulfate ions, a serum sample to which had been added $Na_2S^{35}O_4$ *in vitro* was subjected to electrophoresis. The S^{35} activity contained in this mixture was four times the amount found in the plasma of rats injected with 600 μc of $Na_2S^{35}O_4$. It was observed that little, if any, S^{35} was associated with serum proteins. Presumably, most of the free labeled sulfate ion had migrated off the paper into the buffer solution. The results of this experiment confirm that some of the injected labeled sulfate becomes bound *in vivo* either to a plasma protein or to a prosthetic group.

When a stain, specific for protein-bound carbohydrate, is substituted for tetrabromphenolsulfonphthalein, two deeply stained spots are observed, one at the position corresponding to the S^{35} activity peak (i.e., between the α_1 globulin and albumin peak), and the other several millimeters from the original toward the anode. This observation suggests that sulfate is associated with a carbohydrate prosthetic group.

Experiments are now in progress to determine more specifically the distribution of radioactive sulfate in the serum and plasma of normal and X-irradiated rats.

Uptake of $S^{35}O_4$ by Megakaryocytes and Platelets Following Whole-Body Irradiation in Rats

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

It has been shown[12] that the megakaryocytes of rat bone marrow concentrate S^{35}-labeled sodium sulfate and that the circulating platelets also accumulate the isotope but at a slower rate. It was suggested that the S^{35}-labeled platelets were derived at least in part from the labeled megakaryocytes. The present experiments were undertaken in an attempt further to elucidate the effects of whole-body irradiation on megakaryocytes and on platelet production and utilization.

Male Sprague-Dawley rats were irradiated with 750 r of X rays, and afterward injected intravenously with 300 μc of carrier-free $Na_2S^{35}O_4$ 24 hr prior to their sacrifice (at 1, 2, 3, 4, and 24 days after irradiation). The rats were exsanguinated from the abdominal aorta under nembutal anesthesia and the blood was separated by differential centrifugation into plasma, platelets, and red blood cells. Duplicate samples of plasma and of platelet suspensions were counted for their radioactivity in a gas flow counter, and activities were corrected for self-absorption. One femur from each rat was cracked open longitudinally and fixed in 95% alcohol for sections of bone marrow. Smears of bone marrow were made from the other femur. The sections and smears were covered with stripping film (Kodak, Ltd.) and exposed for about 30 days. After development of the film, the slides were stained with Giemsa stain.

The megakaryocytes from rats sacrificed 1, 2, 3, 4, and 24 days after irradiation accumulated sulfate in a seemingly normal manner, as judged by autoradiograms. The platelets were also labeled (Table 33). Within each experiment the plasma and platelet activities of the experimental animals were often lower than those of the controls, especially on the third and fourth days postirradiation; however, low platelet and plasma activities were not always correlated with each other. Variations from one experiment to another in the activity of plasma and platelets are as yet not fully explained.

The observed uptake of sulfate by the megakaryocytes after whole-body irradiation indicates that the metabolic processes involving sulfate incorporation were functioning in the megakaryocytes remaining at the times studied. Whether there was a quantitative effect on sulfate uptake was not apparent in the autoradiograms. It is evident that the platelets became labeled, and it is likely that the tagged platelets were derived at least in part from labeled megakaryocytes, although previous work has shown that some platelet labeling may occur in the blood stream. These studies are being extended and will include other time intervals postirradiation.

Studies of Soft Beta Self-Absorption in $BaSO_4$, Tissue Homogenates, Plasma, and Platelet Suspensions

B. Anderson T. T. Odell

These studies with a gas flow proportional counter were carried out in the execution of other experiments with S^{35}, since it was necessary in counting the radioactivity of various materials to correct for self-absorption. The results are pre-

[12] T. T. Odell, Jr., W. D. Gude, and F. G. Tausche, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 55.

TABLE 33. CONCENTRATION OF $S^{35}O_4$ IN PLASMA AND PLATELETS OF IRRADIATED RATS

Time postirradiation (days)	Experiment no.	Act./plt. × 10^7		Peripheral platelet count		Plasma act./100 μl	
		Control	Irrad.	Control	Irrad.	Control	Irrad.
1	2	11.1	14.3	940,000	850,000	7,264	9,149
1	2	17.8	11.7	1,170,000	1,010,000	9,537	6,773
1	4	8.6	5.4	1,025,000	880,000	1,490	11,630
1	4		6.8		980,000		14,560
2	2	20.5	17.6	1,075,000	858,000	5,947	8,522
2	2	13.6	23.5	775,000	619,000	7,719	4,503
2	4	11.4	4.2	1,110,000	900,000	7,071	11,447
2	4		5.5		1,055,000		8,061
2	4		3.2		950,000		7,360
3	2	16.6	10.9	980,000	435,000	6,581	2,771
3	2	19.2	11.6	1,015,000	880,000	6,429	3,142
3	3	9.9	6.7	945,000	930,000	6,924	3,413
3	3	8.9	4.6	880,000	1,045,000	6,403	3,280
3	4	8.5	5.0	1,170,000	870,000	11,805	1,917
4	1	13.1	4.1			18,656	7,077
4	1	13.2	5.2			23,895	13,128
4	1	11.5	5.8			17,811	10,129
4	6	9.2	3.8	1,375,000	605,000	6,353	5,674
4	6		4.9		580,000		11,057
4	6		4.4		810,000		4,576
24	3	9.4	16.4	1,457,500	685,000	6,294	6,945
24	3		14.8		930,000		5,323
24	3		19.3		1,160,000		7,474
24	3		16.7		1,055,000		5,931

sented at this time, because it is thought that the information will be helpful to others working on related problems.

Efficiency of a Gas Flow Proportional Counter in the Measurement of 0.1-100 mμc of S^{35} as Carrier-Free Sulfate in Distilled Water. A standard containing 100 mμc of S^{35} as carrier-free Na_2SO_4 in 100 μl was prepared from a solution whose concentration was known within ±20%. Serial dilutions were made from this standard to give concentrations of 77, 7.7, 0.77, and 0.08 mμc of S^{35} in 100 μl, and 100-μl aliquots of these standards were spread on planchets over an area having a diameter of about 40 mm. Thus the total area covered by the samples was always 12.5 cm^2. After drying in air, the aliquots were counted. Similar sets of standards were run with each set of absorption studies and are summarized in Table 34.

S^{35} Counting Efficiency in 45% Liver and Kidney Homogenates and in $BaSO_4$. Liver from a rat anesthetized with nembutal and exsanguinated from the abdominal aorta was homogenized in a Potter-Elvehjem homogenizer with enough water to yield a 45% homogenate after the addition of the $S^{35}O_4^{--}$ standard solution. The final homogenate (45% of wet liver by weight) contained 10 mμc of S^{35} in every 100 μl. This experiment was repeated three times. A kidney homogenate of the same concentrations with respect to wet tissue and activity was prepared with the last liver homogenate. Water standards were used in each of the three experiments (Table 33). The theoretical wet weight of

TABLE 34. CARRIER-FREE $S^{35}O_4^{--}$ COUNTED IN WATER

S^{35} on planchet (mμc ± 20%)	Standard 1: Platelet Expt. 1, liver homogenates 1 and 2		Standard 2: Platelet Expt. 2, plasma		Standard 3: Platelet Expt. 3, liver and kidney homogenates	
	(cts/min)	(cts/min/mμc)	(cts/min)	(cts/min/mμc)	(cts/min)	(cts/min/mμc)
0.05			43	860		
0.08	96	1200				
0.1			93	930		
0.2			173	865		
0.5			446	892		
0.77	946	1229				
1.0			883	883	1,322	(1322)
1.92	2,355	1227				
2.5			2,217	887	2,888	1155
3.85	4,552	1182				
5.0			4,589	918	5,922	1184
7.5					8,804	1174
7.7	9,009	1170				
	9,555	1241				
10.0			8,698	870	11,793	1179
					12,128	1213
15.4	18,620	1209				
20.0			17,633	881	24,520	1226
					23,764	1188
					25,268	1263
23.1	27,979	1211				
30.0					35,672	1189
30.8	36,353	1180				
38.5	44,631	1159				
40.0					47,756	1194
50.0			43,042	861	55,454	1109
					53,582	1072
					57,786	1156
60.0					67,622	1127
77.0	92,904	1207				
	89,727	1165				
100.0			87,798	878		
		$\bar{x}$ = 1198		$\bar{x}$ = 884		$\bar{x}$ = 1174
		6.8% precision		7.9% precision		16.3% precision

tissue per square centimeter was calculated for each aliquot. The efficiencies, as compared to water standards are plotted against milligrams per square centimeter of wet tissue, give a curve similar to that in Fig. 25.

In the third experiment, the dry weights of the tissues on the planchets were determined by weighing the planchets before the homogenates were put on them and again after the homogenate aliquots had been allowed to dry overnight. Comparison of these weights with the theoretical wet weights showed that in the liver 40% of the wet weight remained after air-drying overnight, whereas in the kidney only 25% remained. In the problem of correcting for self-absorption in counting large numbers of homogenates of various organs, it

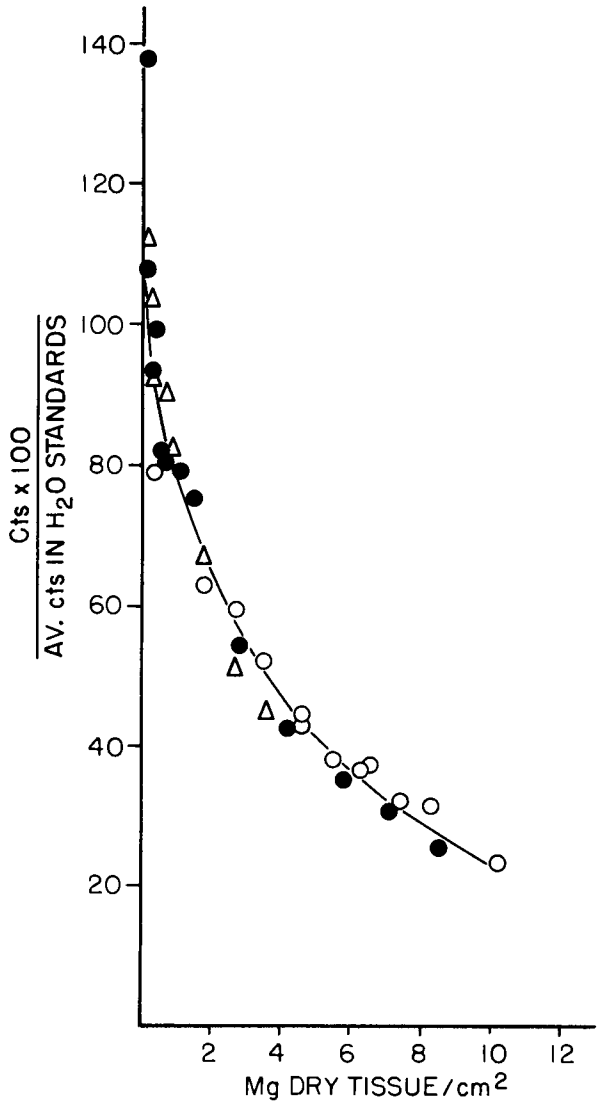

Fig. 25. S^{35}-labeled sulfate counting efficiency in tissue homogenates in barium sulfate. ●, Liver homogenate; Δ, kidney homogenate; O, $BaSO_4$.

would be helpful to have conversion factors (from wet to dry weights) so that it would be necessary only to weigh the tissues used in making the homogenates.

A sodium sulfate standard was prepared in such a way that 1.0 ml contained 1.0 mμc of S^{35} and also contained enough carrier SO_4^{--} to yield 11.7 mg of $BaSO_4$ in the standard gravimetric determination. Six aliquots of this solution ranging from 3 to 8 ml were carried through the gravimetric determination. The $BaSO_4$ was transferred from the grossed crucible onto a planchet where it was wet with water and evenly distributed over the counting area of the planchet with a stirring rod. The crucible was tared and the exact weight of the $BaSO_4$ on the planchet was thus ascertained. After drying, the samples were counted and the efficiency of counting in $BaSO_4$ relative to water standards was calculated. This experiment was repeated on different size aliquots to extend the curve at either end. Figure 25 shows the efficiency of S^{35} counting in $BaSO_4$ and in the liver and kidney homogenates plotted against milligrams dry weight per square centimeter.

Efficiency of S^{35} Counting in Rat Plasma. A series of plasma samples were prepared in such a way that varying amounts of activity ranging from 0.05 mμc of S^{35} were contained in a given volume. Aliquots of 200 and 100 μl were taken from each sample, spread on a planchet, and allowed to air-dry. The samples were then counted, along with a series of water standards, and the counting efficiencies were calculated. The average efficiency in 100 μl of plasma was 90.5% and in 200 μl 76.3% (Table 35).

Efficiency of S^{35} Counting in Rat Platelet Suspensions. A platelet suspension was prepared in such a way that it contained 100 mμc of S^{35} per ml and had a platelet count of 19,590,000 platelets/mm^3. Fractional concentrations of both activity and platelets were achieved by making serial 1:1 dilutions from this suspension. Platelet counts were made on each suspension, and 200 μl aliquots (except for two 100-μl aliquots) of each suspension were counted for efficiency determinations. The counting efficiencies were calculated relative to the same amount of activity in water. This experiment was repeated except that the standard $S^{35}O_4^{--}$ solution was made up in saline instead of water; however, the amount of $S^{35}O_4^{--}$ solution was only 10% of the total volume in either case, so that this difference should be negligible. A third experiment was carried out with one platelet suspension containing 8,370,000 platelets/mm^3. Aliquots of different sizes were taken from it so that fractional amounts of activity in corresponding numbers of platelets were counted. In all experiments, efficiencies greater than 100% were obtained when counting 200-μl samples of platelet suspensions containing less than about 6 million platelets/mm^3; variation in results increased with decreasing numbers of platelets. The values above 100% are attributed to backscatter.

TABLE 35. EFFICIENCY OF S^{35} COUNTING IN RAT PLASMA

S^{35} on planchet (mμc)	$\frac{\text{Counts 100 } \mu\text{l plasma}}{\text{Counts } H_2O \text{ standards}} \times 100$	$\frac{\text{Counts 200 } \mu\text{l plasma}}{\text{Counts } H_2O \text{ standards}} \times 100$
0.05	88.6	
0.1	90.9	76.1
0.2		73.9
0.5	92.0	
1.0	87.0	76.0
2.0		80.5
5.0	94.4	
10.0	90.1	74.5
20.0		74.1
50.0	92.7	
100.0	88.1	78.9
	$\bar{x} = 90.5$	$\bar{x} = 76.3$

Distribution of Ca^{45} in the Blood Cells and Bone Marrow of Rats

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

The presence of calcium in platelets was reported many years ago.[13–15] Although its chemical form in the platelet is unknown, the importance of calcium in the coagulation of the blood and in the interendothelial cement of the blood vessel wall is well established. Since it has been shown that platelets incorporate *in vivo* C^{14}-labeled formate, S^{35}-labeled methionine, and S^{35}-labeled sodium sulfate, and that the megakaryocytes of the bone marrow (and certain myeloid cells) selectively accumulate S^{35}-labeled sulfate,[16,17] observations were made on the radioactivity of platelets and of bone marrow following the injection of Ca^{45} to determine whether calcium is similarly accumulated by these elements.

Male Sprague-Dawley rats, weighing 350 to 400 g, were injected intravenously (jugular vein) with 200 μc of Ca^{45} at pH 4.6, and were sacrificed by exsanguination from the abdominal aorta under nembutal anesthesia at 20, 44, 68, 92, and 116 hr and 8 and 10 days after injection. Separation of the blood elements was accomplished by differential centrifugation. The platelets and red blood cells were washed twice with 10 ml of saline and then resuspended in saline. Duplicate samples of plasma, washed platelets, and washed red blood cells, as well as one sample of the platelet and RBC washes, were measured for their activities in a gas flow counter. One femur of each rat was cracked open and fixed in 95% alcohol for bone marrow sections, and bone marrow smears were made from the other femur.[18] The sections and smears were covered with stripping film (Kodak, Limited) and exposed for 30-60 days, after which the film was developed and the slides stained with the Giemsa method.

The activity of the platelets paralleled the activity of the plasma (Fig. 26). The erythrocytes showed a small variable activity throughout the 10-day period, without any apparent increase during

[13] M. Aynaud, *Compt. rend. soc. biol.* 76, 480-481 (1914).

[14] F. Haurowitz and J. Sladek, *Z. Physiol. Chem.* 173, 233-234 (1928).

[15] G. Endres and L. Herget, *Z. Biol.* 88, 451-464 (1929).

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

[17] T. T. Odell, Jr., W. D. Gude, and F. G. Tausche, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 55.

[18] W. D. Gude and T. T. Odell, Jr., *Stain Technol.* 30(1), 27-28 (1955).

the first few days after injection (Table 36). The activities of the first platelet and RBC washes also followed the plasma, as anticipated, presumably owing largely to trapped plasma; the second washes contained relatively little activity. The autoradiograms of the bone marrow smears and sections revealed no localization of activity in any of the cells.

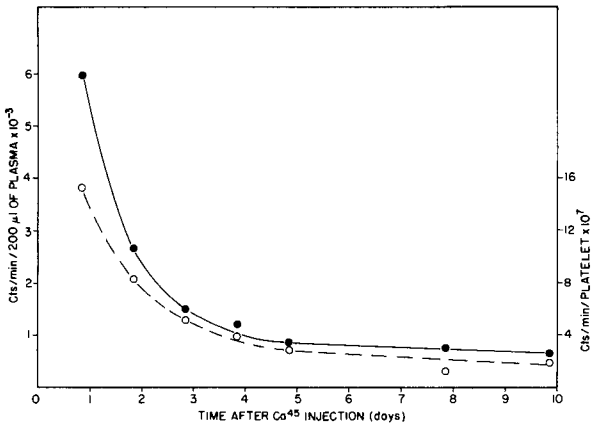

Fig. 26. Radioactivity of plasma and platelets after intravenous injection of Ca^{45}. Each point represents duplicate samples from each of 2 rats (1 rat at 10 days). ●, Plasma; O, platelets.

It is apparent that the injected Ca^{45} did not accumulate in the platelets, but rather the activity of platelets and plasma declined in a parallel manner; this is interpreted to indicate that calcium diffused freely in and out of the platelets, maintaining a calcium equilibrium between platelets and plasma. This is in contrast with the gradual accumulation of formate-C^{14}, methionine-S^{35}, or $Na_2S^{35}O_4$ previously observed in platelets after intravenous injection into rats; in the latter cases, increase in platelet activity during the 3 days following injection, while the plasma activity was falling, indicated incorporation of these substances into the platelets (probably by way of the megakaryocytes). The absence of Ca^{45} activity in the megakaryocytes also contrasts with the previous

TABLE 36. RADIOACTIVITY OF ERYTHROCYTES AND ERYTHROCYTE AND PLATELET WASHES OF RATS INJECTED WITH Ca^{45}

Time after injection[a] (days)	cts/min/RBC × 10^7	cts/min/200 µl 1st RBC wash	cts/min/200 µl 2nd RBC wash	cts/min/200 µl 1st plt. wash	cts/min/200 µl 2nd plt. wash
1	4.23	433	32	94	20
	3.24	460	26	88	12
2		239	23	34	8
	6.84	157	5	45	14
3	4.85	97	14	14	0
	1.03	108	9	26	6
4	3.40	69	13	23	8
	1.25	82	9	16	0
5	2.98	66	9	24	7
	1.73	49	18	26	13
8	3.95	43	10	22	11
	1.32	46	9	21	13
10	7.84	48	32	23	17

[a]Less 4 hr.

experiments in which S^{35} sulfate was seen to concentrate in these cells.

Although the erythrocytes appeared to contain some radioactivity, the amount was small and variable throughout the 10-day period studied, so that no definite conclusions about RBC uptake are warranted at this time.

Effect of Thyroxine on Succinic Acid Dehydrogenase and Cytochrome Oxidase in Cellular Fractions of Rat Liver After Partial Hepatectomy

S. R. Tipton P. W. Rueff, Jr.

Succinic dehydrogenase, cytochrome oxidase, protein, and deoxyribonucleic acid were measured in fractions of rat liver at the time of: (1) partial hepatectomy, where about 68% of the liver was removed, and (2) sacrifice, after 4 days of post-operative liver regeneration. Eight rats on normal stock diet were used as controls; the experimental group (eight rats) received daily doses of 0.25 mg of thyroxine per 100 g of body weight for 4 days before partial hepatectomy and during the 4 days of regeneration. Enzyme activities were measured spectrophotometrically by the methods of Cooperstein *et al.*[19,20] Ten per cent homogenates were fractionated into nuclear, mitochondrial, and supernatant portions by differential centrifugation at forces of 600 g for nuclear, and 9000 g for mitochondrial fractions.

Thyroxine treatment resulted in an increase in the activity of liver succinic acid dehydrogenase and cytochrome oxidase per milligram of protein, in the total activity (per 10 ml of 10% homogenate) in the whole homogenate, and in the mitochondrial fraction at operation and at sacrifice (Tables 37, 38). The protein levels in the liver fractions were not affected by the thyroxine (Table 39). Since the mitochondrial protein level was not affected and the enzyme activity was increased, it appears that thyroxine has a specific effect on the activity of these enzymes and not merely a general action on the mitochondrial protein. Thyroxine treatment had no significant effect on the rate of restoration of liver protein in the 4-day regenerative period after partial hepatectomy.

[19] S. J. Cooperstein, A. Lazarow, and N. J. Kurfess, *J. Biol. Chem.* **186**, 129-139 (1950).

[20] S. J. Cooperstein and A. Lazarow, *J. Biol. Chem.* **189**, 665-670 (1951).

TABLE 37. THE ACTIVITY OF SUCCINIC DEHYDROGENASE AND CYTOCHROME OXIDASE IN UNITS PER MINUTE PER MILLIGRAM OF PROTEIN

Treatment		Homogenate	Mitochondrial	Nuclear	Supernatant
		Succinic dehydrogenase			
Control at	operation	1.10 ± 0.2[a]	2.50 ± 0.17[a]	1.10 ± 0.25[a]	0.06 ± 0.03[a]
	sacrifice	1.50 ± 0.2	3.50 ± 0.15	1.40 ± 0.3	0.10 ± 0.04
Thyroxine-treated at	operation	1.40 ± 0.15	3.50 ± 0.12	1.40 ± 0.3	0.30 ± 0.08
	sacrifice	1.80 ± 0.21	5.50 ± 0.19	1.80 ± 0.25	0.50 ± 0.1
		Cytochrome oxidase			
Control at	operation	1.40 ± 0.12	3.51 ± 0.2	1.80 ± 0.3	0.10 ± 0.04
	sacrifice	2.31 ± 0.22	5.30 ± 0.25	3.10 ± 0.32	0.20 ± 0.05
Thyroxine-treated at	operation	1.90 ± 0.1	4.60 ± 0.32	3.10 ± 0.18	0.20 ± 0.04
	sacrifice	2.62 ± 0.15	6.70 ± 0.37	3.70 ± 0.25	0.20 ± 0.03

[a] Standard deviation.

TABLE 38. TOTAL ACTIVITY MEASURED IN ACTIVITY PER MILLIGRAM OF PROTEIN PER MINUTE TIMES MILLIGRAMS OF PROTEIN IN 10 ml OF 10% HOMOGENATE

Treatment		Homogenate	Mitochondrial	Nuclear	Supernatant
		Succinic dehydrogenase			
Control at	operation	287.9 ± 28.1[a]	179.0 ± 32[a]	90.2 ± 9.2[a]	8.8 ± 1.1[a]
	sacrifice	321.7 ± 33	167.0 ± 29	100.1 ± 9.8	11.1 ± 1.4
Thyroxine-treated at	operation	384.0 ± 25.3	235.8 ± 31	135.3 ± 11.3	10.5 ± 0.9
	sacrifice	445.2 ± 33.5	214.3 ± 27	180.9 ± 14	11.5 ± 1.2
		Cytochrome oxidase			
Control at	operation	448.0 ± 26	227.0 ± 24	160.3 ± 12.2	22.7 ± 1.6
	sacrifice	663.1 ± 27	305.1 ± 20	278.9 ± 8.5	28.6 ± 0.9
Thyroxine-treated at	operation	596.3 ± 21.1	319.6 ± 17	247.2 ± 11.1	313 ± 0.8
	sacrifice	779.8 ± 25.4	408.0 ± 21	332.9 ± 15	21.9 ± 1.1

[a]Standard deviation.

TABLE 39. TOTAL PROTEIN IN 10 ml OF 10% LIVER HOMOGENATE

Treatment		Homogenate	Mitochondrial	Nuclear	Supernatant
Control at	operation	319.0	62.8	85	168.6
	sacrifice	300.0	51.2	84.5	176.9
Thyroxine-treated at	operation	316	68	87	168.0
	sacrifice	304.3	55	85	154.2

A Method for Satisfactory Histological and Cytological Staining of Autoradiograms Prepared with Stripping Film

W. D. Gude T. T. Odell

In the preparation of autoradiograms with stripping film, the emulsion overlying the tissue section or smear has a tendency to stain intensely with some of the commonly used dyes (hemotoxlyin, toluidin blue, fuchsin) and to interfere with proper histological and cytological differentiation. In order to overcome these defects, the following procedure has been developed with which well-stained autoradiograms have been consistently obtained.

After development of the emulsion, the slides are washed for 15 min in water; the short rinsing time lessens the hazard of loosening and swelling of the film. Following this, they are placed in distilled water buffered to pH 6.6-6.8 for 1 hr and then thoroughly air-dried. When dry, they are stained for 1-2 hr in Wolbach's Giemsa mixture,[21] buffered to pH 4.8-5.2, consisting of: 2 ml of Giemsa stock solution, 3 ml of absolute methyl alcohol, 100 ml of tap water, and 0.5-1.0 ml of citric acid (M/10) in 25% methanol[22] or sodium phosphate (M/5) in 25% methanol.[22] As when staining other preparations with Giemsa, periodic

[21]F. B. Mallory, *Pathological Technique*, p. 195, Saunders, Philadelphia, 1938.

[22]R. D. Lillie, *Histopathologic Technic*, Blakiston, Philadelphia, 1948.

examination of the slide under the microscope may be necessary for proper differentiation. It is better, however, to dry the slide before checking the intensity of coloring of the tissue. If further staining is desired, the slide may be returned to the solution and differentiated to the desired color. The pH of the Giemsa solution may be readily adjusted by small additions (0.5-1.0 ml) of acid or basic buffer in order to produce the desired contrast between purplish-blue nuclei and reddish-pink cytoplasm.

The use of "subbed" glass slides which have a thin coating of chrome-alum-gelatin mixture (recommended by Kodak, Ltd.) helps in preventing loosening of the stripping film during development, rinsing, and staining. This coating has been found not to interfere with the application of smears of bone marrow or blood cells.

Solubility of Liver Protein in Two Homogenizing Fluids

F. G. Tausche N. G. Anderson[23]

A comparison was made of the percentage of total liver protein soluble in two homogenizing fluids, 0.24 M sucrose and 0.1 M phosphate buffer (pH 7.8). Rats were killed by decapitation; the thorax and abdomen were opened, and a cut was made in the decending aorta through which the liver was immediately perfused with cold homogenizing fluid. A 5% liver homogenate was prepared over a 3-min period in a Potter-Elvehjem homogenizer suspended in an ice bath. Samples of the whole homogenate were frozen for total protein determinations. The remaining liver homogenate was centrifuged at 25,000 $\times g$ for 180 min, and the supernatant was removed and sampled for total protein determinations. The total protein of whole homogenate and of supernatant was determined with the biuret reaction and was expressed in terms of milligrams of bovine serum albumin.

The percentage of the whole protein which dissolved in the homogenizing fluids is presented in Table 40. (The data include observations made on normal rats and on rats pretreated with thyroxine as part of another experiment.) In group I (four normal rats) an average of 31.99% of the available total protein was soluble in the 0.24 M sucrose and 37.58% in the 0.1 M phosphate buffer. In group II (five rats which had received 50 μg of thyroxine per day for 4 days), 22.48% was soluble in the sucrose and 31.30% in the phosphate buffer. These differences, tested with an analysis of variance method, were found to be significant below the 1% level. Thus it is evident that an appreciably larger amount of total protein was soluble in 0.1 M phosphate buffer than in 0.24 M sucrose, a difference to be borne in mind in future experiments necessitating use of these homogenizing fluids.

TABLE 40. PERCENTAGE TOTAL PROTEIN IN WHOLE HOMOGENATE SOLUBLE IN HOMOGENIZING FLUID

	0.24 M Sucrose (%)	0.1 M Phosphate buffer (pH 7.8) (%)
Group I	32.89	38.15
Normal rats	31.09	37.00
Av	31.99	37.58
Group II	22.59	31.63
Rats received	25.71	30.98
50 μg thyroxine/day for 4 days	19.14	
Av	22.48	31.30

Incorporation of S^{35}-Labeled Sulfate in Healing Wounds, Megakaryocytes, and Platelets of Scorbutic Guinea Pigs

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

It is well known that vitamin C deficiency interferes with the formation of collagen fibers[24] and polysaccharide in the ground substance of connective tissue.[25,26] In addition, there is severe capillary fragility[27] and a tendency toward thrombocytopenia[28] in scurvy. Because S^{35}-labeled sulfate is selectively incorporated into the sulfomuco-

[23] General Physiology group.

[24] S. B. Wolbach and P. Howe, *Arch. Pathol.* 1, 1-24 (1926).

[25] J. R. Penney and B. M. Balfour, *J. Pathol. Bacteriol.* 61, 171-178 (1949).

[26] I. Gersh and H. R. Catchpole, *Am. J. Anat.* 85, 457-522 (1949).

[27] E. P. Ralli and S. Sherry, *Medicine* 20, 251-340 (1941).

[28] A. K. Presnell, *J. Nutrition* 8, 69 (1934).

polysaccharides of connective tissue[29] and into megakaryocytes and platelets,[30] the following experiments were undertaken to compare the uptake of $S^{35}O_4^{--}$ in the healing wounds, megakaryocytes, and platelets of scorbutic and of normal guinea pigs.

Twelve days after the start of a scorbutigenic regimen, scorbutic guinea pigs and controls were subjected to experimental wounding. Four days later they were injected with 17 μc of $S^{35}O_4$/100 g of body weight, and the next day they were sacrificed by exsanguination. At the time of death, tissues were excised from the wound for histologic and autoradiographic study, in addition to specimens of bone marrow. Platelet counts were also performed, and the blood was collected and fractionated by differential centrifugation into platelet, plasma, and erythrocyte fractions.

The wounds of the nonscorbutic controls were observed to be moderately well organized by the fifth postoperative day. Maturing fibroblasts were surrounded by numerous reticulin fibers, and there was abundant intercellular ground substance which stained metachromatically with dilute toluidin blue. Autoradiograms indicated selective concentration of radiosulfur in the newly formed connective tissue; the concentration was minimal in the vicinity of immature fibroblasts and maximal in the intercellular space in areas of active collagen formation.

By contrast, the wounds of the scorbutic guinea pigs were poorly organized and totally deficient in metachromatic ground substance and collagen formation. Radiosulfur was present only in trace amounts, if at all.

The platelet counts were slightly reduced in the scorbutic animals, but the specific radioactivity of the platelets was essentially the same as in the controls (Table 41). Likewise, radiosulfur was observed in megakaryocytes in similar concentrations in scorbutic and in nonscorbutic animals, as judged by autoradiograms of bone marrow. Plasma activity was generally lower in the experimental guinea pigs than in the controls.

These observations provide information about the utilization of sulfate in the formation of connective tissue, presumably for the production of intercellular sulfomucopolysaccharides,[31] since sulfur, when given as sulfate, does not enter appreciably into compounds such as methionine, cysteine, and taurin.[32] The deficient incorporation of sulfate into the wounds of the scorbutic guinea

[29] D. D. Dziewiatkowski, R. E. Benesch, and R. Benesch, *J. Biol. Chem.* **178**, 931 (1949).

[30] T. T. Odell, W. D. Gude, and F. G. Tausche, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 55.

[31] K. Meyer and M. M. Rapport, *Science* **113**, 596-599 (1951).

[32] H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.* **130**, 67-80 (1939).

TABLE 41. SCORBUTIC AND NORMAL GUINEA PIGS INJECTED WITH $Na_2S^{35}O_4$

	Body wt. at time of $S^{35}O_4^{--}$ inj. (g)	Peripheral platelet count	cts/min/100 μl plasma	cts/min/av platelet $\times 10^7$
Control	424	865,000	2651	6.0
	458	765,000	2456	6.6
	490	680,000	3468	8.0
		$\bar{x}$ = 770,000	$\bar{x}$ = 2858	$\bar{x}$ = 6.9
Scorbutic	425	505,000	1803	4.2
	359	565,000	2709	5.4
	375	460,000	1778	6.1
	352	520,000	1786	6.9
	347	365,000	2073	5.0
	401	400,000	2085	4.6
		$\bar{x}$ = 469,000	$\bar{x}$ = 2039	$\bar{x}$ = 5.4

pigs is consistent with the observations of Friberg and Ringertz[33] and supports the view that scurvy interferes with the formation of sulfomucopolysaccharides. The essentially normal uptake of sulfate by scorbutic megakaryocytes and platelets fails to disclose evidence of any metabolic defect which might be correlated with the observed thrombocytopenia and hemorrhagic state.

There are several possible explanations for the lower average plasma activity in the scorbutic animals; the difference may be real or a result of the experimental design. In injecting the isotope, it was assumed that the ratio of blood (and plasma) volume to body weight remained constant; therefore, a specified amount of sulfate was injected for every 100 g of body weight. It is possible, however, that losses in body weight and in blood volume in the scorbutic guinea pigs were not proportional so that the injected activity may have been diluted more in the circulatory system of the scorbutic animal. On the other hand, it is conceivable that sulfate was cleared more rapidly from the plasma of scorbutic guinea pigs. Solution of this problem will require further investigation.

Studies of Cumulative Injury from Divided Doses of X Rays and Fast Neutrons

F. P. Conte M. Slater[34]
G. S. Melville P. Ledford

Previous investigations with paired doses of X radiation suggested that rats[35] and mice[36] recover at an exponential rate following whole-body irradiation. Other experiments with fractionated neutron irradiation[37,38] have suggested that recovery occurs less readily after exposure to neutrons than after gamma or X irradiation. The present study was undertaken to determine the rate of recovery following exposure to fast neutrons and to compare it with that occurring after X irradiation.

Female RF mice, 10-15 weeks old, were randomly grouped for irradiation in lots of 10 to 20 at each dose level. The fast neutron irradiation was performed in the ORNL 86-inch cyclotron, at rate of approximately 70 rep/min, in a facility described earlier.[39] In this experiment, in addition to the dosimetric methods employed in earlier single dose lethality studies[40] all exposures were continuously monitored by a tissue-equivalent chamber connected to an integrating instrument (modified Victoreen Radocon). X irradiation was carried out at approximately 75 r/min, with maximum backscatter; other factors were as follows: T.S.D. 81.0 cm, 30 ma, 3.0 mm of Al extreme filtration (beryllium window), hvl 0.55 mm of Cu. Further details of the X irradiation are given elsewhere.[41] Except for one series of single exposures (controls), all mice received two equal doses separated by intervals of 2-8 days.

Following a single exposure, the observed LD_{50}/30 days was approximately 355 rep of fast neutrons (cyclotron radiations) and 560 r of X rays (Table 42, Fig. 27, 28); these results are in agree-

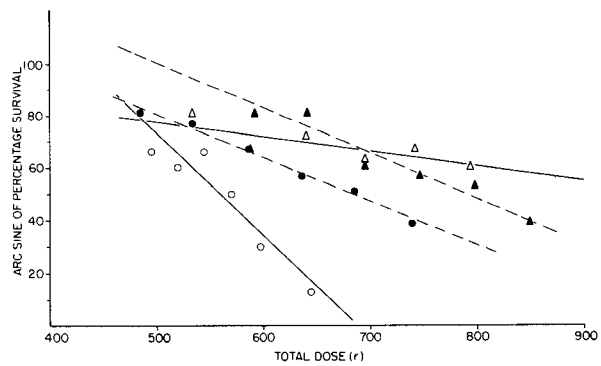

Fig. 27. Survival of RF mice exposed to single and paired doses of X rays. The authors are grateful to A. W. Kimball and G. J. Atta of the Mathematics Panel for these mathematical angular transformations, plotted from the results (Table 43) according to the best fit within the experimental error by least-squares analysis. O—, Control; ● - - - -, 2 days; Δ—, 5 days; ▲ - - - -, 8 days.

[33] U. Friberg and N. R. Ringertz, *Exptl. Cell Research* 6, 527-528 (1954).

[34] Biophysics section.

[35] C. W. Hagen, Jr. and E. L. Simmons, MDDC-1210, Univ. of Chicago Rep., CH-3815 (1947).

[36] E. Paterson, C. W. Gilbert, and J. Mathews, *Brit. J. Radiol.* **25**, 425-433 (1952).

[37] P. S. Henshaw, E. F. Riley, and G. E. Stapleton, *Radiology* **49**, 349-360 (1947).

[38] T. C. Evans, *Radiology* **50**, 811-834 (1948).

[39] A. C. Upton, F. P. Conte, G. S. Hurst, W. A. Mills, A. W. Kimball, and P. Ledford, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 55.

[40] G. S. Hurst, W. A. Mills, F. P. Conte, and A. C. Upton, submitted for publication.

[41] A. C. Upton, F. P. Conte, G. S. Hurst, and W. A. Mills, submitted for publication.

TABLE 42. THIRTY-DAY SURVIVAL OF MICE EXPOSED TO SINGLE OR PAIRED DOSES OF FAST NEUTRONS OR X RAYS

X rays				Fast neutrons			
Time interval	Total dose (r)	No. alive / No. exposed	Percentage survival	Time interval	Total dose (rep)	No. alive / No. exposed	Percentage survival
Single exposure	494	8/12	66.6	Single exposure	286	9/10	90.0
	520	9/12	75.0		323	7/10	70.0
	545	8/12	66.6		340	3/20	15.0
	571	7/12	58.0		347	6/19	31.6
	597	3/12	12.5		377	0/20	0.0
	644	0/12	0.0		413	0/20	0.0
2 days	485	20/20	100.0	2 days	330	10/10	100.0
	536	19/20	95.0		372	9/18	50.0
	587	17/20	85.0		390	0/18	0.0
	638	14/20	70.0		398	11/20	55.0
	688	12/20	60.0		434	2/19	10.0
	740	8/20	40.0		474	0/20	0.0
5 days	538	20/20	100.0	5 days	344	10/10	100.0
	589	17/20	85.0		388	8/10	80.0
	641	18/20	90.0		410	8/10	80.0
	692	16/20	80.0		416	10/10	100.0
	743	17/20	85.0		454	8/10	80.0
	794	15/20	75.0		496	9/10	90.0
8 days	592	20/20	100.0	7 days	378	9/9	94.4
	644	20/20	100.0		416	10/10	100.0
	695	15/20	75.0		438	1/10	10.0
	747	14/20	70.0		448	8/10	80.0
	798	13/20	65.0		486	0/10	0.0
	850	8/20	40.0		532	0/10	0.0

ment (within 10%) with the figures obtained in earlier acute lethality experiments with the same facilities and mice of the same age, sex, and strain. With fractionation of the dose, the $LD_{50}/30$ days increased in proportion to the time between exposures. In both the neutron-exposed and X-irradiated mice, the recovery, as judged from residual injury,[35] appeared to be exponential as a function of time; this was true, however, only for intervals of 2, 7, and 8 days. When paired doses of either neutrons or X rays were separated by an interval of 5 days, the $LD_{50}/30$ days was much greater than anticipated. For all intervals of fractionation, the survival slopes of the neutron-exposed mice closely resembled those of the X-irradiated animals.

The observed lethality data of mice exposed to paired doses of X rays at the 2- and 8-day intervals are consistent with earlier observations and with the concept of exponential recovery from irradiation.[35,42] The close resemblance of the survival slopes of neutron-exposed mice to those of X-irradiated animals strongly suggests that under the conditions of these experiments recovery from neutron irradiation and from X irradiation, as measured by $LD_{50}/30$ days, proceed similarly; precise comparison, however, is unwarranted because of the relatively large degree of statistical

[42] H. A. Blair, Univ. of Rochester Rep., UR-312 (1954).

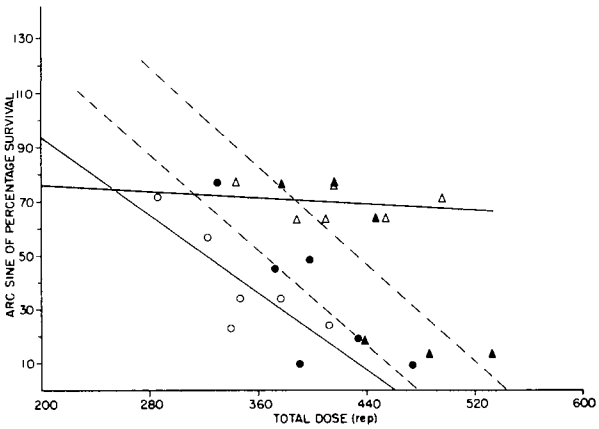

Fig. 28. Survival of RF mice exposed to single and paired doses of fast neutrons (cyclotron radiations).

variation in the neutron results. In view of earlier observations[37,38] significant differences in recovery may well be encountered at dose rates other than those employed in this study and with different biologic parameters as the basis for comparison.

The high degree of survival observed when the paired doses were separated by 5 days is significant. Analyzed in terms of residual injury, the mice were almost entirely recovered at 5 days; whereas, at 8 days, recovery was much less complete. This suggests that recovery may not occur at an exponential rate within the first few days postirradiation. The explanotion is currently under study; it is conceivably related to variation in the rates of mitotic and physiologic regeneration occuring in different organs following whole-body irradiation. On the other hand, it is noteworthy that recovery was assayed in these experiments in terms of resistance to a second dose of radiation and that the latter does not actually measure repair of injured tissues.

Studies of Cataract Induction by Fast Neutrons

K. W. Christenberry P. Ledford
G. S. Melville F. F. Wolff
G. S. Hurst[43]

The following experiments were undertaken to gain information about threshold doses of X rays and fast neutrons for cataract induction in several species and to learn about the relative effectiveness of X rays, gamma rays, and neutrons of different energies for lens injury.

RF mice 12-18 weeks old, rats of a Wistar subline 8-14 weeks old, and Dutch hybrid and albino rabbits 20-30 weeks old were exposed to 250-kvp X rays, Co^{60} gamma rays, and fast neutrons of approximately 2.5-Mev (Po-B)[44] and 14-Mev (T + d)[45] energies (Table 43). Mice and rats were exposed in groups of 10-20, and rabbits in lots of 3-10, per dose level. After irradiation, the animals were examined periodically with the slit lamp for lens opacities.

The effects of X rays on the lens of the mouse were not detectably different from those of gamma rays at the doses studied; a dose of 333 r resulted in a moderately severe cataract (++) within the first 6-8 months. A single exposure to 100 r had a less marked cataractogenic action, whereas the effects of 33 r were barely detectable. The lens opacities produced by 333 r and by 100 r of X or gamma rays were indistinguishable from those caused by 35.4 and 11.6 rep, respectively, of 2.5-Mev neutrons (Fig. 29), suggesting an RBE of approximately 9. When a dose of 100 r of gamma

[43]Health Physics Division.

[44]Source provided by K. Z. Morgan, Health Physics Division.

[45]The Cockcroft-Walton generator was made available by J. L. Meem and R. G. Cochran, Physics Division.

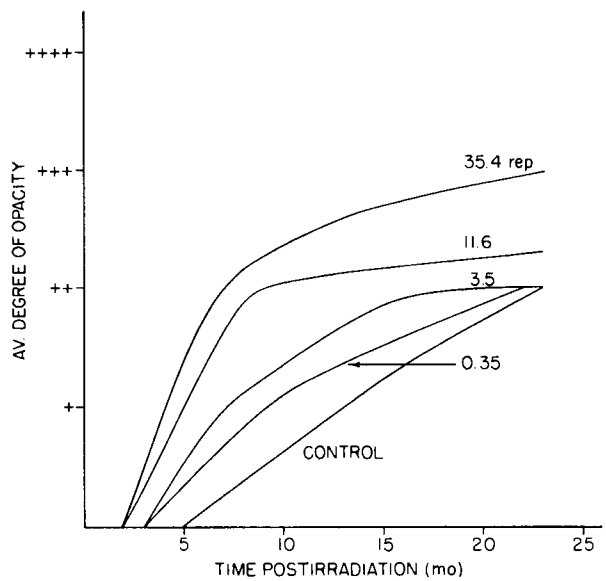

Fig. 29. Lens opacities in RF mice exposed to 2.5-Mev neutrons.

TABLE 43. RADIATIONS GIVEN TO THE LENS IN CATARACT STUDIES

Animals	250-kvp X-ray dose (r)	Co^{60} gamma-ray dose (r)	2.5-Mev neutron dose (rep)	14-Mev neutron dose (rep)
RF mice	33	33	0.354	
	100	100	1.16	
	333	333	3.54	
		100 (10 r daily)	11.6	
		100 (10 r 3-day interval)	35.4	
		100 (10 r weekly)	35.4 (3.54 rep daily)	
			35.4 (3.54 rep 3-day interval)	
			35.4 (3.54 rep weekly)	
Wistar rats	333		35.4	13.8
	100		11.6	1.38
	33		3.54	1.38
	10		1.2	
Rabbits	333		35.4	13.8
	100		11.6	2.77
	33		2.3	1.38

rays or 35.4 rep of fast neutrons was given in ten equal fractions at intervals of 1, 3, or 7 days, the resulting opacification of the lens progressed slightly more slowly than when the same total dose was administered in a single brief exposure.

In the rat, exposure of the lens to 333 r of gamma rays or 13.8 rep of 14-Mev neutrons failed to produce opacities of the lens distinguishable from those in the nonexposed controls; however, irradiation with 35.4 or 11.6 rep of 2.5-Mev neutrons appeared to be slightly cataractogenic (Fig. 30). No opacities have been observed in the rabbits (Table 43), which have been followed for 28-33 months since exposure.

These results confirm the relatively high radiosensitivity of the lens of the rodent and the relatively high biological effectiveness of neutrons for the production of lens injury. The data indicate that detectable lens damage may result from as little as 1-3 rep of fast neutrons in mice but that the rat probably requires ten times as much radiation for comparable injury; the results indicate also that the lens of the rabbit may tolerate 35 rep of 2.5-Mev neutrons without appreciable harm. Evidence is presented suggesting that fractional exposures to gamma rays or fast neutrons are not completely additive in their cumulative effects on the lens, in confirmation of earlier findings.[46] These preliminary data do not permit comparison of the relative effectiveness of neutrons of different energies.

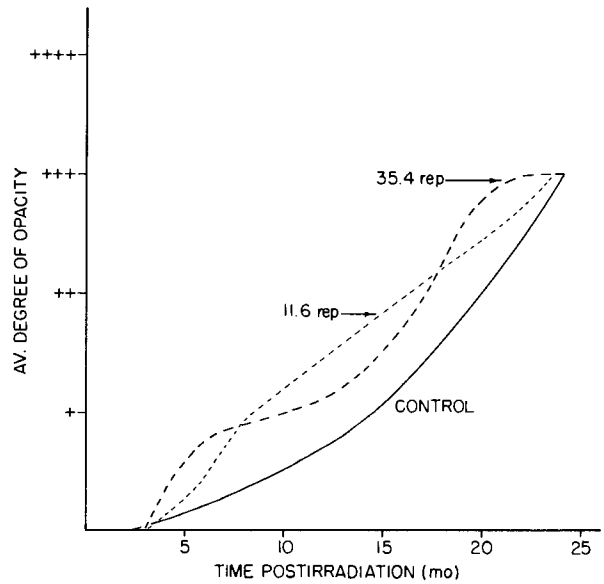

Fig. 30. Lens opacities in Wistar rats exposed to 2.5-Mev neutrons.

[46] T. C. Evans, E. F. Riley, and T. J. Leinfelder, Proc. Fifth Conf. Radiation Cataracts, Natl. Res. Council, Washington, D. C., March 26, 1954.

TRACER STUDIES ON INTERMEDIARY METABOLISM

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E. J. Bell[1]
M. V. Long
H. Sherwood

A Flavoprotein Peroxidase from *Streptococcus faecalis*

M. I. Dolin

A flavoprotein peroxidase has been isolated in a high state of purity, and is the first example of a nonporphyrin peroxidase. In addition, this enzyme has the property of forming an enzyme-substrate complex which can be measured spectrophotometrically, thus making it possible to carry out direct spectrophotometric studies on equilibrium binding and kinetics.

Previous work from this laboratory[2,3] has established the existence of a cyanide- and azide-resistant peroxidase for DPNH in extracts of *Streptococcus faecalis*. By the technique of zone electrophoresis[3] on extracts that had been purified about 50-fold with ammonium sulfate and gel procedures, it was possible to demonstrate that the peroxidase activity was directly proportional to the flavinadenine dinucleotide (FAD) content of the enzyme. From the flavin to protein ratio, it became apparent that extensive purification (at least 2000-fold) would be necessary in order to reach purity. The necessary large amounts of starting material (vacuum-dried cells) were obtained by growth of the organisms on a pilot plant scale at one of the large pharmaceutical companies. With this material it was possible, by the techniques mentioned, to bring the enzyme to apparent electrophoretic homogeneity. The best preparations obtained so far have an FAD content of 0.66%, which is equivalent to a molecular weight of 120,000, if 1 mole of flavin per mole of enzyme is assumed. The turnover number, based on the flavin content, is 5000. In the oxidized state, the enzyme has a typical flavin spectrum, with maxima at 370 and 450 mμ. There is no hematin component present. On the addition of DPNH, the 450-mμ band is shifted to about 445 mμ, the extinction at 450 mμ is reduced about 20%, and a new broad absorption band appears in the region 520-600 mμ. On the addition of peroxide, the spectrum of the native oxidized enzyme is regenerated. With a 100-fold excess of DPNH, in the absence of peroxide, the spectrum is stable for at least 1 hr; however, in oxygen-saturated solution and in an atmosphere of 100% oxygen, it is possible to show that there is an autooxidation that takes place at 1/100 the rate of the peroxidation.

The addition of excess hydrosulfite to the enzyme in the presence of DPNH results in only a 10% further reduction of the 450-mμ band, however, in the absence of DPNH, hydrosulfite reduces the 450-mμ band about 80% and no new absorption band appears above 520 mμ (Fig. 31). These results indicate that in the presence of DPNH, the bound flavin is complexed in such a manner that it can no longer react chemically with hydrosulfite. These results may be interpreted as follows: DPNH combines with the flavoprotein (FP) to form a complex which is only partially reducible with hydrosulfite and which is rapidly oxidizable with peroxide, but which is only slowly autooxidizable in the presence of molecular oxygen. This complex may be either (1) $DPNH_2 \cdot FP$ ($DPN \cdot FPH_2$), which

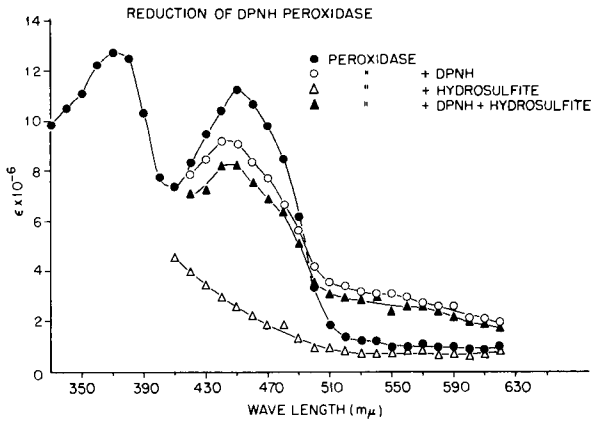


Fig. 31. Reduction of DPNH peroxidase. ●, peroxidase; O, peroxidase + DPNH; Δ, peroxidase + hydrosulfite; ▲, peroxidase + DPNH + hydrosulfite.

[1] Research Participant.

[2] M. I. Dolin, *Arch. Biochem. and Biophys.* 1955, in press.

[3] M. I. Dolin, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, pp. 67-68.

is a half-reduced system and may have the properties of the product of one electron transfer, or (2) the fully reduced system $DPNH_2 \cdot FPH_2$. Perhaps both are possible.

That at least the fully reduced complex may exist is shown by experiments in which graded levels of DPNH are added to the hydrosulfite-reduced enzyme. Under these conditions, a typical saturation curve is obtained on plotting ΔE_{450} against DPNH concentration (Fig. 32). Thus the concentration of the enzyme substrate complex (ES) can be directly determined, under equilibrium conditions. From this data it is possible to calculate the thermodynamic constants for the binding of DPNH to the reduced enzyme. The value for k is 4.4×10^4 which is equivalent to $\Delta F = -6350$ calories. The values for DPNH binding to the oxidized enzyme, as determined from kinetic data in which the concentration of ES is inferred from V_{max}, are of the same order of magnitude.

From these results it is possible to state that the peroxidation is not a simple two-step reaction, with reduced FP as a free intermediate. The mechanism must involve a ternary complex as follows:

1. $DPNH_2 + FP \longrightarrow DPNH_2 \cdot FP\ (DPN \cdot FPH_2)$
2. $DPNH_2 \cdot FP + H_2O_2 \longrightarrow DPNH_2 \cdot FP \cdot H_2O_2$
3. $DPNH_2 \cdot FP \cdot H_2O_2 \longrightarrow DPN + FP + 2\,H_2O$

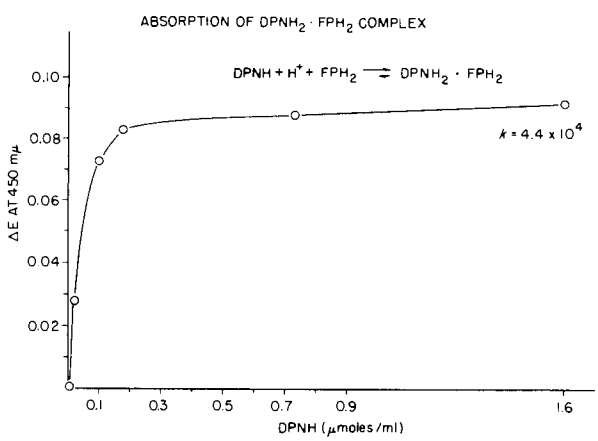


Fig. 32. Absorption of $DPNH_2 \cdot FPH_2$ ($k = 4.4 \times 10^4$).

The peroxide complex would be extremely unstable and would decompose immediately into enzyme and products. The possibility that a metal is involved in the reaction, either for binding substrates, or for electron transfer, or both is indicated in the spectrophotometric experiments that show the creation of a new absorption band in the presence of DPNH, and from the fact that the enzyme will react with a one-electron oxidant (ferricyanide). Neutron activation analysis appears to rule out the presence of molybdenum, copper, or manganese.

The isolation of DPNH peroxidase in a high state of purity definitely establishes that the enzyme is a flavoprotein and not a porphyrin. This is the first example of a peroxidase which is not a porphyrin enzyme and, unless the system is unique, it may indicate the existence of a new class of enzymes, the flavoprotein peroxidases.

In any case, this system should be a useful tool for studying enzyme mechanism, especially flavoprotein catalysis, since it is possible to determine directly, by spectrophotometric means, the formation of an enzyme-substrate complex.

Further work on this system is in progress in order to (1) clarify the mechanism, especially with regard to metal catalysis, (2) determine whether enzymatic catalysis is involved in the synthesis of holoenzyme from apoenzyme,[3] and (3) determine whether coupled phosphorylation (or phosphate turnover) is involved in this or any of the other oxidases[3] present in extracts of *S. faecalis*.

Succinic Acid Decarboxylase System

S. F. Carson E. F. Phares

A resolution and partial purification of succinic acid decarboxylase enzyme systems from *Propionibacterium* and *Veillonella* cultures was reported previously.[4] The present report describes additional purification and certain properties of the enzymes and enzyme systems.

Figure 33 is a composite diagram representing a number of starch (zone) electrophoresis runs carried out on a *Propionibacterium* preparation. Enzymes of the succinic decarboxylase system shown in Fig. 33 consist of the succinate-activating and -decarboxylating enzymes. Additional

[4] S. F. Carson and E. F. Phares, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, pp. 69-72.

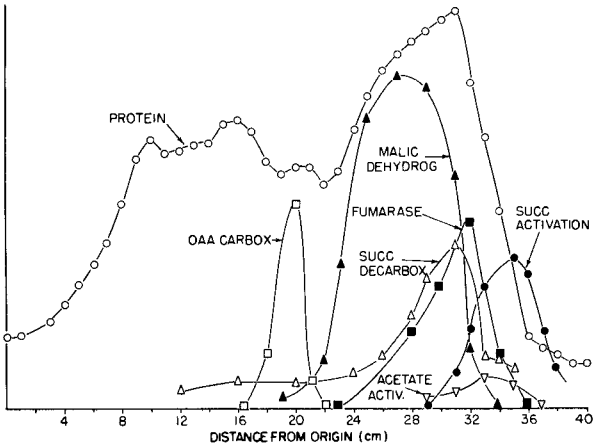


Fig. 33. Relative positions of protein and some enzymes from starch electrophoresis of *Propionibacterium* extract. Complete conditions used in these separations are described in *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 72.

enzymes were found, some of which may be closely connected to the system under study: oxalacetic (OAA) carboxylase, fumarase, and malic dehydrogenase. No evidence of lactic dehydrogenase or a "malic enzyme" was found. The "CO_2-producing enzyme," which converts the C_1 residue from the decarboxylation reaction to CO_2, is found in a resolved state in the *Veillonella* particle fraction. Reactions involving these enzymes will be described.

The succinyl-CoA decarboxylase system exhibits a number of properties of a sulfhydryl enzyme. It is protected during storage by sulfhydryl compounds, such as BAL; it can be reactivated by the addition of cysteine; typical sulfhydryl inhibitors, such as mercury, copper, and arsenite inhibit 100, 75, and 50% respectively in 1×10^{-2} M concentration.

There was no inhibition from cyanide, azide, or atabrine; pretreatment with hydrogen gas, and incubation in a hydrogen atmosphere did not inhibit CO_2 production. This latter result is in contradistinction to experiments reported in a discussion by Krampitz[5] in the 1952 Oak Ridge Symposium ("Some Aspects of Microbial Metabolism"). The inhibitor data indicate that oxidation-reduction reactions such as occur in fatty acid oxidation systems probably do not participate in the succinate decarboxylation reactions.

Combining the succinyl-CoA decarboxylase and the *Veillonella* particle fraction does not complete the system unless a raw bacterial extract from either *Propionibacterium* or *Veillonella* is present as well. The results show that the *Propionibacterium* extract contains a richer supply of the missing material than *Veillonella* (Table 44).

Data presented in Table 45 were obtained from similar experiments (as shown in Table 44) with the important exception that the *Propionibacterium* and *Veillonella* extracts were heated in a 100°C bath for 10 minutes. The results show that the missing factors are heat stable and are present in *Propionibacterium* extract.

Experiments were designed to couple the C_1 fragment (resulting from succinate decarboxylation) to known CO_2 fixation systems, specifically OAA and malic carboxylases. DPNH oxidation, however, was observed upon incubation of the *Propionibacterium* extract with only pyruvate and succinyl-

[5] *J. Cellular Comp. Physiol.*, 41, Supp. 1, 32 (1953).

TABLE 44. ADDITION OF RAW EXTRACTS TO PURIFIED ENZYME FRACTIONS

Vessels	1	2	3	4	5	6	7	8
Veillonella extract	+				+	+		
Veillonella particle fraction		+					+	+
Propionibacterium extract			+		+		+	
Propionibacterium purified enzyme				+		+		+
CO_2 produced; μl/30 min	8	1	1	2	90	30	45	3

All vessels contained 100 μmoles of potassium phosphate buffer at pH 6.5, 0.4 μmole of succinyl-CoA, 50 μmoles of potassium succinate, and 8 μmoles of cysteine. All chemicals were adjusted to pH 6.5. Incubation: 30°C; N_2 atmosphere.

TABLE 45. ADDITION OF BOILED EXTRACTS TO PURIFIED ENZYME FRACTIONS

Vessels	1	2	3	4	5	6	7
Boiled *Veillonella* extract					+		+
Veillonella particle fraction		+	+	+		+	
Boiled *Propionibacterium* extract				+	+	+	
Propionibacterium purified enzyme	+		+	+	+		+
CO_2 produced; μl/30 min	4	4	10	18	6	7	6

All vessels contained 100 μmoles of potassium phosphate buffer at pH 6.5, 0.4 μmole of succinyl-CoA, 50 μmoles of potassium succinate, and 8 μmoles of cysteine. All chemicals were adjusted to pH 6.5. Incubation: 30°C; N_2 atmosphere.

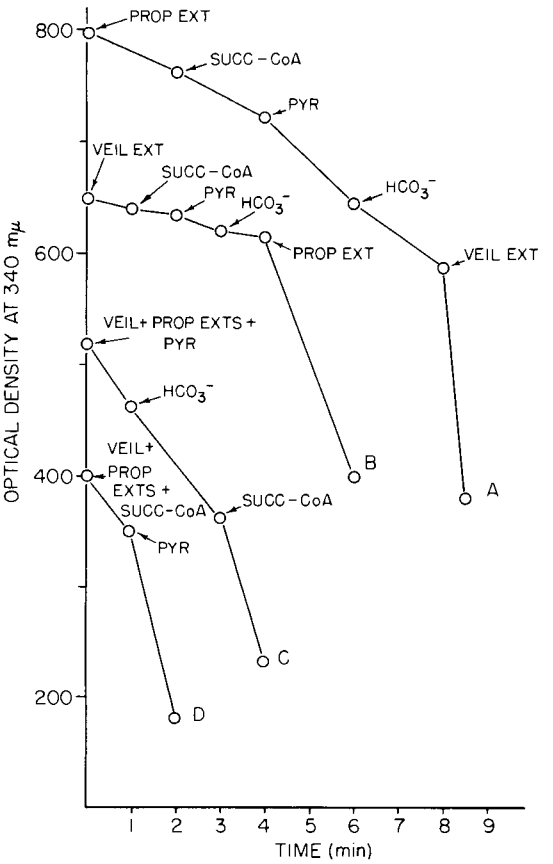


Fig. 34. DPNH oxidation by *Propionibacterium* and *Veillonella* extracts. Each cuvette contained 0.2 ml of M/10 potassium phosphate, pH 6.5, ~0.03 μmole of DPNH plus the following amounts and volumes of the indicated additions: *Propionibacterium* extracts (~20 mg of protein/ml), 0.01 ml; *Veillonella* extracts – supernatant from ultracentrifuge – (20 mg of protein/ml), 0.01 ml; succinyl-CoA, 0.16 μmole in 0.01 ml; potassium pyruvate, 0.1 μmole in 0.01 ml; $KHCO_3$, ~5 μmoles in 0.005 ml.

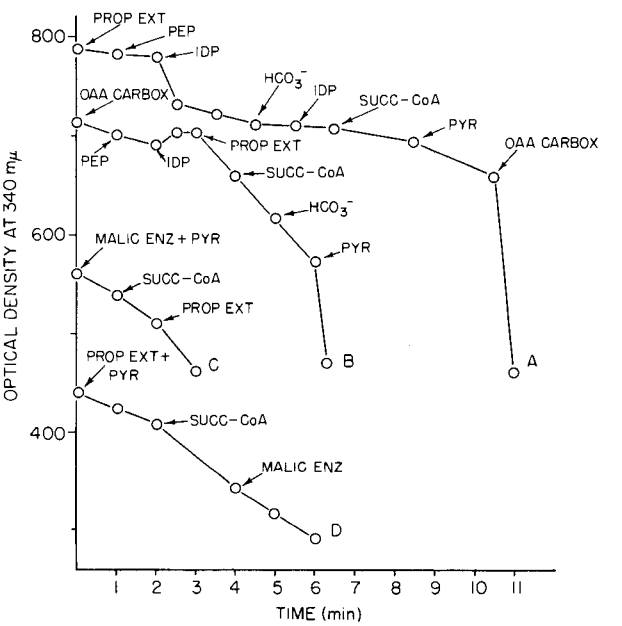


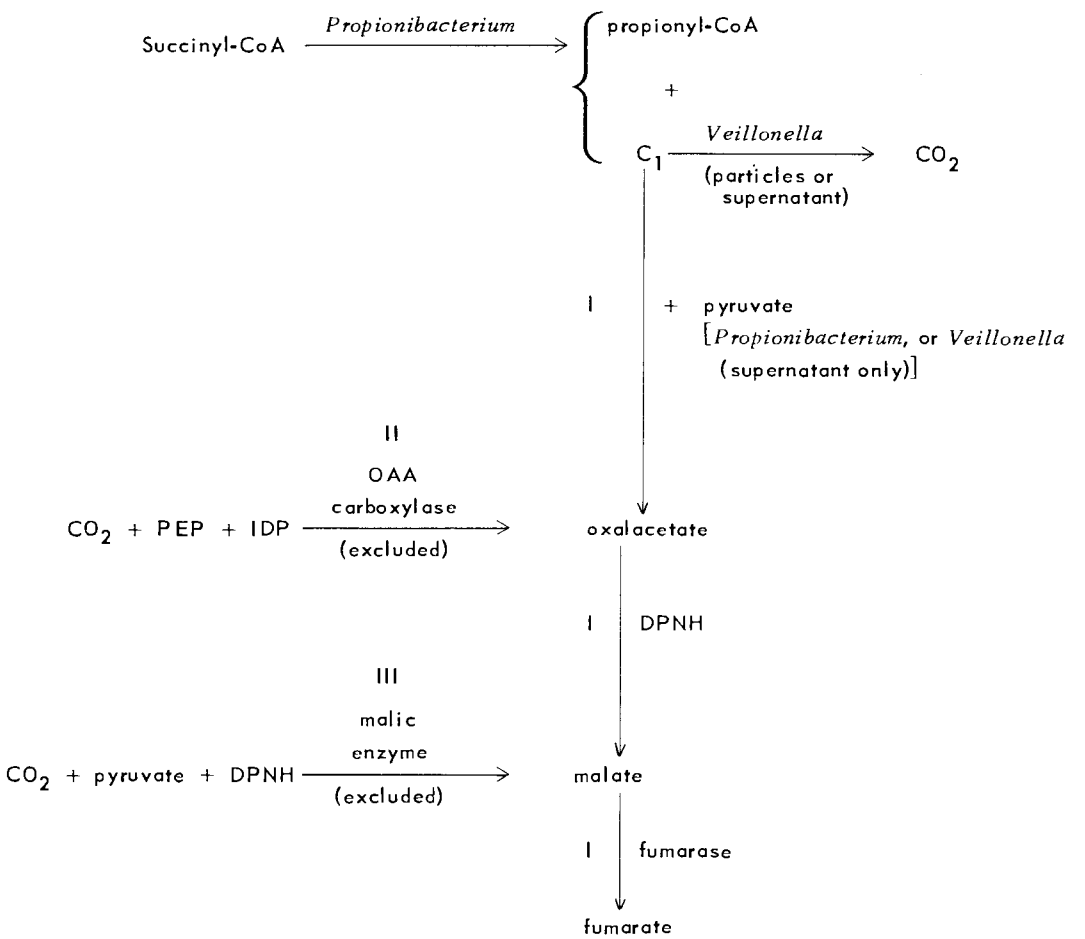
Fig. 35. Nonparticipation of OAA and malic carboxylases in the succinic decarboxylase coupled DPNH oxidation. Each cuvette contained 0.2 ml of M/10 potassium phosphate (pH 7.4 for curves A and B, and pH 6.5 for curves C and D), 0.2 μmole of MnCl, 0.03 μmole of DPNH, and the following amounts and volumes of the indicated additions, which were not described in Fig. 29: OAA carboxylase (chicken liver, 12-fold purified), 0.01 ml; "malic enzyme" (*Lactobacillus casei*) sp. act. 330, lactic dehydrogenase-free, 20 μg in 0.005 ml; phosphoenol pyruvate (PEP), 1 μmole in 0.01 ml; inosine diphosphate, 0.1 μmole in 0.015 ml.

CoA as substrates, and without the addition of enzyme systems from other sources. In addition, it was observed that aged *Veillonella* extracts, which do not decarboxylate succinate, gave a marked stimulation to the observed DPNH oxidation. Figure 34 summarizes the dependencies of the two extracts on pyruvate and succinyl-CoA for this reaction. It is noteworthy that the addition of bicarbonate, even in the presence of pyruvate, does not replace succinyl-CoA.

Figure 35 presents data in which tests were made to couple individual components of OAA and malic carboxylase systems to the C_1 fragment of the succinic decarboxylase system. The addition of phosphoenol pyruvate (PEP), bicarbonate and inosine diphosphate (IDP) gave no sustained effect. (These substances comprise the cofactor and substrate requirements of the OAA carboxylase system.) The subsequent addition of succinyl-CoA and pyruvate gave the usual stimulation of DPNH oxidation. The marked effect of OAA carboxylase on the system when pyruvate is present (curve A), is anomalous because of the presence of lactic dehydrogenase (LDH) in the OAA carboxylase preparation. In curve B a different order of additions was used, and the marked stimulation of DPNH oxidation by addition of the *Propionibacterium* extract is undoubtedly caused by its content of malic dehydrogenase. In curves C and D the nonparticipation of malic enzyme is demonstrated.

The experiments described have ruled out known carboxylation systems (OAA and malic carboxylases) as participants in the reaction involving the C_1 fragment (from succinyl-CoA decarboxylation) and pyruvate with DPNH oxidation.

The following diagram indicates the reactions as far as they are understood at present.



In further support of the exclusion of known carboxylation reactions (II and III), the following activators, some of which are known to be essential for reactions II and III, have no effect on DPNH oxidation in the system involving reaction I: Mn^{++}, ATP, PEP, IDP, HCO_3^-; also propionyl-CoA, acetyl-CoA, and CoA do not stimulate.

BIOCHEMISTRY

STUDIES ON NUCLEIC ACIDS AND ENZYMES

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M. H. Jones
A. B. Ottinger

Biochemical Studies on Bacteriophage

E. Volkin L. Astrachan
M. H. Jones

Considerable evidence has accumulated which indicates that during the process of bacterial virus infection there is both a physical and a functional partition of the virus deoxyribonucleic acid (DNA) and protein. Our approach is to investigate the biochemical properties of the separate entities in relation to the function of the whole virus.

DNA. The combined action of pancreatic deoxyribonuclease and snake venom diesterase is known to hydrolyze thymus DNA quantitatively to the mononucleotides. However, we have found that with bacteriophage T4 DNA the enzymes, while liberating some 80% of the other three bases of the DNA as mononucleotides, yield only 15% of hydroxymethylcytosine (HMC) mononucleotide. The remainder of these bases are found as more complex polynucleotides. Many of these polynucleotides have been separated and partially identified by the usual ion-exchange procedures. It was thus determined that there was at least one HMC residue per polynucleotide, which indicates the relative resistance of these internucleotide linkages. In addition, the unique observation was made that glucose was present in the HMC mononucleotide and the HMC polynucleotide portions in equimolar quantities with the HMC residues. The sugar was identified as glucose, by chromatography in the borate system and by the hexokinase-firefly luminescence system. The sugar has been tentatively allocated to the 5-hydroxymethyl group as an O-glycoside because of its essentially complete liberation as the free sugar under controlled acid hydrolysis, without simultaneous destruction of the HMC.

Analyses from other laboratories have revealed that the base composition of DNA's (and the amino acid composition of the proteins) from the even-numbered bacteriophages are precisely the same. Since it is known that the three even-numbered bacteriophages are genetically different, and it is assumed that the DNA is primarily responsible for the genetic properties, it follows that these properties are determined by some more subtle difference in the DNA structure. Such a difference could reasonably be the nucleotide sequences in the DNA chains.

The resistance of glucose-hydroxymethylcytosine internucleotide linkages to enzymic attack offers a rational approach to quantitation of some of the preexisting nucleotide sequences in the DNA chains. Another approach designed to determine pyrimidine sequences in the chain involves controlled acid hydrolysis and ion-exchange isolation of the fragments liberated. This method is being investigated first with the readily available thymus DNA. To date, at least a score of polynucleotide peaks have been isolated and are currently being identified. Since these substances contain no purines, the relative frequencies of certain pyrimidine sequences may be elucidated.

Protein. The protein part (ghosts), obtained by osmotically shocking the virus, maintains the ability to alter profoundly the metabolism of *Escherichia coli*. Under varying conditions, one may observe the effect of "ghosts" to inhibit *E. coli* RNA synthesis, inhibit cell multiplication, or cause cell lysis. We are investigating the mechanism involved in the inhibition of RNA synthesis.

Our approach is based on the information that inorganic phosphate is esterified by ghost-treated *E. coli* at a normal rate even though RNA synthesis is inhibited. Identification of the newly synthesized phosphate compounds will provide information about pathways for RNA synthesis in addition to clues about the inhibition of RNA synthesis.

After completion of preliminary experiments, ion-exchange procedures were devised for the separation of newly synthesized acid-soluble phosphate compounds which appear in the supernatant growth medium. The incorporation of P^{32} inorganic phosphate was used to detect the new synthesis of phosphate compounds.

Present data indicate that the esterified P^{32} found in the supernatant medium is incorporated

[1]Research Participant.

into both nucleotide-like and non-nucleotide compounds. The establishment of a relation between these compounds and RNA synthesis awaits their identification.

Catalytic Reduction of Pyrimidine Nucleotides

W. E. Cohn D. G. Doherty
A. B. Ottinger

The discovery of glucose in T2 bacteriophage raised the question as to whether this sugar was in place of or in addition to the expected deoxyribose of the 5-hydroxymethylcytosine with which it was associated. Direct methods of assaying for the sugars of the pyrimidine nucleotides are not available; the glycosidic linkage is relatively stable to the conventional orcinol and diphenylamine tests. Furthermore, these and similar tests are destructive in nature and would not lend themselves to the isolation of fragments containing glucose or a pentose. The identification of pentoses in the pyrimidine nucleotides was accomplished in minimal degree by Levene and his collaborators, using hydrogen reduction of the base to labilize the glycosidic linkage. With the catalysts, materials, and methods available in their day, it would not be possible quantitatively to reduce microgram amounts of a rare nucleotide and recover, or identify unequivocally, the sugar components. Accordingly, having new catalysts and methods at hand, we have undertaken a study of the catalytic reduction of pyrimidine nucleotides by hydrogen.

With Baker's rhodium-on-alumina catalyst, cytidylic, uridylic, and thymidylic acids are reduced by 1 atmosphere of H_2 at reasonably rapid rates (e.g., complete reduction in 30 minutes to a few hours, depending on factors to be discussed). Reduction is measured by the disappearance of the characteristic ultraviolet absorption spectrum which depends upon the C_4,C_5 double bond which is being saturated, by the uptake of 1 mole of H_2 per mole base thus disappearing, and by the appearance of a characteristic orcinol (or diphenylamine) reaction in amount also equivalent to the loss in ultraviolet absorption. On complete reduction, 1 mole of H_2 has been consumed, all UV absorption is gone, 1 mole of reducing power has appeared, and all ring nitrogen and phosphate are still organically bound (the 6-amino group of reduced cytidylic acid is easily hydrolyzed and some of it appears as NH_3). Dihydrocytidylic and dihydrouridylic acids behave on ion-exchange columns in a fashion very similar to their parents and thus may be analyzed and recovered in similar fashion. However, evidence is accumulating to indicate the appearance of alkali lability in the 1,6 bond. In about 30 hr in 0.1 N NaOH, or overnight in 0.02 N, dihydrouridylic acid is converted to a substance which chromatographs as a more acidic material. This new material, in contradistinction to dihydrouridylic acid itself, is very sensitive to dilute acid; at pH 1-2, it is rapidly and largely converted (a few hours) to ribose phosphate and an acidic nitrogen-containing substance. The amount of nitrogen thus released is twice the amount of ribose phosphate found. From its behavior as a weak acid, in contradistinction to NH_3 or urea, it has been concluded that it is β-ureidopropionic acid (in the formula, R = H)

$$NH_2-CO-NR-CH_2-CH_2-COOH,$$

and that its immediate parent, derived from dihydrouridylic acid by alkali, is the ribose phosphate derivative of this (I,R = ribose phosphate bound through the 1 carbon atom as usual).

This reaction has been used to prepare ribose 2-phosphate from uridylic acid *a* and ribose 3-phosphate from the *b* isomer, thus verifying the above procedures as well as the earlier conclusion that the isomerism was as stated. It has also been used to reduce a small amount ($\sim$1 μmole) of 5-hydroxymethyldeoxycytidylic acid, prepared by Volkin, and to get a diphenylamine reaction (deoxyribose test) equivalent, within 80%, to the phosphate content. The amount of material was too small, however, to show definitely the equivalent presence of glucose, which is known from other tests, to be present. This and other possibilities for the new reaction in nucleic acid chemistry are under investigation.

Ion-Exchange Behavior of Undegraded Nucleic Acid Preparations

J. X. Khym W. E. Cohn
A. B. Ottinger

Work in many laboratories has led to the conclusion that polynucleotides are bound to strong-base anion exchangers with a force roughly proportional to their phosphate content, moderated to some degree by the specific bases present, much

as are the pyro di- and triphosphates. It has also been observed that whole nucleic acids, once bound on such an exchanger, are not eluted except after *in situ* degradation. From these observations, it has been surmised that whole nucleic acids are quickly and quantitatively absorbed by strong-base anion exchangers. Quantitative break-through experiments with undegraded DNA and RNA samples at controlled pH and salt concentration, and with exchangers of varying divinylbenzene (DVB) content, have now shown that conditions exist under which little or no absorption occurs. Calf liver RNA absorbs strongly, at pH values above 7 and in low salt concentrations, on the conventional 8 and 10% DVB exchangers, but not on 24% (flow rates about 0.10-0.60 ml/cm^2/min, mesh size 200 and up); below 7, a large degree of break-through occurs. Thymus DNA does not absorb even at pH values as high as 9.5. After boiling, however, the same DNA exhibits a degree of absorption which seems to be proportional to the time of boiling. Any fraction of either nucleic acid preparation which does absorb is essentially irreversibly bound. The mechanism does not appear, therefore, to be one of simple ion exchange.

Besides its interest from the standpoint of ionic and ion-exchange properties, this phenomenon is being investigated for its application to several practical problems: the separation of DNA and RNA, the separation of RNA's of differing composition, and the detection of partially degraded DNA. Preliminary evidence of usefulness in all three directions has been obtained.

Studies on Enzyme-Substrate Binding

D. G. Doherty R. Shapira
E. Eavenson

A previous semiannual report[2] described the background and initial work leading to the discovery of carbon-carbon bond hydrolysis by α-chymotrypsin. The enzymic hydrolysis of ethyl-5-(p-hydroxyphenyl)-3-ketovalerate has now been extensively studied and the nature and kinetics of the reaction clearly defined. At the optimum pH 7.9 the reaction rate was found to be monomolecular and proportional to the enzyme concentration. Determinations of rates at a series of enzyme and substrate concentrations (Fig. 36) enabled the

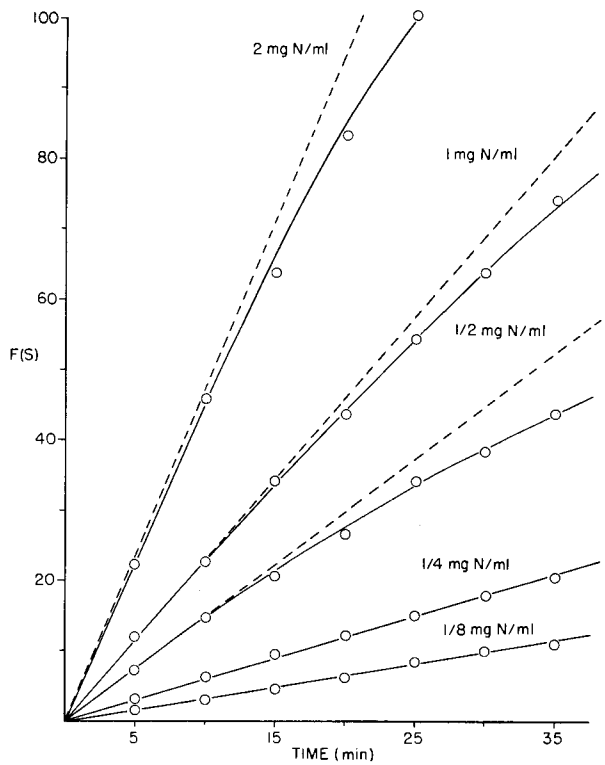


Fig. 36. α-Chymotrypsin-catalyzed hydrolysis of ethyl-5-(p-hydroxyphenyl)-3-ketovalerate at 25°C and pH 7.9 V_0 in units of 10^{-6} moles/min; 0.02 M tris(hydroxymethyl)aminomethane HCl.

estimations of the kinetic constants K_s and k_3 to be made. At enzyme concentrations of ½-2 mg of protein N/ml, the values were $70 \pm 3 \times 10^{-3}$ and $2.5 \pm 0.3 \times 10^{-3}$ moles/min/mg of protein N/ml, respectively. At two dilute enzyme concentrations (¼ and ⅛ mg of protein N/ml), the same k_3 of $40 \pm 3 \times 10^{-3}$ moles/min/mg was obtained. This difference may be easily explained by competitive inhibition by the hydrolysis products of the reaction. If no such inhibition took place, a plot of $K_s \ln \{[S]_0/[S]\} + [S]_0 - [S]$ versus time should yield a straight line passing through the origin and having a slope of k_3 E. The data presented in Fig. 37 show clearly by the negative deviation from the calculated slope of the experimental points at the three higher enzyme concentrations that the reaction is competitively inhibited by the split product. In contrast, the experimental values at ¼ and ⅛ mg of protein N/ml are in good agreement with the calculated slope. Similar determinations

[2] *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p. 74.

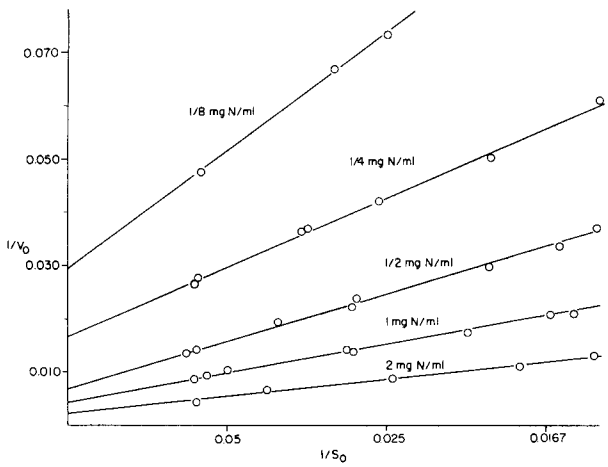


Fig. 37. Plot of F (S) = K_s ln $[S]_0/[S]$ + $\{[S]_0 - [S]\}$ in units of 10^3 moles versus time for α-chymotrypsin and ethyl-5-(p-hydroxyphenyl)-3-ketovalerate at 25°C and pH 7.9; $[S]_0 = 60 \times 10^{-3}$ M.

with the free acid, 5-(*p*-hydroxyphenyl)-3-ketovaleric acid, gave a K_s of $14 \pm 2 \times 10^{-2}$ M and a k_3 of 0.8×10^{-3} moles/min/mg of protein N/ml. These results are in accord with the constants for the ester hydrolysis since the keto group of the keto ester is relatively more polarized than the keto group of the keto acid. This is reflected in its greater affinity for the enzyme (K_s 0.04 versus K_s 0.14) as well as the more rapid decomposition of the ES complex (k_3 2.5×10^{-3} versus k_3 0.8×10^{-3}). Apparently, the major factor in the hydrolysis of a bond, provided that the specific structural requirements for the enzyme have been fulfilled, is whether the activation energy for bond rupture can be sufficiently lowered by attachment on the active center of the enzyme to bring about an exchange of groups. The enzyme reaction observed here is very similar to the nucleophilic double displacement reactions observed in the hydrolysis of acetoacetic esters by the hydroxyl anion. The β-keto ester system is an ideal one to test this hypothesis and perhaps to provide data on the upper limit of bond strengths susceptible to enzyme action, since the strength of the carbonyl-carbon bond can be readily varied by either substituting other electrophyllic groups for the carboxylate group or by replacing the hydrogens with electrophyllic or electrophobic groups. Synthetic procedures for the preparation of such substrates are at present under development in this laboratory.

Microbial Utilization of Heparin

D. G. Doherty J. F. Christman

With a single exception in which no products were isolated, enzymic attempts to degrade heparin have been unsuccessful. The search for enzymes capable of producing chemically identifiable fragments under mild conditions has led to an examination of microbiological sources. No previous record exists of any microorganism which utilizes heparin as a substrate. The use of elective culture techniques with a chemically defined medium and heparin as a sole source of carbon, nitrogen, sulfur, or a combination of these was chosen as the most promising approach. Media of this nature, inoculated with soil or mud, were incubated either aerobically or anaerobically at room temperature. When growth occurred, the culture was transferred to a second liquid culture and then plated on a solid medium. Of the 97 cultures isolated, 34 had a pronounced ability to utilize heparin as a nutritional substrate. Table 46 shows the taxonomic relationships of the organisms and their respective abilities to utilize heparin.

The data presented in Table 46 show that in each case an organism which utilizes any portion of the heparin molecule also utilized the sulfate as a nutritional source of sulfur. Thus it seems highly probable that some of the sulfate groups must be removed before any other enzymic attach on heparin can occur. It may also be noted that all the organisms isolated were gram negative, which emphasizes the marked difference between the two main divisions of microorganisms. In addition, a great majority of the organisms are easily recognized as members of the genus *Escherichia*, possibly owing to the initial conditions of isolation in which only inorganic salts and heparin served as sources of nitrogen and sulfur. If one establishes an anaerobic elective culture, using inorganic sources of nitrogen and sulfur with dextrose as an energy source, members of the *Escherichia* invariably result; this may explain the high proportion of this family represented. There appears to be little correlation between the constitution of the original elective culture and the organism isolated. One further point of interest is the observation that organisms which can specifically degrade heparin are extremely widespread in nature. Several representative gram-negative short rods of the genus *Aerobacter* which

TABLE 46. HEPARIN-DEGRADING ABILITY OF FRESHLY ISOLATED MICROORGANISMS

Organism	No. of strains isolated	Ability to utilize the element in heparin		
		Carbon	Nitrogen	Sulfur
Aerobacter aerogenes	5	+++	+++	++++
Aerobacter chloacae	1	–	+	++++
Aerobacter (species unknown)	5	–	–	++++
Paracolobactrum intermedium	1	–	–	+++
Paracolobactrum aerogenoides	5	–	–	++++
Pseudomonas fluorescens	1	–	–	+++
Achromobacter liquifaciens (Type 1)	4	++	–	+++
Achromobacter liquifaciens (Type 2)	2	+	++	+++
Bacterium sp. (Type 1)	4	+	++	++++
Bacterium sp. (Type 2)	1	–	+	++++
Bacterium sp. (Type 3)	4	–	–	++++
Bacterium sp. (Type 4)	1	+++	+	+++
TOTAL	34	16	14	34

have been in pure culture at least 10 years exhibit marked ability to utilize heparin as a sole source of sulfur.

Although a number of methods have previously been published in the literature for the colorimetric analysis of heparin, none of these were well suited to the particular assays which were required. All of these methods, originally designed to determine the concentration of heparin in blood plasma, depended on the presumed formation of a heparin-dye complex with a corresponding shift in the absorption maximum of the solution. Our system, on the other hand, contains little protein and rather high concentrations of inorganic materials. Assays, following classical approaches, resulted in generally complete precipitation of the complex. To avoid this difficulty, gum arabic was introduced as a protective colloid. This modification has resulted in assays which are stable for over 48 hours. The general procedure is as follows:

To each tube add 1.0 ml of 1% aqueous gum arabic and 1 ml of sample; shake thoroughly. Then add 1 ml of 0.05% aqueous toluidine blue O (prepared as described below) and shake thoroughly. Dilute with 10 ml of distilled water, and read with Evelyn colorimeter, using a 490-mμ filter, with a dye blank set at zero optical density. The toluidine blue O is prepared by grinding 1.0 g of dye with three 10-ml portions of distilled water, diluting to 100 ml, and filtering. Exactly 5.0 ml of this solution is diluted to 100 ml.

A standard curve, showing a linear response in the range of 10-100 μg of heparin, is shown in Fig. 38.

Structure and Activity in Radiation-Protective Sulfhydryl Compounds

D. G. Doherty R. Shapira
W. T. Burnett[3] E. Eavenson

The study of structure and radiation-protective activity previously reported[2] has been extended to sixty compounds. No other compound was found with a protective activity greater than S,β-aminoethylisothiouronium·Br·HBr (AET bromide). It remained clearly superior to β-mercaptoethylamine (MEA) in twenty trials with five C_3H mice per group per trial. Table 47 shows that a significant increase in the 30-day survival was obtained with AET bromide, injected intraperitoneally, at a dose level equimolar to MEA. MEA, in contrast to AET

[3]Radiation Protection and Recovery section.

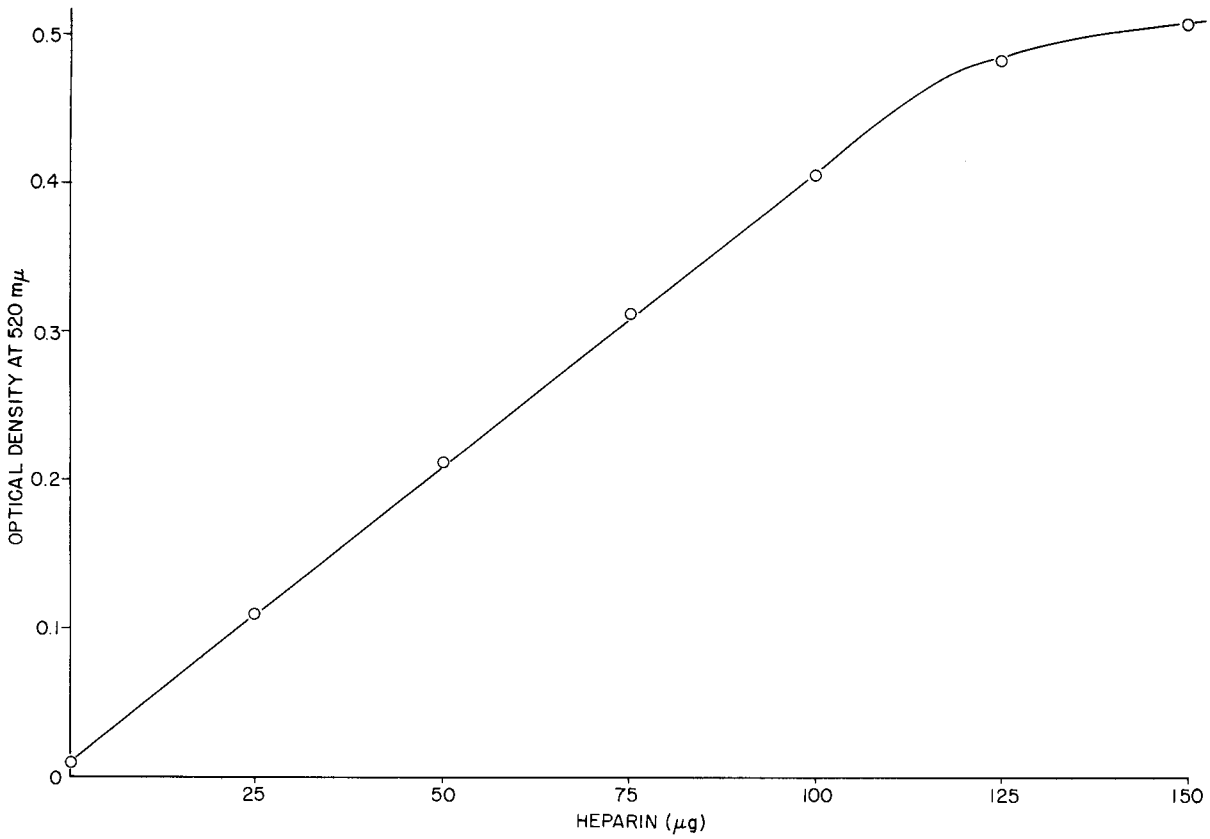


Fig. 38. Determination of toluidine blue with heparin.

bromide, required a near-toxic dose for protective activity. Some tentative conclusions that can be drawn from an analysis of the relation of structure to activity of the compounds studied are: (1) Compounds demonstrating maximum protective activity have (a) a free amino group, (b) a free or potential sulfhydryl group, and (c) a separation of the amino and sulfhydryl groups by not more than three carbon atoms. (2) Sulfides, disulfides, and sulfones were relatively inactive. (3) Substitution of secondary, tertiary, and quaternary amino, acyl amino, ureido, and guanidino groups for the primary amino group resulted in a loss of activity. The stability and probably slow rate of metabolism of AET bromide may account for its superior protective action.

TABLE 47. SURVIVAL OF IRRADIATED MICE TREATED WITH β-MERCAPTOETHYLAMINE AND S,β-AMINOETHYLISOTHIOURONIUM·Br·HBr

Treatment before 800 r of X rays	Survival on ith day[a] (%)	Survival on 30th day (%)
Saline	28 (29/105)[b]	0 (0/105)
AET bromide, 8.8 mg/mouse	78 (82/105)	47 (49/105)
Saline	23 (9/40)	0 (0/40)
MEA, 3.6 mg/mouse	82 (33/40)	10 (4/40)

[a]See Burnett, Doherty, and Kimball, this report, for explanation of ith day.
[b]Number of survivors/number treated.

PLANT BIOCHEMISTRY[1]

N. E. Tolbert
L. P. Zill
A. R. Krall
F. B. Gailey[2]
C. W. Nystrom[3]
P. C. Kerr
D. A. Mondon

Carbon Dioxide Fixation by Etiolated Plants After Exposure to White Light

N. E. Tolbert F. B. Gailey

Since the first report on these investigations,[4] the work has been extended. Plants grown in total darkness (etiolated plants) exist without chlorophyll or the photosynthesis process. When placed in the light, the etiolated plants have been shown to acquire, over a period of 1-2 days, the ability to photosynthesize. During this greening period, adaptation or synthesis of some of the enzymes for catalyzing photosynthesis may occur. The biochemistry of this process is of plant physiological interest. In addition, it may provide a system to study the effects of radiation on enzyme formation.

Seven-day-old etiolated Thatcher wheat plants were exposed to 600 ft-c of continuous white light, after which the total chlorophyll, rate of $C^{14}O_2$ fixation during a 10-min photosynthesis period, and the C^{14} products which formed were measured. Rapid chlorophyll synthesis began after the plants had been in the light for 2-3 hr but appreciable photosynthetic CO_2 fixation required an additional 1-2 hr (Fig. 39). The amount of total chlorophyll in the leaves increased rapidly until the plants had had 12 hr of light but leaves did not become saturated until after about 32 hr of light.

Before photosynthetic CO_2 fixation began there was a measurable dark-type fixation of $C^{14}O_2$ into malic, aspartic, and glutamic acids (Fig. 40). When photosynthetic CO_2 fixation became appreciable, after 4-5 hr of light, the first products to become labeled were phosphoglyceric acid and alanine. At this stage of greening, the C^{14} did not move rapidly into hexose, sucrose, or pentose sugars but rather piled up in the C_3 state as measured by the percentage C^{14} in each compound. Increase in the rate of C^{14} incorporation into sucrose and hexose phosphates became substantial after the plants had another hour of light, and

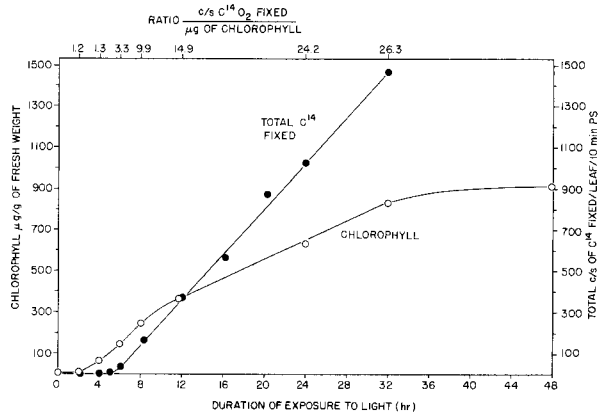


Fig. 39. Chlorophyll formation and $C^{14}O_2$ fixation during greening of etiolated wheat.

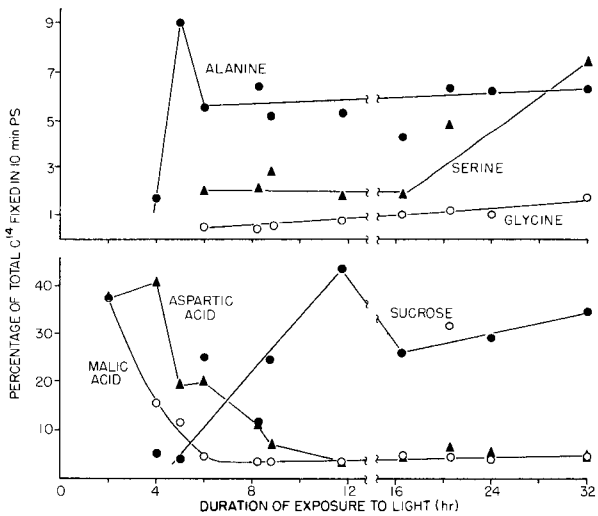


Fig. 40. Products from $C^{14}O_2$ fixation during greening.

[1]Abbreviations used in this report are: ATP, adenosine triphosphate; HDP, hexose diphosphate; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; AMP, adenosine monophosphate; ADP, adenosine diphosphate; PGA, phosphoglyceric acid; UDPG, uridine diphosphoglucose; DNP, dinitrophenol.

[2]Research Participant (summer 1954).

[3]ORINS Fellow (arrived Feb. 1, 1955).

[4]See Plant Biochemistry, *Biol. Semiann. Prog. Rep., Aug. 15, 1954*, ORNL-1766.

continued to increase until the plants had had 9-12 hr of light. From this time on, the percentage incorporation of the $C^{14}O_2$ into sucrose and the components of the path of carbon in photosynthesis remained constant.

Total fixation of $C^{14}O_2$ began to increase at a rapid linear rate when photosynthetic fixation began, around the fourth hr of light and continued to increase during the next 24 hr or longer. When the percentage incorporation of the C^{14} into the photosynthesis products became constant, after a total of 9-12 hr in the light, the total fixation in the 10-min test was not more than 25% of the rate found in the fully greened plant after 32 hr of light.

These results suggest that, after the plant had had 4-5 hr of light, the enzymic process and precursors were present to commence slow photosynthetic fixation forming PGA which also labeled alanine through pyruvic acid. Why these C_3 compounds accumulated C^{14} activity at this stage of greening is not known. The plant may not yet have had the ability, as represented by active enzymes, to reduce the triose to sucrose, or the pools of C_3 compounds may have had to be saturated before equilibriums would favor the synthesis of sucrose. In either case, this condition existed for 1 hr or more during greening.

It is significant that, after 9-12 hr of greening, when the percentage incorporation of C^{14} into sucrose had reached a normal equilibrium, the total CO_2 fixation rate was far from saturated and, in fact, was functioning at only about one-fourth its normal rate. This indicates that by this time the reactions in the reduction of PGA were functioning at about equilibrium conditions of normal steady-state photosynthesis. Increasing total fixation after 12 hr must be owing to other processes slower to reach normalcy. The data indicate that regeneration of the CO_2 acceptor, ribulose diphosphate, was one of these limiting factors and that another was continued chlorophyll synthesis. On the basis of the increasing chlorophyll efficiency ratio for total CO_2 fixation with increasing time of greening, other factors which would be limiting photosynthesis rates after 9-12 hr of greening could be postulated as involved in the efficiency of the chloroplast unit.

In a study of the reasons for changes in the amount of C^{14} in the constituents of the path of carbon in photosynthesis during the first hours the etiolated plant acquires the ability photosynthetically to fix $C^{14}O_2$, more detailed analyses of the organic phosphate esters of this cycle have been made and under conditions whereby the amounts of these intermediates have been artifically raised by prior feeding of them to the etiolated plant for 24 hr before exposure to light. In separate experiments, plants were sprayed with water (the control), 0.3 M glucose, 0.3 M ribose, 0.1 M mixture of 20% sedoheptulose and 80% sedoheptulosan, or 0.1 M glycolic acid.

In Table 48 are summarized the relative amounts of C^{14} which appeared during the 10-min photosynthesis period with $C^{14}O_2$ in the products from an etiolated Thatcher wheat plant which had been fed one of the above compounds for 24 hr and then exposed to 3 hr of light. Similar data have been collected for each hour of the experiments from 2-25 hours. Accumulation of all the results indicate that one limiting factor during this greening process was the availability of ribulose

TABLE 48. RELATIVE AMOUNTS OF C^{14}-LABELED PRODUCTS AFTER EXPOSURE TO 3 HOURS OF LIGHT

Products	Solutions sprayed on leaves prior to light exposure				
	Water	Glucose	Ribose	Sedoheptulose-sedosan	Glycolic acid
Aspartic acid	+++	++++	++++	+++	+++
Malic acid	++	+++	+++	++	++
PGA	+	++	++	0	+
HP area	Trace	+++	++++	Trace	+
UDPG area	Trace	+	+	0	Trace
Sucrose	Trace	+++	+++	Trace	+

diphosphate, which is the immediate precursor for photosynthetic carboxylation. For the water-sprayed controls, only traces of C^{14} activity accumulated in ribulose diphosphate after the leaves had been in the light for 1 day. Spraying the plants with ribose increased the amount of photosynthesis during the earliest hours after it began, and the amount of ribulose diphosphate and sedoheptulose phosphate which was labeled with C^{14} after 25 hr of light. Sedoheptulose spray, itself, was inhibitory, probably because of the sedoheptulosan accompanying the free sugar. Glycolic acid was without effect except in stimulating incorporation of C^{14} into serine.

An Unidentified Phosphorus Compound in Plants

N. E. Tolbert P. C. Kerr

The presence, in large amounts, of an unidentified phosphorus compound in the plant xylem sap for phosphate transport has been reported.[4] Physiological distribution of the compound elsewhere in plants has been observed. The results indicate that the unknown is the major phosphorus containing compound besides inorganic phosphate in the roots of barley seedlings, that it is transported to the leaves along with inorganic phosphate, and is completely used up for metabolism in the leaf.

In Table 49 is reported the total counts per second of P^{32} in the root or leaf of young barley plants of various ages from 2 to 22 days. Radioactive decay of the P^{32} is corrected back to the first day of the experiment. In these experiments, P^{32}-labeled phosphate was a part of the nutrient solution in which the roots of the seedlings were bathed. Seedlings were harvested on each day and chromatographically analyzed for the unidentified compound. In Table 50 is reported similar data on etiolated barley plants grown in total darkness.

TABLE 49. DISTRIBUTION OF THE UNIDENTIFIED P^{32}-LABELED COMPOUND IN GREEN BARLEY PLANTS

Age of plant (days)	Activity (cts/sec) in:	
	Roots	Leaves
2	152	76
3	636	222
4	641	546
8	1039	561
15	2707	43
22	3156	143

TABLE 50. DISTRIBUTION OF THE UNIDENTIFIED P^{32}-LABELED COMPOUND IN ETIOLATED BARLEY PLANTS

Age of plant (days)	Activity (cts/sec) in:	
	Roots	Leaves
4	256	22
5	248	177
6	758	984
8	691	788
10	690	853
12	450	538
14		1705
21	481	3248

The data show that, as the normal green plants grew older, a large amount of phosphate was accumulated by the roots in the unidentified compound. We have reported[4] a simultaneous vigorous transport of large quantities of this substance by the xylem flow from the roots to the leaves. However, as seen in column 2 of Table 49, the substance did not accumulate in the leaf. Presumably, it was used for metabolic purposes in the leaf.

In the etiolated plant, accumulation of the unidentified substance in the roots began, but when the plant failed to develop into a normal plant and growth was arrested, the presence of the substance in the roots decreased to a very small value, as compared to the roots of the green plant of the same age. However, other experiments have shown that the transport of the unidentified substance out of the roots by the xylem flow to the leaves was occurring in the etiolated plant. In the leaf of the etiolated plant, growth was arrested and starvation became more severe as the dark period increased. As a result, the unidentified substance accumulated in the etiolated leaf since it was not needed by the non-growing leaf.

An interesting analogy is available in nitrogen metabolism in plants to furnish a working hypothesis for the function of this unidentified substance. In a higher plant, two main forms of nitrogen are transported from the roots to leaves – inorganic nitrate and amide nitrogen in glutamine and asparagine. In the leaves, both are used for synthesis of protein in plant growth. However, when growth ceases in an etiolated plant, the amides accumulate in large amounts in the leaf. Likewise with phosphate metabolism in plants, there would appear to be two storage forms of phosphorus moving to the leaves from the roots. If metabolism is normal the unidentified form is used for growth; if metabolism is arrested, as in the etiolated plant, the unidentified compound is not utilized but accumulates in the leaf. The unidentified substance is not ortho or metaphosphate, or polyphosphates. Identification of the unidentified substances is under investigation.

Excretion of Glycolic Acid by Algae During Photosynthesis

N. E. Tolbert P. C. Kerr
L. P. Zill D. A. Mondon

Glycolic acid is known to be present in plants and algae and probably is formed as a side reaction from the path of carbon in photosynthesis. When *Chlorella* and normal steady-state photosynthesis with $C^{14}O_2$ were used, large amounts of radioactivity appeared in the supernatant solution. Analysis of the supernatant from culture conditions by paper chromatography indicated that all the C^{14} was present as glycolic acid. The radioactivity in the supernatant constituted 3-10% of the total C^{14} fixed within a few minutes of photosynthesis. At pH values below 3, the amount of C^{14} excretion into the supernatant was increased several-fold, but this was caused by excretion of sucrose and other cell components. As much as 30-40% of the total C^{14} fixed in a photosynthesis period of less than 1 min was excreted into the supernatant as glycolic acid, provided that photosynthesis was continued with nonlabeled carbonate. Excretion of the glycolic acid was dependent on CO_2 or carbonate, and did not occur in a nitrogen or nitrogen-oxygen atmosphere. In the latter cases, large amounts of glycolic acid accumulated inside the cells. C^{14}-glycolic acid excreted into the supernatant was continually being reabsorbed and metabolized. For this work, quantitative methods of paper chromatographic analysis have been developed for glycolic acid, which is fairly volatile in usual procedures used in paper chromatography, by developing the papers between sheets of glass, followed by spraying the paper with alkali.

The Effect of Ultraviolet and γ Radiation on the Loss of Cellular Constituents of *Chlorella*

L. P. Zill D. A. Mondon
N. E. Tolbert P. C. Kerr

The loss of cellular constituents by organisms in aquatic cultures after exposure to ionizing and nonionizing radiation is known. However, investigation of these materials has been limited by the availability of methods for their detection and measurement. Thus far, the separation technique of paper chromatography has not been applied to the determination of the number of compounds lost and their possible identification. The loss of these compounds becomes important when radiation effect on intracellular processes is being studied. *Chlorella* cells, fully labeled with P^{32} or C^{14}, have been prepared and used for this study. Figures 41 and 42 present data on the gross losses of phosphorylated and C^{14}-labeled compounds after ultraviolet and gamma irradiation. Paper chromatography of the phosphorylated compounds lost after gamma-radiation indicates that the primary compound affected is orthophosphate with only small amounts of organophosphates present. However, with ultraviolet irradiation, some fifteen organophosphates are found in the culture fluid. Identification of certain of these compounds may become possible upon completion of the chromatographic investigation of the C^{14} compounds, since procedures for the identification of the C^{14}-labeled compounds are better established. After long exposures to ultraviolet (180 min) over half of the labeled constituents (C^{14} compounds) have been lost from the cells. The loss of compounds with short exposures is significant and must be considered in any interpretation of intracellular changes.

A Manometric Actinometer for the Ultraviolet Spectrum

L. P. Zill D. A. Mondon

The previous report on this project[4] described an actinometer which depended on the manometric

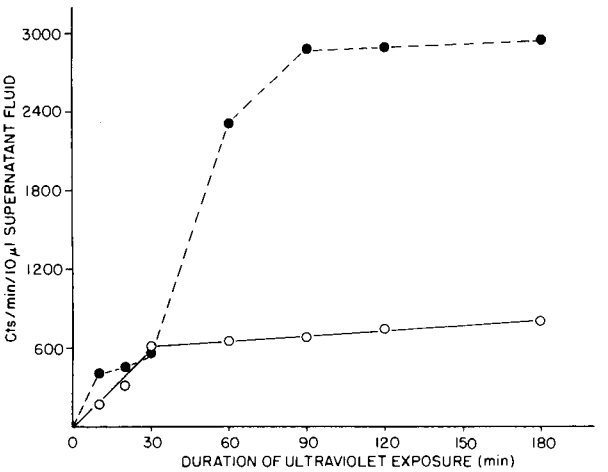


Fig. 41. Loss of cellular constituents (expressed as activity in supernatant fluid) with increasing exposure to ultraviolet radiation. A G.E. 15-watt germicidal lamp (2537 A) was used at a distance of 6 mm from the 1-inch-diameter quartz exposure vessel. ●, C^{14} compounds lost; O, P^{32} compounds lost.

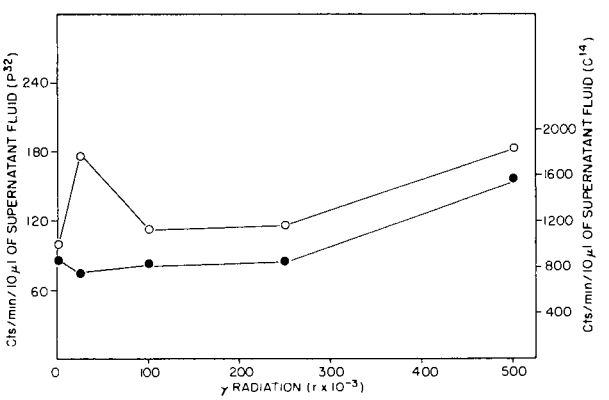


Fig. 42. Loss of cellular constituents (expressed as activity in supernatant fluid) with increasing exposure to gamma radiation. O, phosphate compounds (left scale); ●, C^{14} compounds (right scale).

measurement of evolved gases as a direct and accurate indication of the amount of incident ultraviolet light. With a constant ratio of uranyl sulfate to oxalic acid (1:5), it was found that increases in the absolute concentrations of these materials led to increased gas production until a threshold was reached. This was attributed to increased absorption of light. However, spectrophotometric measurement showed that all the concentrations investigated exhibited infinite absorption of the incident light. The increased gas production has now been found to be caused by the more subtle changes which occurred in the rates of the two reactions which produce the CO and CO_2. It has further been found that this increased gas production parallels the decrease in pH owing to the absolute concentrations of the uranyl sulfate and oxalic acid. Figure 43 illustrates this information and also the increased production of CO as evidence for a rate change in one or both of the reactions being measured. Although the gas production varies with changes in the concentrations of the reactants, it is constant and reproducible for any given concentration. The original concentrations employed by Leighton and Forbes have therefore been chosen for routine use since quantum efficiencies were determined with them. Close correlation between observed gas production and that calculated from permanganate titration of the actinometer fluid

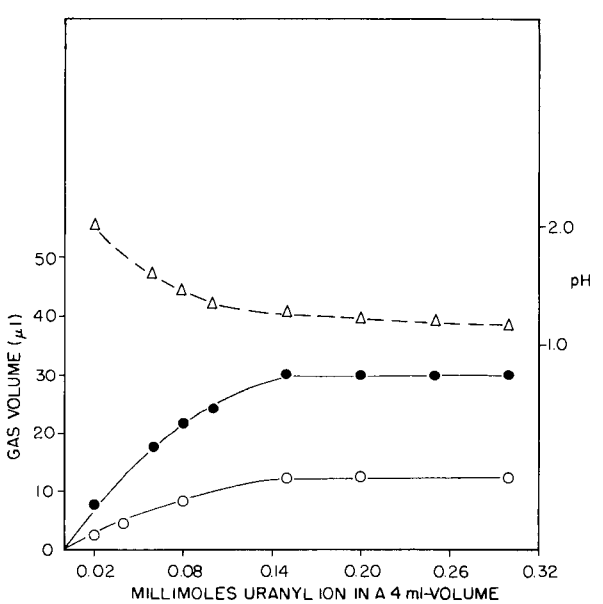


Fig. 43. Variation of total gas production (●), CO (O), and pH (Δ) with the absolute concentration of uranyl sulfate and oxalic acid. The relative concentration of uranyl ion with respect to oxalate ion is 1:5 for all samples. Light intensity and geometry of exposure are the same for all points indicated.

can be obtained by use of the proper ratio of CO to CO_2 for any given concentration. With an actinometer solution 0.01 M in uranyl sulfate and 0.05 M in oxalic acid ("standard solution" of Leighton and Forbes) it is possible to calculate a standard curve[4] relating the gas production to the light quanta or calories. This curve is given in Fig. 44.

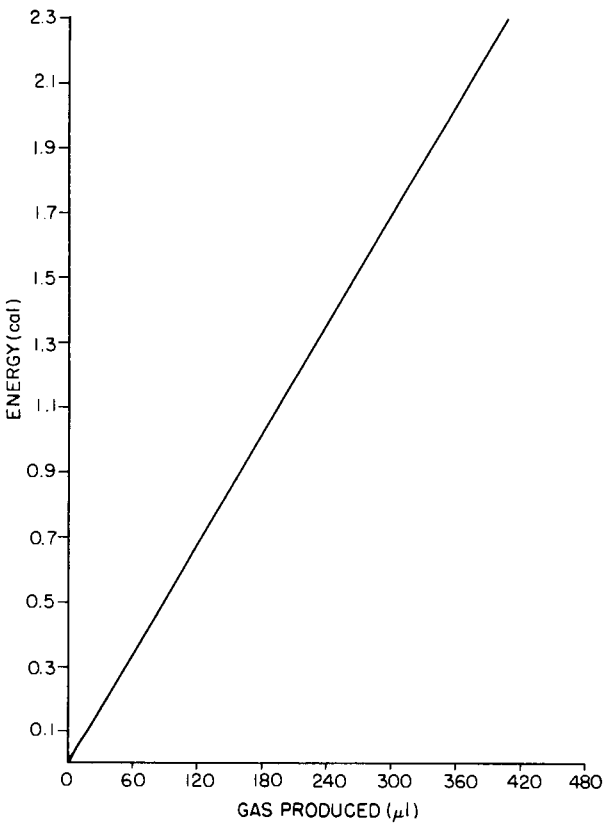


Fig. 44. Energy in calories equivalent to total gas production of standard actinometer fluid.

This report concludes this project. Full experimental details will be presented in a paper being prepared for publication.

Effects of Ionizing and Nonionizing Radiations for Photosynthesis in Plants

L. P. Zill D. A. Mondon
N. E. Tolbert P. C. Kerr

The manifestations of radiation damage in the living cell are diverse and often indirectly related to the primary site of action. In view of the lack of knowledge concerning the primary site it is necessary to study indirect effects in the hope that this will lead to knowledge concerning the primary effect of radiation. This project is concerned with the effect of radiation on photosynthesis as a process which can reflect radiation damage either to the system directly or to the formation of the enzymes needed to catalyze the process. Photosynthetic rates, as measured by the fixation of $C^{14}O_2$ have been used as indicators of the overall effect on the photosynthetic cycle. To determine the radiation sensitivity of normal photosynthesis, experiments were carried out with green Thatcher wheat. With 10-min exposure to ultraviolet (at the particular geometry used) or 100,000 r of gamma radiation there was 70-80% reduction in photosynthetic rates. Increased exposures up to 30 min of ultraviolet or 500,000 r of gamma rays produced no further significant effect on the CO_2 fixation rate. Paper chromatographic investigation of the distribution of C^{14} in the photosynthetic products formed directly after radiation showed a significant increase in glycine and serine and a decrease in sucrose.

After exposure to 100,000 r of gamma radiation, plants were permitted to sit in the light for increasing periods of time. Their ability to incorporate $C^{14}O_2$ photosynthetically recovered almost completely in 24 hr from the 80% inhibition immediately after irradiation. Glycine and serine decreased significantly below normal at 5 hr after irradiation. Interpretation of the changes in glycine and serine are not as yet possible, since there may be alternative explanations for their variations.

Changes in Phosphate Distribution in Barley Associated With Inhibition of Photosynthesis by Carbon Monoxide

A. R. Krall

It has been reported[4] that CO inhibited CO_2 fixation, and that this inhibition was reversed rapidly by yellow light and more slowly by red light. The specificity of the reversal for yellow light was taken as an indication that cytochrome oxidase was involved in at least the recovery from the inhibition.

It has now been possible to show that ATP showed an increase in content of P^{32} label before any other phosphorylated intermediate when the plant was recovering from the CO inhibition. This

recovery of ATP was most pronounced with yellow light (589 mμ), which will cause specific dissociation of a cytochrome oxidase-carbon monoxide complex. The recovery of ATP was less in red light (640 mμ) even though the red light is known to be far more effective in promoting photosynthesis under normal conditions.

The experiments on ATP recovery were run in higher CO concentrations than those used previously and the experiments were terminated before all the plant phosphates had equilibrated with the P^{32}-labeled inorganic phosphate. Table 51 shows a comparison of the percentage of P^{32} found in the various fractions separable by the ion-exchange method[5] from alcohol extracts of labeled barley under normal photosynthetic conditions and under photosynthesis in CO-CO_2 mixtures in red and in yellow light. It is seen that about one-half the phosphate in a normally photosynthesizing leaf is esterified whereas only 5% is esterified in the inhibited leaf in red light. About three times as high a percentage of the P^{32} taken up is esterified in the leaf exposed to yellow light. About ten times as high a percentage of the label was found in ATP in the yellow as the red light, which shows that the levels of label in all the other esters follow rather than precede that of ATP. It may be concluded from these experiments that CO does inhibit steady-state photosynthesis, and that this inhibition is removed by yellow light. White light is no more effective than yellow light in removing this inhibition under the conditions used here, a result to be expected if the action spectrum of the reversal showed a peak in the yellow as does reversal of the CO inhibition of cytochrome oxidase.

These results strongly implicate cytochrome oxidase as a participant in the normal steady-state photosynthetic machinery. Since cytochrome oxidase is not a part of the chloroplast,[6] this is further indication that phosphate bond energy is generated outside the chloroplast and that this spatial separation from the chloroplast may be the reason for the many peculiar effects known as the "induction phase" of photosynthesis.

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

[6] J. H. McClendon, *Am. J. Botany* 40, 26 (1953).

TABLE 51. EFFECT OF RED AND YELLOW LIGHT ON THE DISTRIBUTION OF P^{32} IN PLANT PHOSPHATES

Eluting agent number	Major component	Experimental conditions		
		Air, 2000 ft-c of white light	99% CO-1% CO_2, 1000 ft-c of red light	99% CO-1% CO_2, 1000 ft-c of yellow light
		Percentage of P^{32} eluted		
1	Unknown	1.4	0.12	
2	Inorganic	50.4	94.3	84.2
3	G-6-P	4.3	1.46	3.53
4	F-6-P	2.2	0.26	1.07
5	Unknown	5.6	0.92	0.95
6	AMP	1.3	0.32	
7	PGA	20.4	1.32	3.8
8	HDP	8.5	1.18	4.9
9	ATP	5.5	0.13	1.52
Total P^{32} (cts/min $\times 10^{-3}$)		2459	2008	663

The ends of eight cut barley leaves weighing 0.92 g were placed in 2 ml of water containing 95 μc of P^{32} in each experiment. The twoinhibition experiments were flushed, in series, with the same gas for 1 hr 15 min in light after 15 min flush in darkness. Eluting agents are those of Khym and Cohn.[5]

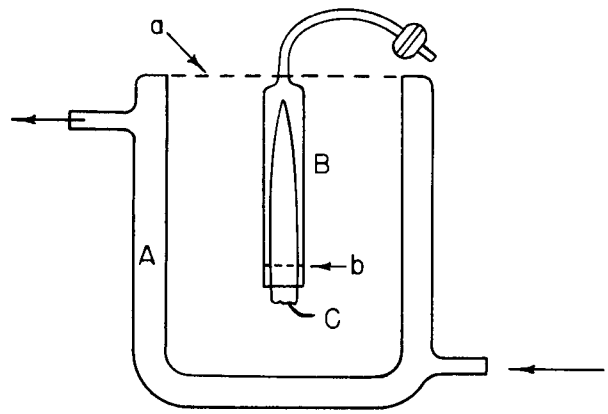
Light Catalyzed Fixation of Carbon Monoxide by Barley Leaves

A. R. Krall N. E. Tolbert

Carbon monoxide is slowly oxidized to CO_2 in animal tissue. Warburg has proposed that it is hydrated by carbonic anhydrase to yield formic acid. Other work from this laboratory has outlined the main pathways of formate metabolism in plants. Thus it may be possible to decide from the nature of products formed whether CO enters the plant metabolism as formate, as CO_2, or as neither of these precursors.

Carbon monoxide labeled with 25% C^{14} was synthesized from CO_2 by the zinc reduction method[7] adapted to 2 ml total volume. The gas was washed with 6 N alkali for a short time to assure freedom from CO_2 and then forced into a narrow glass tube which was inverted over water and in which the leaf was placed (Fig. 45). Neither CO_2 nor oxygen was present in this gas mixture. The leaf was illuminated at various intensities and kept at a constant temperature by the water bath. At the end of the uptake period the leaf was pulled from the bottom of the tube through the water, and quickly immersed in boiling 30% ethanol. Compounds labeled with C^{14} were separated by paper chromatography and autoradiography. The percentages of C^{14} fixed into each compound are reported in Table 52. The total fixation of $C^{14}O$ per hour per leaf is reported (Table 53) as determined by counting aliquots of the plant extract. The significant variations are the increased rates found on illumination compared

[7] R. B. Bernstein and T. I. Taylor, *Science* 106, 498 (1947).



A – COOLING BATH

B – EXPOSURE TUBE ($1\frac{1}{2}$ ml vol)

C – CUT BARLEY LEAF

a – WATER LEVEL IN BATH

b – WATER LEVEL INSIDE TUBE

Fig. 45. Carbon monoxide fixation chamber. A, cooling bath; B, exposure tube ($1\frac{1}{2}$-ml vol.); C, cut barley leaf; a, water level in bath; b, water level inside tube.

TABLE 52. DISTRIBUTION OF C^{14} IN PRODUCTS OF $C^{14}O$ UPTAKE BY BARLEY LEAVES

Products	Experimental conditions						
	Dark, 12°C, 120 min (%)	20 ft-c, 12°C, 27 min (%)	600 ft-c, 12°C, 25 min (%)	2000 ft-c, 12°C, 80 min (%)	10,000 ft-c, 12°C, 39 min (%)	2000 ft-c, 28°C, 25 min (%)	2000 ft-c, 12°C, 45 min[a] (%)
Serine	Trace	42	47	41	34	34	10
Glycine		3	8	3	4	3	21
Sucrose	Trace	3	17	7	17	17	38
Glyceric acid		3	Trace	3	2	11	Trace
Alanine		10	Trace	3	11	5	Trace
Phosphate area	Trace	37	29	29	24	13	29
Other		3	15	15	7	11	2

[a] This leaf was infiltrated with 1.5×10^{-4} M DPN before the experiment and then allowed to exist until it regained normal appearance.

TABLE 53. EFFECT OF LIGHT, TEMPERATURE, AND 2,4-DINITROPHENOL ON RATE OF $C^{14}O$ UPTAKE

Experimental conditions		Length of exposure (min)	Rate[a] of uptake [(cts/sec)/hr]	Total C^{14}[b] per chromatogram (%)
Light (ft-c)	Temperature (°C)			
Dark	12	120	16	
20	12	27	405	3.8
600	12	25	470	14.8
2,000	12	80	470	14.8
10,000	12	39	390	8.2
2,000	28	25	995	11.9
2,000 (with DNP)	12	45	485	4.2

[a]Determined by counting an aliquot of the leaf extract.

[b]Total activity counted on paper chromatogram with a G-M tube. No attempt was made to place equal size aliquots on each chromatogram.

with that rate found in darkness and the increased rate found at the higher temperature. Infiltration with DNP, which uncouples oxidative phosphorylation, did not affect the rate of $C^{14}O$ fixation but did shift the C^{14} among the products.

Serine contained the highest percentage of the fixed C^{14} in all experiments except in the DNP-inhibited leaf. Glyceric acid contains only a small portion of the label in experiments run at 12°C, but a substantial portion in the experiment run at 28°C. Sucrose contained appreciable label in all but the shortest time and lowest light-intensity experiments. DNP shifted the labeling pattern toward sucrose and increased the amount of label in glycine. This shift in labeling caused by DNP would indicate a participation of phosphorylation in serine formation, which was inhibited.

The data indicate that CO was fixed by barley leaves in the light, but not in the dark, at a rate which was temperature dependent. Serine was the predominant product in the normal leaf indicating that $C^{14}O$ was being first converted to HCOOH or H_2CO and that this conversion and subsequent fixation were dependent on light energy from the photosynthetic process.

In control experiments with $C^{14}O_2$, sucrose was the only compound which accumulated label when fixation proceeded as slowly as in the $C^{14}O$ experiments. Thus the distribution of labeled observed with $C^{14}O$ cannot be explained by an assumption of oxidation of CO to CO_2 and then photosynthetic CO_2 fixation. However it has not been determined whether the CO is first hydrated to formic acid or reduced to formaldehyde. Both of these latter compounds are precursors for the β carbon of serine, which then would be converted to sucrose through pyruvate. Degradation of serine and sucrose to determine the distribution of the C^{14} within these products will be done to test the validity of this hypothesis.

PHYSIOLOGY

GENERAL PHYSIOLOGY

C. W. Sheppard
N. G. Anderson
M. L. Anderson
P. J. Mathias
E. C. Horn[1]
M. E. House[2]

High-Resolution Gradient Differential Centrifugation

N. G. Anderson M. L. Anderson

The technique of high-resolution gradient differential centrifugation[3] has been developed into a precision analytical technique for the quantitative separation of nuclei, mitochondria, microsomes, and soluble constituents of tissue homogenates. The discreteness of the separation obtained with one centrifugation is indicated in Fig. 46.

[1]Research Participant.

Fig. 46. Separation of brei constituents achieved in one centrifugation.

For accurate work it is necessary to employ sector-shaped centrifuge tubes since 25% of the sedimentable particles hit the sides before reaching the bottom in a parallel-walled centrifuge tube where the distance from the axis of rotation to the bottom of the tube is twice the distance from the axis to the meniscus. Modified sector tubes with a useful volume of 65 ml have been blown in suitably machined graphite molds. The tubes and supporting accessories are shown in Fig. 47. Particles at various levels in the tube are recovered by introducing a dense fluid to the bottom of the tube through a stainless steel needle and removing the gradient fluid as it rises to the upper constriction. These tubes are especially useful for isolating large particles such as nuclei. For work at higher rational speeds (up to 3200 rpm), a second design without the constricted top has been developed. The gradient is sectioned by raising it into a sliding device such as is shown in Fig. 48. Sections of any volume up to 5 ml may be obtained.

[2]Technical assistant supported by research grant (RG-4203) from the National Institutes of Health, U. S. Public Health Service.

[3]For brief description, see *Biol. Semiann. Prog. Rep. Feb. 15, 1954*, ORNL-1693, pp. 68-69.

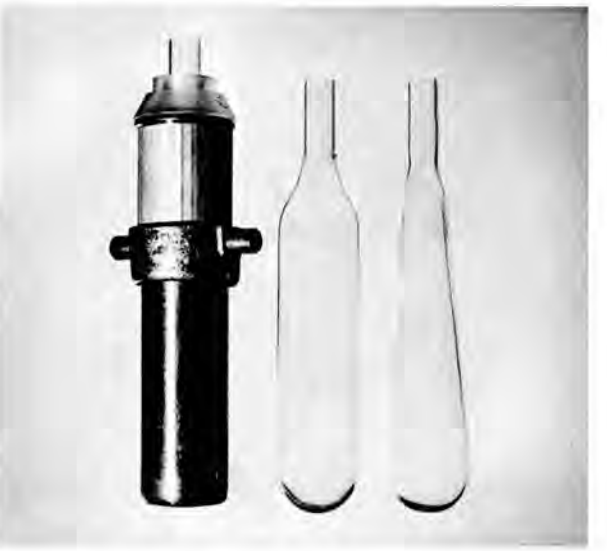


Fig. 47. Type A sector tubes and supporting accessories.

Fig. 48. Gradient sectioning device for use with Type B sector tubes.

Methods for accelerating and decelerating the centrifuge have been improved by the use of a printing chronograph to record the time at which speeds which are multiples of 100 rpm are reached. Plots of controlled acceleration and deceleration curves are shown in Fig. 49, where the line width indicates the variation seen. The thin line on the deceleration graph indicates the deceleration observed when the centrifuge is switched off at 3000 rpm (uncontrolled deceleration). By integration of the area under the curves the equivalent centrifugation at the plateau speed is obtained, and the entire centrifugation may then be expressed in terms of equivalent time at that one speed.

An improved mechanical gradient engine has been constructed which delivers smooth reproducible gradients (Fig. 50). Mixing of the two solutions of which the gradient is composed is accomplished in a 1.2-ml chamber with a magnetic mixer. The shape of the concentration versus delivery curve may be varied by changing the radii of the piston driving arms.

The distribution of acid-soluble nucleotides in rat liver has been examined. Preliminary data suggest that a considerable fraction of these compounds is desorbed from the mitochondria as they sediment out of the soluble layer. This finding has been checked by studying the distribution of acid-soluble nucleotides between particulate and nonparticulate fractions in homogenates containing 20, 10, 5, and 2½% liver. These were separated by high-speed centrifugation without the use of gradient techniques. After 3 hrs' centrifugation at 26,000 × *g*, 30% more acid-soluble 2600 A absorbing material per milligram of tissue was obtained in the 2½% brei supernatant than fluid in the supernatant fluid from the 20% brei. This confirms the observation that nucleotides are easily lost from particulates during dilution.

A Test of Specific Uptake from Organ Homogenates by Homologous Organs in the Young Mouse

E. C. Horn M. E. House

Specific responses (weight increase, increased mitotic rate) of embryonic organs to homologous organ transplants have been demonstrated.[4] With the use of tagged transplanted material, it has been shown that an increase in weight under such circumstances can best be interpreted as the incorporation from the grafts of tissue-specific proteins or of protein constituents larger than amino acids.[5]

One of the obvious shortcomings of embryonic tissues as experimental material is that it is difficult, and at times impossible, to gather amounts of material sufficient for qualitative and quantitative analysis. This project was begun with the primary objectives of (1) confirming the tissue-specific transfer of materials in experimental animals large enough to allow for the fractionation of tissues and (2) possibly characterizing more critically the nature of the materials involved in this transfer to the host organs.

The removal of particulates from the peritoneal cavity to the lymphatics through stomata in the lining seroendothelial membrane has been conclusively demonstrated in a wide variety of mammals, including the mouse.[6] Studies on the

[4] P. Weiss, *Yale J. Biol. Med.* **19**, 235-278 (1947).

[5] J. D. Ebert, *Proc. Natl. Acad. Sci. U. S.* **40**, 337-347 (1954).

[6] L. Allen and E. Vogt, *Am. J. Physiol.* **119**, 776-782 (1937).

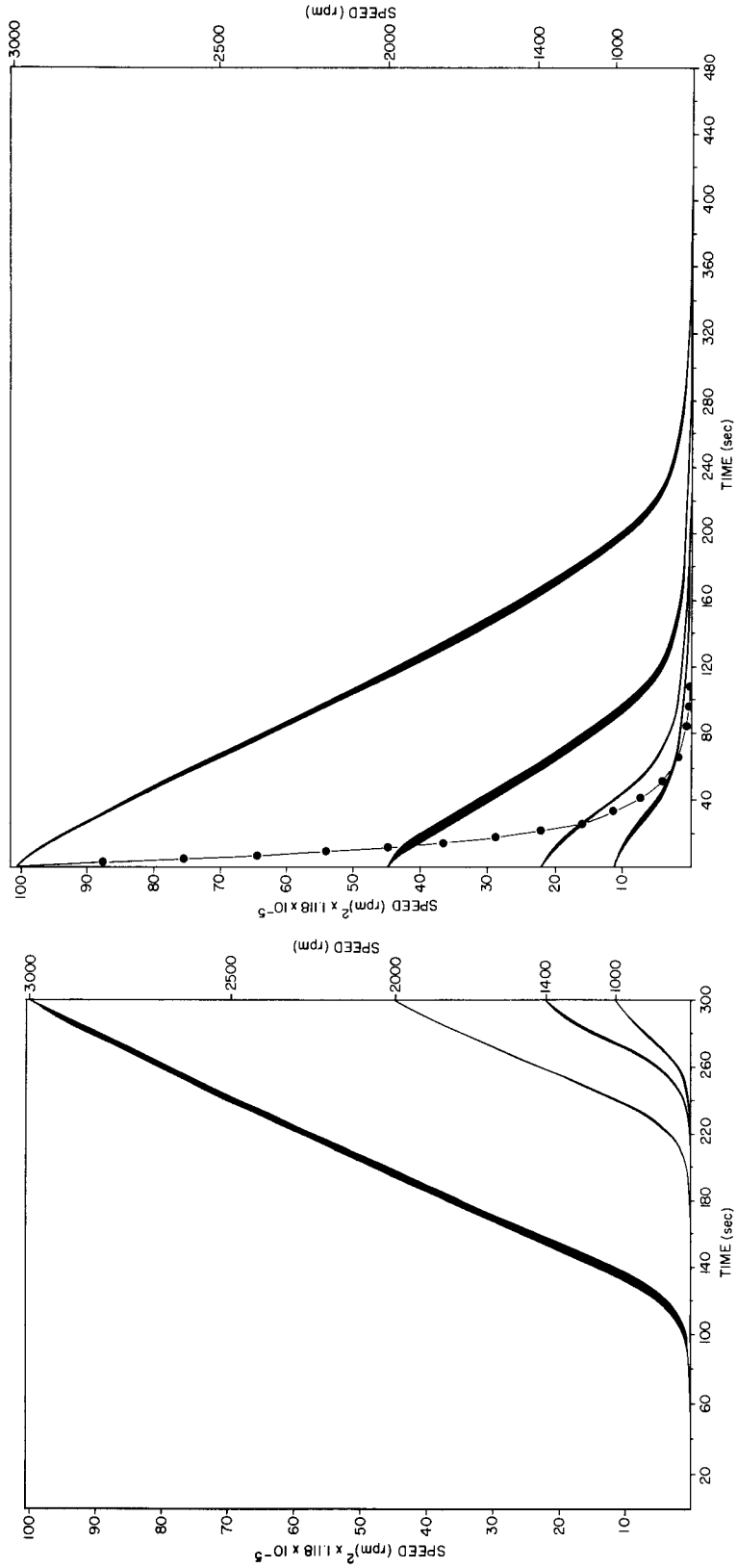


Fig. 49. Acceleration and deceleration curves.

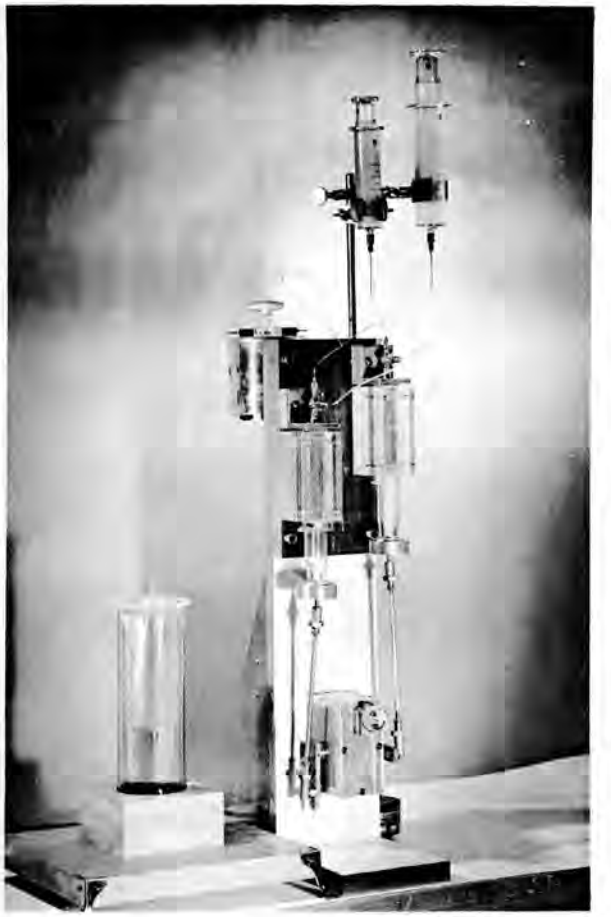


Fig. 50. Gradient engine.

uptake of intraperitoneally injected tagged plasma and tagged homologous erythrocytes from the peritoneal cavity have revealed rapid transfer to the circulation of sizeable quantities in 8-12 hr both in normal[7] and in ascitic dogs.[8] From these experiments it was concluded that sufficient quantities of tagged materials from homogenates of mouse organs would pass to the general circulation for demonstration of a specific incorporation, if such existed, to be achieved in this period of time by this method.

Radioactive carbon was incorporated into plant proteins[9] (either oat seedlings or *Chlorella*) which were in turn intubated as a suspension into fasted adult female mice (C_3HRI) over a period of <12 hours. Approximately 12 hr after the last intubation these adults were sacrificed, their livers, kidneys, spleens, and thymuses pooled, weighed, and homogenized in a known volume of iced 0.14 M NaCl solution. Measured aliquots of the various homogenates were then injected intraperitoneally into young (6-week-old) mice of the same strain and sex as the donor. Other measured aliquots were plancheted for counting. In most experiments the young mice were sacrificed after 6-12 hr and their various organs were pooled, weighed, and homogenized; measured amounts of these homogenates were plancheted for counting. Dry weights, in addition to the calculated wet weights, were obtained for the samples plancheted.

With one donor tissue (liver) crude fractionation at 0°C of the homogenate into whole cells and nuclei, mitochondria and microsomes, and supernate was accomplished. The injection of these three fractions intraperitoneally into young mice and the later assay of the host organs were carried out as for the whole homogenates.

All counts were made in a methane flow chamber and corrected for background and self-absorption. Activity is expressed in counts per minute per milligram dry weight of tissue.

Table 54 summarizes the data from a typical experiment. Under the conditions of the experiment there is no consistent indication of specific incorporation, by the host organs, of radioactive materials from the injected homologous tissue homogenates. In other experiments in which host mice were sacrificed 24 hr after injection of donor tissue homogenates, activities of recovered host tissue homogenates were too low for the accumulation of dependable data in spleen and thymus.

Noteworthy in these data are the indications of consistently high uptake by the host spleen when activity is expressed as counts per unit dry weight of tissue. Such results might be thought to substantiate the common notion that the spleen, per milligram of tissue is a more effective organ than the liver for removing foreign bodies and foreign protein.[10] Actually, data from different sources are conflicting on this point. After intraperitoneal injection, accumulation of colloidal silver is essentially the same in the mouse liver as in the

[7] C. R. Snead, *Arch. Surg.* **56**, 249 (1948).

[8] F. W. McKee, W. G. Wilt, R. E. Hyatt, and G. H. Whipple, *J. Exptl. Med.* **91**, 115 (1950).

[9] Grateful acknowledgement is made of indebtedness to Drs. N. E. Tolbert and A. R. Krall for supplying the incorporated material.

[10] F. M. Burnet and F. Fenner, *The Production of Antibodies*, 2nd ed., MacMillan, Melbourne, 1949.

TABLE 54. ACTIVITY OF HOST ORGANS ACCORDING TO INJECTED DONOR MATERIAL[a]

Host organ assayed	Type of host by injected material						
	Kidney	Liver	Spleen	Thymus	Liver: whole cells and nuclei	Liver: mitochondria and microsomes	Liver supernate
Kidney	13.76	10.42	4.92	1.29	12.27	8.65	3.42
Liver	9.67	10.00	3.33	2.34	12.59	11.42	4.66
Spleen	13.72	16.11	8.78	5.27	14.81	21.61	6.24
Thymus	5.67	7.89	2.03	1.31	7.14	32.96	4.23

[a]Activity expressed as counts per minute per milligram (dry weight) of tissue homogenate assayed.

spleen, on a unit dry weight basis.[11] Colloidal gold[12] and trypan blue,[13] administered intravenously to mice, is accumulated in the first 12 hr more effectively by the liver than by the spleen on a dry weight basis. Finally, the mouse liver on a dry weight basis has been shown to take up in a 24-hr period twice as much injected tagged tobacco mosaic virus as the spleen, although the same experiment in the rabbit showed the spleen to be the more effective organ.[14]

The Effect of Nucleoprotein on Ascites-Produced Mortality in the Mouse

E. C. Horn M. E. House

Evidence is accumulating which points to specific differences in the nuclei of different tissues of the same organism. Embryonic amphibian nuclei from different presumptive areas of the gastrula have been shown to lose progressively their early ability to participate in normal differentiation within oocyte cytoplasm.[15] Subtle chemical differences have been described between the nuclei of ox liver and thymus and between those of rat liver and rat hepatoma.[16] It is quite conceivable that the one finding is related causally to the other. To investigate this possibility, Ehrlich ascites tumor cells have been exposed *in vivo* to various substances extracted from their nuclei.

Relatively large quantities (ca 100 ml) of ascites tumor suspension have been harvested from tumor-bearing mice, washed free of ascites fluid with saline, and their nuclei isolated by blending in 1% citric acid.[17] After extensive washing, the nuclei are extracted with neutralized distilled water to yield a highly viscous preparation of nucleoprotein. Histone has also been extracted from such nuclear preparations with dilute acid. Such extractions have been lyophilized after dialysis and isoelectric precipitations to give an easily water-soluble residue with low nucleic acid content.

The viscous nucleoprotein (NP) preparations have been injected intraperitoneally into mice, with no further treatment. The histone has been dissolved in distilled water as needed. All preparations were made and kept prior to injection at temperatures close to 0°C.

Table 55 shows the results as daily survivors of three groups of mice, one of which received 0.2 ml of ascites suspension, another 0.5 ml of a NP preparation, and the other which received 0.2 ml of the same ascites suspension followed 4 days later with 0.5 ml of NP. No significant change in the death rate of the treated animals versus the controls is evident.

[11]J. C. Gammill, B. Wheeler, E. L. Carothers, and P. F. Hahn, *Proc. Soc. Exptl. Biol. Med.* 74, 691-695 (1950).

[12]L. Wish, J. Furth, C. W. Sheppara, and R. H. Storey, *Am. J. Roentgenol. Radium Therapy* 67, 628-640 (1952).

[13]C. D. Stevens, A. Lee, P. H. Stewart, P. M. Quinlin, and P. R. Gilson, *Cancer Research* 9, 139-143 (1949).

[14]R. L. Libby and C. R. Madison, *J. Immunol.* 55, 15-26 (1947).

[15]R. Britts and T. J. King, *J. Exptl. Zool.* 122, 485-506 (1953); *Anat. Record* 120, 723-724 (1954) Abst.

[16]H. J. Cruft, C. M. Mauritzen, and E. Stedman, *Nature* 174, 580-585 (1954).

[17]L. Goldberg, E. Klein, and G. Klein, *Exptl. Cell Research* 1, 543-570 (1950).

When the twelve animals which had received the single 0.5 ml NP injection alone were challenged with an ascites suspension 35 days after the first injection, the results were strikingly different as shown in Table 56.

Table 57 summarizes the results when the time interval between the single intraperitoneal NP injection and the ascites challenge is varied. From these mortalities it is evident that, during the first 2 weeks after the NP injection, an ascites challenge results in deaths at a rate comparable to that seen in the controls. Roughly 2 weeks later, the NP injected animals respond quite differently to an ascites challenge, dying significantly earlier than the controls. At 5 weeks after NP injection the results are essentially the same as those at 4 weeks. Two and one-half months after NP injection, the treated animals show a lower mean survival than the controls but the difference is poorly marked.

Results from experiments which combine varied doses of NP with varied time intervals to challenge are not fully tabulated but preliminary analysis shows a dose dependency.

The injection of other substances has been assayed in a manner less thorough than but comparable to that presented for NP. Mice injected with histone from chicken erythrocytes and protamine (commercial) do not show a decrease in mean survival when challenged with ascites suspensions. Histone from ascites cells is presently being tested more thoroughly because preliminary experiments suggest that it may be effective in lowering mean survival.

With the demonstration of antigenicity of nucleoprotein and of deoxyribonucleic acid from calf thymus,[18] the interpretation of the main results of these experiments is presently considered to rest on the unequivocal demonstration of an immune reaction in mice injected with nucleoprotein.

A preliminary report of this work has been submitted for publication.[19]

Biological Acetylation Studies

P. J. Mathias C. W. Sheppard

For the determination of acetylcholine-synthesizing ability of tissue preparation in experiments reported by us elsewhere,[20] acetylation of choline

[18] U. Blix, C. N. Iland, and M. Stacey, *Brit. J. Exptl. Pathol.* **35**, 241-251 (1954).

[19] E. C. Horn, *Biochim. et Biophys. Acta* (in press).

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

TABLE 55. SURVIVAL OF MICE INJECTED WITH ASCITES SUSPENSION FOLLOWED BY NUCLEOPROTEIN CHALLENGE 4 DAYS LATER

Injection	No. of mice	Days →	No. of survivors in indicated no. of days after ascites injection								
			4	5	6	7	8	9	10	11	12
Ascites control	10		9	9	8	7	7	7	7	4	3
NP control	12		12	12	12	12	12	12	12	12	12
Ascites + NP 4 days later	12		12	12	11	11	11	11	10	7	4

TABLE 56. SURVIVAL OF MICE INJECTED WITH NUCLEOPROTEIN FROM ASCITES TUMOR CELLS AND CHALLENGED WITH ASCITES SUSPENSION 35 DAYS LATER

Injection	No. of mice	Days →	No. of survivors in indicated no. of days after ascites injection								
			4	5	6	7	8	9	10	11	12
Ascites control	11		11	11	11	11	11	8	7	7	7
NP + ascites 35 days later	12		12	3	0						

TABLE 57. SURVIVAL OF MICE INJECTED WITH NUCLEOPROTEIN FROM ASCITES TUMOR CELLS AND CHALLENGED WITH ASCITES SUSPENSIONS AT VARIOUS INTERVALS

Injection	Interval (days)	No. of mice	Days →	No. of survivors in indicated no. of days after ascites injection[a]															
				4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Control	13	12		12	12	12	12	11	11	9	8	7	Terminated					
Experimental		12		12	10	10	10	10	9	8	8	5	Terminated					
Control	25	12		12	12	11	11	9	5	5	5	3	3	3	2	2	1	0
Experimental		12		12	7	4	4	2	1	1	1	0						
Control	34	12		12	12	12	12	11	10	7	7	Terminated						
Experimental		12		12	7	7	7	5	5	4	2	Terminated						
Control	78	10		10	10	9	9	9	9	8	8	8	8	8	8	7	2	0
Experimental		12		12	11	11	11	11	10	8	8	8	8	6	4	1	0	

[a]These experiments were conducted with more dilute suspensions of ascites cells, hence the increase in mean survival time when compared with those of the previous tables.

by rat brain preparations was used as a control system. Systems utilizing both acetate and citrate were found to yield more consistent results than systems utilizing either one of these substrates. The results of further experiments reported here may offer some explanation for this observation. Balfour and Hebb[21] have reported that both acetate- and citrate-utilizing systems exist in crude brain preparations and that the requirements of the two systems differ in some respects. Our result was not inconsistent with their report, if our improved yields might be the result of combined syntheses through two different mechanisms. However, the high cysteine requirement reported by them for one system and the strict anaerobiosis and/or high cysteine requirement reported by others[22] was apparently not in agreement with our finding that synthesis in systems containing added acetate, citrate, and highly purified coenzyme A (CoA) occurred equally well in the presence or absence of cysteine, either aerobically or anaerobically. Further investigation of some of the properties of the system seemed to be of interest.

With the exception of the two irradiation experiments, the results reported here were obtained from experiments performed on acetone-dried powder from a single preparation of rat brain. Different preparations have been variable in their behavior, but different extracts from this one preparation were consistent.

Water-clear enzyme solutions were prepared from homogenates of acetone-dried powder, subjected to high-speed centrifugation, and used without further purification. Acetylations were carried out as previously reported with minor modifications.

In Table 58 the rates of acetylcholine synthesis in micrograms of acetylcholine (ACh) per gram of rat brain powder per hour resulting from the addition of citrate, acetate, CoA, and cysteine are compared with the rate observed in the "standard" acetate + citrate + CoA system. The following may be seen from the table: The lack of a cysteine effect in the "standard" system, enhanced synthesis in the presence of cysteine in the citrate and/or acetate and the acetate + CoA systems, reduced synthesis, in the presence of cysteine in the CoA system, and synthesis

[21]W. E. Balfour and C. Hebb, *J. Physiol.* 118, 94-106 (1952).

[22]W. Feldberg, *Methods in Medical Research*, Vol. 3, Year Book Publishers, Chicago, 1950, pp. 95-106.

TABLE 58. ACETYLCHOLINE SYNTHESIS BY RAT BRAIN EXTRACTS. EFFECT OF ACETATE, CITRATE, AND CoA WITH AND WITHOUT CYSTEINE

(Modified system/"standard" system)

	Synthesis (μg of ACh/g of rat brain powder)/hr	
	Without cysteine	With cysteine
Acetate	<380	525/1584
Citrate	<380	1012/1584
CoA	1500/1584	1200/1584
Acetate + citrate	695/1584	1233/1584
Acetate + CoA	1695/1584	1920/1584
Citrate + CoA	616/1584	616/1584
Acetate + citrate + CoA	1584	1584
None of above additions	<380	437/1584

unaffected by cysteine in the CoA + citrate system.

It seems possible from these results that the cysteine-insensitive acetate + citrate + CoA synthesis may represent the net synthesis by cysteine potentiated, cysteine-insensitive, and cysteine-inhibited mechanisms for acetylcholine synthesis in the crude brain preparation.

Recent interest in the possible effect of irradiation on CoA had previously encouraged us to determine the effect of X irradiation on the enzymic acetylation of choline by rat brain, a system which is highly dependent on the activity of CoA. Results of these early experiments showed that CoA activity in this system was unaffected by as much as 19 kr and that there was no demonstrable effect on the activity of the system as a whole. These results were reported in the November 1953 Biology Division Bimonthly Report. In view of the more recent results it seemed possible that the irradiation effect on acetylcholine synthesis by brain preparations, might exist in one of the systems other than the CoA + acetate + citrate system which was used.

Synthetic activity of an irradiated and a control enzyme solution was tested in the cysteine-sensitive citrate system. Acetylcholine synthesis by the control samples incubated with and without cysteine exceeded synthesis in the irradiated samples. Cysteine added to the irradiated enzyme solution partially restored its activity.

A similar effect of cysteine has been observed in frozen, stored enzyme preparations, i.e., the CoA + acetate + citrate system was unaffected by storage with cysteine; synthesis by the acetate + CoA and the acetate + citrate systems was partially protected by storage with cysteine and was partially restored by cysteine addition immediately before incubation.

BIOPHYSICS

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

MOLECULAR BIOPHYSICS

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

***Tradescantia* Studies.** Several aspects of the radiation response of *Tradescantia* pollen not previously studied but fundamental to many more elaborate radiobiological studies of this material have been investigated. These include the determination of the temperature coefficients and time/intensity relation for X-ray-induced aberrations as well as a search for photorecovery processes following ultraviolet treatment of growing pollen tubes. This work essentially completes the characterization of what may be called basic constants of the radiation response of pollen. A small although definite temperature effect has been found for X-ray-treated dry pollen in the range 10-30°C. This temperature dependence for aberration production is considerably less than that determined for microspores and reported by other investigators. No time/intensity effects have been observed in growing pollen tube cultures exposed to X rays. This result is in agreement with our previous results for dry pollen. Similar negative results have been found for photorecovery after ultraviolet treatment of growing pollen tube cultures.

Magnetic Resonance. Resonances in lyophilized plant tissues have been observed verifying the results recently reported by Commoner *et al.*[1] Further study of these preparations is in progress. A continual search is being made to observe resonances caused by free radicals in a living biological system. Several chemical systems, in particular those showing luminescence, are also being examined.

Amphibian Metamorphosis and Radiobiology

J. S. Kirby-Smith D. L. Craig
J. N. Dent[2]

Successful induction of metamorphosis in the neotinic cave salamander *Gyrinophilus palleucus* has been obtained in a cooperative experiment. Two specimens of this species have undergone metamorphosis after being immersed in water containing 1 part in 500,000 of crystalline thyroxin. A third specimen metamorphosed within 3 months when injected three times per week with 0.05 USP units of thyroid-stimulating hormone.

Cytological studies on the tail tips of *G. palleucus* and allied species have been carried out and determinations of chromosome number and chromosome morphology have been made. Irradiation of the regenerating tail tips of these animals has resulted in chromatid, isochromatid, and exchange aberrations morphologically identical to those produced in plant tissues. The sensitivity of this material to X radiation is relatively high, 100 r resulting in an appreciable aberration rate. Preliminary experiments with a small number of animals also indicate that these cells have a large mitotic inhibition after treatment with 100 r. The number of divisions found drops off rapidly with almost no dividing cells seen 16-18 hr following irradiation. Further work on the material designed to investigate its suitability for more elaborate radiobiological studies is in progress.

RADIOLOGICAL PHYSICS

E. B. Darden, Jr. M. L. Randolph

Cockcroft-Walton Fast Neutron Facility. The physics of the fast neutron facility has been advanced toward more precise dosimetry and higher yields under better defined conditions. The intercomparison of dose measurements of the fast neutrons produced by the *d-d* reactor in the Cockcroft-Walton accelerator has been found in agreement to within less than 10% for the two methods of choice: (1) the homogeneous polyethylene-ethylene ion chamber calibrated versus radium and (2) the dose calculation from the neutron flux as measured by counting the companion protons with a proportional counter. The installation of a superior type of ion source[3] has resulted in much higher total beam currents and a beam of

[1] B. Commoner, J. Townsend, and G. E. Pake, *Nature* 174, 689-691 (1954).

[2] Research Participant, General Physiology group (summer 1954).

[3] C. D. Moak, H. Reese, Jr., and W. M. Good, *Nucleonics* 9 (3), 18-23 (1951).

more than 500 μa through a $\frac{1}{4}$-inch aperture to the target. Thus more than 10^8 neutrons/sec have been produced by drive-in on a well-defined spot. The focusing characteristics at 200 kv have been studied systematically. In order to minimize scattering and energy degradation at the target, air-cooled targets are being tested; the results are encouraging and a low scatter target assembly has been put into use. The relations between thin target *d-d* yields, for which precise data are available in the literature, and thick target yields, which we employ to obtain maximum yield, have been studied analytically. Preliminary measurements with lead-lined ion chamber and with photographic film indicate that the gamma-ray dose near the target is certainly much less than 10% of the total dose.

The instrumentation is now essentially complete and understood, and final data for completion of the physical characterization of the facility are being obtained. Efforts to increase the total fast neutron yield by means of zirconium deuteride targets are under way. Some radiobiological experiments with sensitive biological materials could be carried out with currently available neutron yields, but it is felt that the wiser course is to wait until the higher fluxes expected in the near future with improved target conditions are achieved before large-scale biological studies are started.

Gamma-Ray Studies

E. B. Darden, Jr.

Collaboration with the Entomology Research Branch, Agricultural Research Service, U. S. Department of Agriculture, on radiological problems has been continued. The recent, apparently successful, eradication of the screwworm fly on the island of Curacao has been a direct result of this cooperation, and was executed by means of a cobalt source designed, calibrated, and tested by personnel of the Radiological Physics group and the Physiology Section.

The Co^{60} gamma-ray facility of the Biology Division has been partially recalibrated. The new values average 3-4% higher than predicted by decay from the original calibration almost 4 years ago. This difference suggests that the half life of Co^{60} is greater than the previously employed value of 5.3.