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SEMIANNUAL PROGRESS REPORT

FOR PERIOD ENDING AUGUST 15, 1953

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BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

for Period Ending August 15, 1953

Alexander Hollaender, Director

E. J. Slaughter, Technical Editor

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BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications. Results of research in the Biology Division for the period have appeared in 67 open literature publications. Of these, 39 are full-length papers, one a book-length symposium, and 27 are abstracts. Abstracts are not listed here but appear under the title of the lecture in the section "Scientific Society Lectures and Traveling Seminars." In addition to these publications, two project reports have appeared as follows:

C. W. Sheppard and E. B. Darden, *Physical Dosimetry in Typical Biological Experiments with Fast Neutrons from a Cyclotron Source*, ORNL-1559 (1953).

Alexander Hollaender, Director, *Biology Division Quarterly Progress Report for Period Ending Feb. 10, 1953*, ORNL-1497.

AUTHOR(s)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	The mass isolation of whole cells from rat liver	*Science* 117, 627-628 (1953)
_____	On the nuclear envelope	*Science* 117, 517-521 (1953)
_____	Studies on isolated cell components. V. The effects of various solutions on the nuclear envelope of the isolated rat liver nucleus	*Exptl. Cell Research* 4, 306-315 (1953)
Baker, W. K.	Permanent slides of salivary and ganglion chromosomes	*Drosophila Information Service (Cold Spring Harbor)*, No. 26, p. 129, Nov. 1952
_____	V-type position effects of a gene in *Drosophila* virilis normally located in heterochromatin	*Genetics* 38, 328-344 (1953)
Baker, W. K. and E. S. Von Halle	The basis of the oxygen effect on X-irradiated *Drosophila* sperm	*Proc. Natl. Acad. Sci. U.S.* 39, 152-161 (1953)
Billen, D., B. L. Strehler, G. E. Stapleton, and E. Brigham	Postirradiation loss of adenosine-triphosphate from *E. coli* B/r	*Arch. Biochem. and Biophys.* 43, 1-10 (1953)
Brumfield, Robert T.	The effect of ultraviolet irradiation on cell division and elongation in Timothy roots	*Proc. Natl. Acad. Sci. U.S.* 39, 366-371 (1953)
Carlson, J. Gordon, Nyra G. Harrington, and Mary Esther Gaulden	Mitotic effects of prolonged irradiation with low-intensity gamma rays on the *Chortophaga* neuroblast	*Biol. Bull.* 104, 313-322 (1953)
Cohn, Waldo E. and Elliot Volkin	On the structure of ribonucleic acids. I. Degradation with snake venom diesterase and the isolation of pyrimidine diphosphates	*J. Biol. Chem.* 203, 319-332 (1953)
Conger, Alan D.	The effect of boron enrichment on slow neutron-irradiated tissues	*Genetics* 38, 128-133 (1953)

AUTHOR(s)	TITLE OF ARTICLE	PUBLICATION
Delwiche, Eugene A. and S. F. Carson	A citric acid cycle in *Propionibacterium pentosaceum*	*J. Bacteriol.* 65, 318-321 (1953)
Doherty, David G.	The synthesis of glyconyl peptides	*J. Biol. Chem.* 201, 857-866 (1953)
Furth, Jacob	Conditioned and autonomous neoplasms	*Cancer Research* 13, 477-492 (1953)
Furth, J., W. T. Burnett, Jr., and E. L. Gadsden	Quantitative relationship between thyroid function and growth of pituitary tumors secreting TSH	*Cancer Research* 13, 298-307 (1953)
Gaulden, Mary Esther, Marjorie Nix, and Jack Moshman	Effects of oxygen concentration on X-ray-induced mitotic inhibition in living *Chortophaga* neuroblasts	*J. Cellular Comp. Physiol.* 41, 451-470 (1953)
Giles, Norman H., Frederick J. de Serres, and Alvin V. Beatty	The effect of radiation dose fractionation and chromosome frequencies. II. Studies with fast neutrons	*Genetics* 38, 416-420 (1953)
Gude, W. D.	Modified Martins-Mallory stain for mouse pituitary gland	*Stain Technol.* 28, 161-162 (1953)
Hollaender, Alexander (ed.)	Symposium on some aspects of microbial metabolism	*J. Cellular Comp. Physiol.* 41, Suppl. 1 (March 1953)
Hollaender, Alexander and G. E. Stapleton	Fundamental aspects of radiation protection from a microbiological point of view	*Physiol. Revs.* 33, 77-84 (1953)
Khym, J. X. and Waldo E. Cohn	The separation of sugar phosphates by ion exchange with the use of the borate complex	*J. Am. Chem. Soc.* 75, 1153 (1953)
Khym, J. X., D. G. Doherty, E. Volkin, and W. E. Cohn	The identification of the isomeric adenylic acids *a* and *b* as the 2′ and 3′ adenosine phosphates, respectively (Comm. to ed.)	*J. Am. Chem. Soc.* 75, 1262 (1953)
Kirby-Smith, J. S. and D. S. Daniels	The relative effects of X rays, gamma rays, and beta rays on chromosomal breakage in *Tradescantia*	*Genetics* 38, 375-388 (1953)
Kimball, R. F.	Materials and methods in the study of protozoa (Book Review)	*Quart. Rev. Biol.* 28, 75 (1953)
———	The structure of the macronucleus of *Paramecium aurelia*	*Proc. Natl. Acad. Sci. U.S.* 39, 345-347 (1953)
Kimball, R. F. and Nenita Gaither	Influence of oxygen upon gentic and nongenetic effects of ionizing radiation on *Paramecium aurelia*	*Proc. Soc. Exptl. Biol. Med.* 82, 471-477 (1953)
Morse, M. L., A. W. Burke, Jr., and W. T. Burnett, Jr.	The adsorption of oxygen from buffer solutions by cells of *Escherichia coli*, B/r	*J. Cellular Comp. Physiol.* 41, 407-417 (1953)
Noggle, G. R.	Paper chromatography of some ketoheptoses	*Arch. Biochem. and Biophys.* 43, 238-239 (1953)

AUTHOR(s)	TITLE OF ARTICLE	PUBLICATION
Pomper, Seymour	Methionine-requiring mutants of *Saccharomyces cerevisiae*	*J. Bacteriol.* 65, 666-670 (1953)
Pomper, Seymour and D. W. McKee	Mutation of mating type in *Saccharomyces cerevisiae*	*Science* 117, 455-456 (1953)
Schwartz, Drew	Evidence for sister-strand crossing over in maize	*Genetics* 38, 251-260 (1953)
Schwartz, Drew	The behavior of an X-ray-induced ring chromosome in maize	*Am. Naturalist* LXXXVII, 19-28 (1953)
Sheppard, C. W., R. R. Overman, W. S. Wilde, and Ward Sangren	The disappearance of K^{42} from the nonuniformly mixed circulation pool in dogs	*Circulation Research* 1, 284-297 (1953)
Stapleton, G. E., Daniel Billen, and Alexander Hollaender	Recovery of X-irradiated bacteria at suboptimal incubation temperature	*J. Cellular Comp. Physiol.* 41, 345-358 (1953)
Strehler, B. L.	Firefly luminescence in the study of energy transfer mechanisms. II. Adenosinetriphosphate and photosynthesis	*Arch. Biochem. and Biophys.* 43, 67-79 (1953)
Strehler, B. L.	Luminescence in cell-free extracts of luminous bacteria and its activation by DPN (Comm. to ed.)	*J. Am. Chem. Soc.* 75, 1264 (1953)
Taylor, J. Herbert	Autoradiographic detection of incorporation of P^{32} into chromosomes during mitosis and meiosis	*Exptl. Cell Research* 4, 164-173 (1953)
Wheeler, H. E.	Detection of microbial toxins by the use of radioisotopes	*Phytopathology* 43, 236-238 (1953)
Wilde, W. S., J. Ginsburg, and W. G. Walker	The distribution kinetics of intravenous potassium K^{42}	*The Journal-Lancet* 73, 168-169 (1953)
Woods, M. C., Frances Gamble, J. Furth, and R. R. Bigelow	Control of the postirradiation hemorrhagic state by platelet transfusions	*Blood* VIII, 545-553 (1953)
Zill, L. P., J. X. Khym, and G. M. Cheniae	Further studies on the separation of the borate complexes of sugars and related compounds by ion-exchange chromatography	*J. Am. Chem. Soc.* 75, 1339-1342 (1953)

Scientific Society Lectures and Traveling Seminars. Many talks on subjects under investigation in the Biology Division have been given before scientific society meetings and on the Traveling Seminar program. Speakers and their subjects are listed.

Amy, R. L.	Am. Assoc. Anatomists, Columbus, Ohio	A comparative study of the effects of beta, gamma, and X rays on the early development of *Habrobracon* [Abstr., *Anat. Record* 115, 273 (1953)]
Anderson, N. G.	Emory University, Georgia	Studies on the structure of the isolated liver cell nucleus and its envelope

———	University of North Carolina, Chapel Hill	As above
———	Medical College of the State of South Carolina, Charleston	As above
———	School of Medicine, University of Mississippi, Oxford	As above
———	Dept. of Physiology, Tulane University School of Medicine, New Orleans, La.	As above
———	Zoology Dept. University of Tennessee, Knoxville	Studies on isolated nuclei
——— (Tech. Asst., M. L. Anderson)	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago	Constancy of polymerization of DNA in various rat organs [Abstr., *Federation Proc.* **12**, 5 (1953)]
Arnold, W. A.	University of North Carolina, Chapel Hill	Problems in photosynthesis
———	Duke University School of Medicine, Durham, N.C.	As above
———	St. Augustine College, Raleigh, N.C.	As above
———	Zoology Department, University of Texas, Austin	Problems connected with photosynthesis
———	Biology Department, Baylor University, Waco, Texas	As above
———	Texas A & M, Bryan	As above
Atwood, K. C. and F. Mukai	Radiation Research Society, Iowa City, Iowa	Homology patterns of X-ray-induced lethal mutations in *Neurospora*
Baker, W. K.	Department of Zoology, University of Texas, Austin	1. The relationship between heterochromatin and variegation in *Drosophila* 2. The role of oxygen in modifying the effects of X rays on genetic material
Billen, Daniel	Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, L.I.	The effects of X rays on bacteria and the influence of environmental conditions on these effects
Billen, Daniel and Elliot Volkin	Radiation Research Society, Iowa City, Iowa	Observations on several changes in the physiological and cytological character of *Escherichia coli* following X-ray exposure
Billen, Daniel, B. L. Strehler, and G. E. Stapleton	Assoc. S.E. Biologists, Chapel Hill, N.C.	Postirradiation release of adenosinetriphosphate from *Escherichia coli* B/r [Abstr., *J. Tenn. Acad. Sci.* **XXVIII**, 176 (1953)]
Bowen, G. H.	Cold Spring Harbor Symposia, Quant. Biol., Cold Spring Harbor, N.Y.	Studies of ultraviolet irradiation phenomena — an approach to the problems of bacteriophage reproduction

Brumfield, R. T.	Assoc. S. E. Biologists, Chapel Hill, N. C.	Curvatures induced in Timothy roots by ultraviolet radiation [Abstr., *J. Tenn. Acad. Sci.* **XXVIII**, 177 (1953)]
Burnett, W. T., Jr.	Tuskegee Institute, Ala.	Tracer studies in mineral metabolism
Carlson, J. G., N. G. Harrington, and M. E. Gaulden	Assoc. S. E. Biologists, Chapel Hill, N. C.	Effects of prolonged treatment with low-intensity gamma radiation on mitotic activity in *Chortophaga* neuroblast [Abstr., *J. Tenn. Acad. Sci.* **XXVIII**, 178 (1953)]
Carson, S. F.	East Tenn. Sect., The Am. Chem. Soc., University of Tennessee, Knoxville	Enzymatic and chemical methods of degradation of C^{14}-labeled compounds (Abstr., *Branch Chain*, Feb. 1953)
Cohn, W. E.	Department of Biochemistry, Vanderbilt University, Nashville, Tenn.	The separation of biochemically important substances by ion-exchange chromatography
———	Ohio State University, Columbus	1. As above 2. The chemistry of ribonucleic acids
———	Institute of Cancer Research, Philadelphia, Pa.	Recent developments concerning the chemistry of nucleic acids
———	Gordon Research Conference on Ion Exchange, New Hampton, N. H.	Biochemical and medical applications (in, "Symposium on Water Conditioning")
Delwiche, E. A., E. F. Phares, and S. F. Carson	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago, Ill.	Succinate decarboxylation reaction in *Propionibacterium* [Abstr., *Federation Proc.* 12, 194 (1953)]
Dent, J. N. and E. L. Hunt	Am. Assoc. Anatomists, Columbus, Ohio	Iodine uptake and release in larvae of *Hyla versicolor* [Abstr., *Anat. Record* **115**, 299 (1953)]
Doermann, A. H.	Biology Department, University of Rochester, Rochester, N. Y.	Genetic recombination in bacteriophage
———	Biology Department, Vanderbilt University, Nashville, Tenn.	Genetics of bacteriophage
———	Department of Biology, University of Georgia, Athens	As above
———	Cold Spring Harbor Symposia, Quant. Biol., Cold Spring Harbor, N. Y.	The vegetative state in the life cycle of bacteriophage: Evidence for the occurrence, and its genetic characterization
———	University of Michigan Summer Biological Station, Pellston, Mich.	Present views on the genetic organization of the replicating unit of bacteriophage
———	4th Summer Biological Symposium on Radiation Biology, University of Michigan, Ann Arbor	Contribution of radiation experiments to an understanding of bacteriophage (two lectures)
Doherty, D. G.	Am. Chem. Soc., S. E. Section, Miami, Fla.	The hydrolysis of glyconyl peptide derivatives by α-chymotrypsin

_____	Vanderbilt University, Nashville, Tenn.	The synthesis and enzymatic susceptibility of aldonic acid-amino acid derivatives (glyconyl peptides)
_____	Department of Chemistry and Carver Foundation, Tuskegee Institute, Tuskegee, Ala.	Thermodynamic properties of enzyme-substrate complex formation
_____	Department of Chemistry, Florida State University, Tallahassee	As above
_____	University of Florida, Gainesville	As above
_____	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago, Ill.	The synthesis of DL-, D-, and L-glyceryl amino acid derivatives [Abstr., *Federation Proc.* 12, 197-198 (1953)]
Dolin, M. I.	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago, Ill.	Lipoic acid function: α-dicarbonyl oxidation by *Streptococcus faecalis* [Abstr., *Federation Proc.* 12, 198 (1953)]
Dolin, M. I., L. P. Hager, and I. C. Gunsalus	Am. Chem. Soc. 123rd National Meeting, Los Angeles, Calif.	Enzyme systems for the heterolytic cleavage of dicarbonyl compounds (*Abstr. of Papers, 123rd Natl. Meet.*, p. 26C, 1953)
Furth, Jacob	University of Florida, Gainesville	Conditioned and autonomous growth
_____	University of Miami, Miami, Fla.	Carcinogenesis by ionizing radiations in man and animals
_____	Duke University School of Medicine, Durham, N.C.	Conditioned and autonomous growth
_____	Children's Cancer Research Foundation, Boston, Mass.	Monomorphous monochormonal experimental pituitary tumors
Furth, J., W. T. Burnett, Jr. and E. L. Gadsden	7th International Congress of Radiology, Copenhagen, Denmark	Pathogenesis and character of pituitary tumors induced by radioiodine
Furth, J., W. T. Burnett, Jr. and E. L. Gadsden	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago, Ill.	Quantitative relationship between growth of pituitary tumors secreting TSH and thyroid function. TSH bioassay in mice [Abstr., *Federation Proc.* 12, 387-388 (1953)]
Furth, J. and A. C. Upton	7th International Congress of Radiology, Copenhagen, Denmark	Leukemogenesis by ionizing radiation
Gadsden, E. L.	Assoc. S.E. Biologists, Chapel Hill, N.C.	The effect of thyroid hormone on the growth of transplantable pituitary tumors [Abstr., *J. Tenn. Acad. Sci.* XXVIII, 180 (1953)]
Gaulden, M. E.	Department of Zoology, Knoxville College, Knoxville Tenn.	Effects of radiation and chemicals on mitosis and cell structures as observed in living cells

Giles, N. H., Jr., F. J. de Serres, and A. V. Beatty	Radiation Research Society, Iowa City, Iowa	The effect of radiation dose fractionation on chromosome aberration frequencies in *Tradescantia* microspores. II. Studies with fast neutrons
Hollaender, Alexander and G. E. Stapleton	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago, Ill.	New aspects of the oxygen-concentration effect in X-ray inactivation of bacterial suspensions [Abstr., *Federation Proc.* 12, 70 (1953)]
Hollaender, Alexander, G. E. Stapleton, and D. Billen	National Academy of Sciences, Washington, D.C.	The reduction of radiation damage to living cells by treatment after exposure [Abstr., *Science* 17, 468 (1953)]
Hollaender, Alexander	7th International Congress of Radiology, Copenhagen, Denmark	Modification of radiosensitivity by chemical agents
_____	Spring Hill College, Mobile, Ala.	Studies on the mechanisms of the biological effects of radiation
_____	School of Medicine, Louisiana State University, New Orleans	Delayed effects of radiation
_____	Loyola University, New Orleans, La.	Factors modifying sensitivity of living cells to radiation
_____	Department of Physiology, Tulane University, New Orleans, La.	Studies on the mechanisms of the biological effects of radiation
_____	University of Arkansas School of Medicine, Little Rock	As above
_____	Department of Zoology, University of Oklahoma, Norman	As above
_____	Department of Zoology, University of Texas, Austin	Factors modifying sensitivity of living cells
_____	Emory University, Georgia	Studies on the mechanisms of biological effects of radiation
_____	Sigma Xi, University of Virginia, Charlottesville	As above
_____	Samuel Miller Biological Laboratories, University of Virginia, Charlottesville	As above
Hunt, E. L.	Am. Assoc. Anatomists, Columbus, Ohio	Radioiodine uptake duing the development of the chick embryo [Abstr., *Anat. Record* 115, 325-326 (1953)]
Khym, J. X., W. E. Cohn, E. Volkin, and D. G. Doherty	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago, Ill.	The ribose phosphates derived from the isomeric adenylic acids *a* and *b* [Abstr., *Federation Proc.* 12, 229 (1953)]
Kimball, R. F.	Emory University, Georgia	The role of peroxide in the biological effects of X rays on *Paramecium aurelia*

———	Agnes Scott College, Decatur, Ga.	As above
———	Department of Radiotherapeutics, Hammersmith Hospital, London, England	The role of oxygen and peroxide in radiation injury to *Paramecium*
———	Assoc. S. E. Biologists, Chapel Hill, N. C.	The influence of oxygen on X-ray damage to *Paramecium aurelia* [Abstr., *J. Tenn. Acad. Sci.* XXVIII, 182 (1953)]
O'Brien, J. M. and W. S. Wilde	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago, Ill.	The effluographic determination of K^{42} release as related to the action potential of heart muscle [Abstr., *Federation Proc.* 12, 105 (1953)]
Odell, T. T., F. Gamble, and J. Furth	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago, Ill.	The life span of naturally labeled platelets of rats [Abstr., *Federation Proc.* 12, 398-399 (1953)]
Russell, Liane B.	Biology Department, Knoxville College, Knoxville, Tenn.	Radiation hazards in pregnancy
St. Amand, G. and S. R. Tipton	Assoc. S. E. Biologists, Chapel Hill, N. C.	The separation of free neuroblasts and other embryonic cells from grasshopper embryos [Abstr., *J. Tenn. Acad. Sci.* XXVIII, 188 (1953)]
Sheppard, C. W.	National Research Council Symposium on Structure and Cellular Dynamics of the Red Cell, Washington, D. C.	Mechanisms of ion transfer
Stapleton, G. E.	University of Louisville, Louisville, Ky.	Modification of the bactericidal effects of ionizing radiation by postirradiation treatments
———	Soc. Am. Bacteriologists (Gen. Bacteriol. Section), San Francisco, Calif.	Factors affecting the sensitivity of microorganisms to ionizing radiation
Stapleton, G. E. and C. W. Edington	Radiation Research Society, Iowa City, Iowa	Temperature dependence of bacterial inactivation by X rays
Strehler, B. L.	Tuskegee Institute, Tuskegee, Ala.	Photosynthesis – ATP changes and energetics
———	Oceanographic Institute, Florida State University, Talahassee	As above
———	Cancer Research Laboratory, University of Florida, Gainesville	As above
Tipton, S. R. and G. St. Amand	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago, Ill.	The respiratory metabolism of grasshopper embryos (*Chortophaga viridifasciata*) [Abstr., *Federation Proc.* 12, 144 (1953)]
Totter, J. R.	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago, Ill.	The utilization of formate in thymine formation by bone marrow cell suspensions [Abstr., *Federation Proc.* 12, 281 (1953)]

_____	Radiation Research Society, Iowa City, Iowa	Influence of radiation and of aminopterin on the metabolism of formate by rabbit bone marrow *in vitro*
_____	Southeastern Division, University of Texas School of Medicine, Dallas	1. Some studies on the effects of radiation on biological systems 2. Bone marrow metabolism 3. The metabolism of formate
_____	UT–AEC Agriculture Research Program, Oak Ridge, Tenn.	The relationship of folic acid to xanthine oxidase
Tolbert, N. E.	North Carolina Academy of Science, Durham, N.C.	The use of carbon-14 and other isotopes in plant biochemistry
Upton, Arthur C.	University of Tennessee Medical School, Memphis	Effects of ionizing irradiation on mammalian tissues
_____	University of Mississippi Medical School, Oxford	As above
Upton, A. C. and W. D. Gude	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago, Ill.	Changes of dermal connective tissues after total-body irradiation [Abstr., *Federation Proc.* 12, 405 (1953)]
Upton, A. C. and J. Furth	Am. Assoc. Cancer Research, Chicago, Ill.	Inhibition by cortisone of the development of spontaneous leukemia and its induction by radiation [Abstr., *Proc. Am. Assoc. Cancer Research* 1, 57 (1953)]
Upton, A. C., J. Furth, and K. W. Christenberry	Radiation Research Society, Iowa City, Iowa	Late effects in mice of exposure to thermal neutrons compared with X irradiation

Visiting Lecturers. The Biology Division has been privileged to hear seminars given by the following prominent scientists.

Dr. J. H. McClendon	Botany Department, University of Minnesota, Minneapolis	Biochemistry group seminar
Dr. Howard A. Schneiderman	Biology Laboratory, Harvard University, Cambridge	An experimental analysis of the effects of carbon monoxide, carbon dioxide, and oxygen on radiosensitivity
Dr. Ruth McClung Jones	Department of Biology, Winthrop College, Rock Hill, South Carolina	Mammalian Ova
Dr. Gert L. Laqueur	National Institutes of Health, Bethesda, Maryland	Posterior lobe of pituitary hormones and changing concepts of their origin
Dr. C. Levinthal	Department of Physics, University of Michigan, Ann Arbor	Genetics of bacteriophage
Dr. Charles Heidelberger	McArdle Memorial Laboratory for Cancer Research, University of Wisconsin, Madison	Some aspects of nucleic acid biosynthesis *in vivo*
Dr. J. S. Mitchell	Department of Radiotherapeutics, University of Cambridge, England	Laboratory studies and clinical trials of chemical radiosensitizers in radiotherapy

Dr. Jonas S. Friedenwald	Department of Ophthamology, Johns Hopkins University, Baltimore	Studies on diabetic retinopathy
Dr. Clement L. Markert	Department of Zoology, University of Michigan, Ann Arbor	Some aspects of biochemical genetics in *Glomerella*
Dr. T. C. Carter	Institute of Animal Genetics, University of Edinburgh, Scotland	Cytogenetic studies of the mouse, using genetically tagged chromosome translocations
Dr. Cyril L. Smith	University of Cambridge, England	The inactivation of protein monolayers by active radicles
Dr. Hilde Levi	University of Copenhagen, Denmark	Recent work in alpha and beta track autoradiography of biological specimens
Dr. Roger M. Herriott	School of Hygiene and Public Health, Johns Hopkins University, Baltimore	T_2 phage "ghosts"
Dr. L. H. Gray	Radiotherapeutic Unit, Hammersmith Hospital, London, England	Discuss work in the field of radiobiology
Dr. Roy Markham	Molteno Institute, Cambridge, England	Some studies on ribonucleic acids
Dr. Ludwik Gross	Veterans Administration Hospital, Bronx, New York	Experimental mouse leukemia
Dr. Phineas W. Whiting	Zoological Laboratories, University of Pennsylvania, Philadelphia	Radiation genetics in the wasp, *Mormoniella*
Dr. H. S. Roberts	Dept. of Zoology, Duke University, Durham, N.C.	Cinematographic studies of cell division
Dr. Jack M. Siegel	Dept. of Biochemistry, University of Arkansas, Fayetteville	Some reactions medicated by the photosynthetic bacteria
Dr. Malcolm L. Randolph	Dept. of Physics, Tulane University, New Orleans	Resolution of protein molecular weights with the ultracentrifuge
Dr. David M. Morris	Dept. of Biology, North Texas State College, Denton	Some problems of adrenal physiology in the chick
Dr. Edward C. Horn	Dept. of Zoology, Duke University, Durham, N.C.	1. Lamp rays 2. Mouse genetics

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
K. C. Atwood
G. H. Bowen
A. Josephine Bridgman[1]
R. T. Brumfield[2]
A. D. Conger[3]
A. H. Doermann
L. Roberta Lovelace[1]
Drew Schwartz
C. P. Swanson[2]
Sheldon Wolff
Martha C. Chase
Nenita Gaither
Mary Kathryn King
F. H. Mukai
Anita D. Phelps

Dominant Lethals in *Paramecium* and the Effect of X Rays on the Behavior of Nuclei at Conjugation

(Kimball, Gaither, King)

It was pointed out by Kimball[4] that there is little evidence for dominant lethals up to quite high doses of ionizing radiation. The development of stocks differing in three marker genes has made possible a re-examination of this problem. Paramecia of one genotype were irradiated and crossed immediately, or after 10 to 20 fissions, with those of the other. The two members of the conjugant pair were separated after conjugation, a clone was developed from each member, and the source of its nuclei was ascertained.

Normally, at conjugation the two members of the pair fertilize each other so that each produces a hybrid clone. At doses of a few thousand roentgens there is little disturbance in this behavior, but as the dose increases more and more pairs are found in which the unirradiated member fails to get a pronucleus from its irradiated mate. Figure 1 gives a dose curve and shows that oxygen tension during irradiation has little effect on the result.

At all doses, those pairs in which the unirradiated member is a hybrid are, for the most part, normal; and the two clones from the pair grow at the normal rate (Table 1). However, when the unirradiated member fails to receive a pronucleus from its mate, the latter also is likely to be exceptional. Even in this case there are a considerable number of pairs in which the irradiated member is hybrid. This shows that although failing to contribute a pronucleus to its mate, nevertheless, the irradiated member contributed one to itself. In about 30% of these cases, the hybrid clone grew poorly. Since those clones which got their cytoplasm from the irradiated animal but their nucleus entirely from the unirradiated animal grew well, it seems probable that the poor growth in the hybrids is to be attributed to dominant lethal effects in the irradiated component of the hybrid nucleus rather than to cytoplasmic injury. The same amount of disturbance in nuclear behavior occurred when 10 to 20 fissions elapsed between irradiation and conjugation, thus supporting the idea that all the damage is inheritable and probably nuclear.

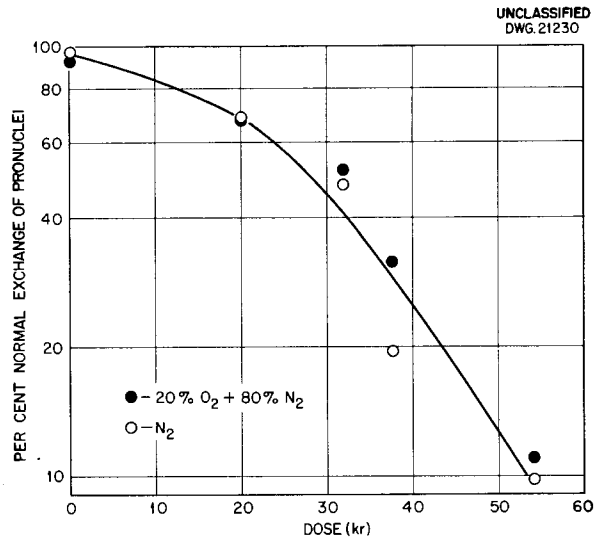

Fig. 1. Relation Between Dose of X Rays and Pronuclear Exchange at Conjugation in *Paramecium aurelia*.

In *Paramecium aurelia*, there are eight products of meiosis formed from the two micronuclei. Normally, only one of these produces the two pronuclei. Thus there is opportunity for nuclear selection. The factors involved in this selection are not known, but it seems quite possible that chromosome aberrations which lead to bridges at anaphase impede the separation of the nuclei containing them and put them at a disadvantage compared with normal nuclei in the same cell. A large portion of the aberrations which would be dominant lethals might be of this kind. Thus, at

(1) Research Participant.
(2) Temporary Employee.
(3) On leave of absence – Fulbright Fellowship.
(4) R. F. Kimball, *Genetics* 34, 412-424 (1949).

TABLE 1. KINDS OF PAIRS AFTER CONJUGATION BETWEEN IRRADIATED AND UNIRRADIATED PARAMECIA

UNIRRADIATED MEMBER			IRRADIATED MEMBER		
Origin of Nuclei	Number of Pairs	Percentage Growing Poorly	Origin of Nuclei	Number of Pairs	Percentage Growing Poorly
Hybrid	417	7	Hybrid	368	12
			Unirradiated	0	
			Irradiated	41	15
			Unknown	8	87
Unirradiated	756	14	Hybrid	282	30
			Unirradiated	66	6
			Irradiated	174	57
			Unknown	234	79
Unknown	43	100	Various	43	63

low doses, segregation products containing dominant lethals would usually be eliminated. At high doses where no normal nuclei are formed, such aberrations might interfere more seriously with the movement of the migratory pronucleus into the unirradiated mate than with the functioning of the stationary pronucleus. Thus dominant lethals would have some opportunity to express themselves in the irradiated member but would have little chance to do so in the unirradiated member.

Role of Oxygen in the Induction of Mutations in *Paramecium*

(Kimball, Gaither, King)

It has been shown[5] that neither H_2O_2 nor irradiated medium in sublethal doses is able to induce detectable amounts of mutation in *Paramecium*. The same result was found when the catalase was partially poisoned with cyanide. These observations have been extended to include H_2O_2 and KCN present during the time of irradiation (Table 2) because Alper[6] has shown that radiation sensitizes bacteriophage to H_2O_2. Table 2 shows no evidence of any effect of H_2O_2 or KCN on the radiation-induced mutation rate.*

Previously, such results might have been attributed to a failure of H_2O_2 to penetrate to the nuclei from outside the cell. However, J. Z. Hearon of the Mathematics Panel has made calculations based upon available data on diffusion, permeability, and catalase activity, and the results show that the average concentration inside a cell of the dimensions of a paramecium must be a very considerable fraction of that outside. Thus it can be concluded that H_2O_2 is not an effective mutagen either by itself or in combination with radiation. This is all the more striking since the concentrations used were more than ten times that to be expected from the X irradiation of pure water with a dose which produces sufficient mutation to cause 50% of the exautogamous clones to be of reduced vigor. Perhaps the most important implication of this finding is that it makes unlikely the belief that any appreciable part of the reduction in the mutagenic effectiveness of X rays by hypoxia is the result of decreased H_2O_2 production.

[5] R. F. Kimball, N. Gaither, and M. K. King, *Biol. Quar. Prog. Rep. Feb. 10, 1952*, ORNL-1244, pp. 18-21.

[6] T. Alper, *Nature* 169, 964 (1952).

*Since this report was written, one experiment has suggested, but not proved, that H_2O_2, when combined with X rays, increases mutation slightly. Even if this is so, the effect is probably too small to account for the action of oxygen; but further work will be required to settle the matter beyond all doubt.

TABLE 2. LACK OF EFFECT OF H_2O_2 AND KCN ON MUTAGENESIS BY X RAYS

DOSE (r)	MATERIAL ADDED	PERCENTAGE OF NORMAL CLONES
None	0.003 M KCl	94.8
	0.003 M KCN	92.8
2800	0.003 M KCl	65.7 ± 2.7
	0.003 M KCN	63.0 ± 2.7
None	0.00018 M H_2O_2	96.6
	None	97.9
2760	0.00018 M H_2O_2	70.4 ± 2.9
	None	71.3 ± 2.8

The above work has made it possible to make unlikely a widely accepted hypothesis but not to replace it. It is always possible to substitute for H_2O_2 other oxygen-dependent active substances such as HO_2 or some organic peroxide which did not react with catalase sufficiently to lead to an appreciable reduction in effect when KCN is present during irradiation. However, the difficulty with the H_2O_2 hypothesis and the work of various members of this Division, which shows that the oxygen effect is quite different for different kinds of genetic effects, suggest that totally different mechanisms must be seriously considered. The one fairly clear point is that only oxygen present during irradiation is important, and so the reaction with oxygen involves some rapid process which must take place either during or very shortly before or after irradiation. This has now been demonstrated for mutations in *Paramecium*, as shown in Table 3.

One other rather surprising finding in *Paramecium* is that the stage of the division cycle at which the irradiation is given does not alter the oxygen effect although the effectiveness of the X rays in producing mutations is very different, as was shown much earlier for beta rays (Table 4). This demonstrates that a great change in sensitivity to radiation can occur without changing the oxygen sensitivity of the process.

Effect of X Radiation on the Genetic Structure of Bacteriophage

(Doermann, Chase)

Previous reports[7,8] dealt with the construction of a genetic map of bacteriophage T4. In continuing these experiments, a new minute (m_{42}) mutant was discovered. The factor responsible for this mutant plaque type is on linkage group II to the left of r_{47}.[7] The characterization of this marker has made possible for the first time crosses involving three linked factors, each of which is distinguishable from the other two. Such a cross was made, and the results locate m_{42} about 13 recombination units to the left of r_{51}. By combining in one stock the appropriate *r* and *tu* markers with m_{42}, crosses are possible which involve three independently segregating markers.

[7] A. H. Doermann and M. B. Hill, *Biol. Quar. Prog. Rep. May 10, 1952*, ORNL-1297, pp. 19-25.

[8] A. H. Doermann and M. B. Hill, *Biol. Quar. Prog. Rep. Nov. 10, 1952*, ORNL-1456, pp. 20-21.

TABLE 3. EFFECT OF OXYGEN DURING IRRADIATION

DURATION AND KIND OF GAS TREATMENT*			PERCENTAGE OF NORMAL CLONES
For 20 min Before Irradiation	For 1 min During Irradiation	For 20 min After Irradiation	
20% O_2	20% O_2	20% O_2	70.5 ± 3.2
N_2	20% O_2	20% O_2	68.5 ± 4.6
N_2	N_2	20% O_2	86.5 ± 3.4

*Ratio of doses for equal effect of $\frac{O_2 \text{ during}}{O_2 \text{ after}} = 2.4$.

TABLE 4. EFFECT OF OXYGEN AND STAGE IN DIVISION CYCLE*

STAGE IRRADIATED	GAS	PERCENTAGE OF NORMAL CLONES	PERCENTAGE EXPECTED IF OXYGEN EFFECT IS THE SAME AT TWO STAGES
Before division	20% O_2	75.8 ± 4.9	77.9
	N_2	87.4 ± 3.7	87.2
After division	20% O_2	40.1 ± 3.7	38.5
	N_2	55.8 ± 4.5	59.1

*Ratios of doses for equal effects, $\frac{O_2}{N_2} = 1.8$; $\frac{\text{after}}{\text{before}} = 3.8$.

The preceding report[9] outlined a plan for the genetic characterization of vegetative phage by X irradiation of infected bacteria. Preliminary to such experiments, it seems necessary to understand the effects of X rays on the genetic structure of the infectious, extracellular phage particle. In these experiments, a nutrient broth suspension of wild-type T4 is irradiated with a dose sufficient to give the desired fraction of survivors as determined by plaque count. Examination of this stock is made by simultaneously infecting a bacterium with a particle from the irradiated stock and one or more unirradiated particles of different genetic constitution. The progeny of this mixed infection is then examined for markers derived from the irradiated parent. These experiments are sufficiently advance to permit certain conclusions:

1. Members of an irradiated suspension, inactive as measured by plaque-forming ability, contribute genetic markers to the progeny of a mixed infection. The efficiency of this contribution decreases with increasing dose of radiation. The shape of the curve of contribution against dose has not yet been determined.

2. The progenies from these mixed infections often contain only two or one of the three markers originally present in the irradiated parent. Among those progenies of mixed infections which contain one or more markers from the irradiated parent, the following relationships are noted. (*a*) When three genetically unlinked markers are studied, it is found that the absence of one from an individual progeny is independent of the absence of the other markers. (*b*) When three linked markers are used, the probability of two being absent simultaneously is correlated with the closeness of their linkage. Thus, m_{42} and r_{51}, which show about 13% recombination, are simultaneously absent from a progeny about half as frequently as either one alone. The markers, r_{51} and tu_{41}, which show about 22% recombination, are simultaneously absent from a progeny only about one-fifth as frequently as either alone. In both cases these frequencies are higher than the product of the frequencies with which the individual markers are absent.

Further experimental analysis of this material is required before a decision can be made between the following interpretations:

1. A single X-ray damage is extensive enough to damage two markers simultaneously.

2. Damages smaller in magnitude than those under (1) above affect two linked markers simultaneously because, as their low recombination value indicates, they are structurally close together and have a low probability of being separated by recombination. They would also show a low probability of becoming separated from any radiation-caused lethal damage occurring near them. Thus the apparent inactivation of two markers with one damage would, in fact, be due only to the failure of separating the damage and the marker by recombination.

It may be pointed out, however, that regardless of its nature, the possibility of producing localized radiation damage within the genetic structure will be of importance in understanding radiation effects at the subcellular level.

[9] A. H. Doermann and Martha Chase, *Biol. Quar. Prog. Rep. Feb. 10, 1953*, ORNL-1497, pp. 13-15.

Adsorption of Bacteriophage to Bacterial Cells

(Bowen)

Studies of the adsorption of bacteriophage particles of type T2r to cells of *Escherichia coli* B have been undertaken as a necessary preliminary to other research on the effects of ultraviolet irradiation. Although the results have not thus far added anything fundamentally new to the information on bacteriophage adsorption already presented in the literature,[10,11] they represent a more complete and accurate set of data than has been available for T2 and they contribute additional evidence to some discussions which have been published.

The experiments have been done by using phage type T2r and *E. coli* B. The bacteria are grown in Difco tryptone broth to a concentration of about 2×10^8 cells/ml, washed, and resuspended in phosphate buffer at the desired concentration. The buffer is about $\frac{1}{14}$ *M* in phosphate, its pH is 6.8, and it contains added salts in amounts chosen to give optimum conditions for adsorption.[12] The composition of the buffer is: KH_2PO_4, 3 g; Na_2HPO_4, 7 g; NaCl, 4 g; $MgSO_4$, 0.2 g; and H_2O, 1000 ml.

An adsorption experiment is performed by mixing phage and bacteria, removing a sample after a measured time interval, rapidly diluting the sample to prevent further adsorption, centrifuging to remove bacterial cells, and assaying the supernatant to determine the number of phage particles remaining unadsorbed to the cells. Low multiplicity of infection is used (less than one particle per ten cells).

The probability per unit time that a phage particle becomes adsorbed is expected to be independent of time and directly proportional to the rate of collision with cells, and hence directly proportional to the bacterial concentration. The fraction, (P/P_0), of particles remaining unadsorbed after time t should be

$$(P/P_0) = e^{-kBt} , \qquad (1)$$

where B is the bacterial concentration. This relation defines a specific rate constant k, which is independent of B.

Experimentally, it has been found that the number of unadsorbed particles does decrease as a very accurate exponential function of time and that a value of k can be determined which is independent of B over a wide range of cell concentrations. The fraction unadsorbed has been followed to a value as low as 10^{-3} without any change being observed in the rate of adsorption, thus demonstrating that the phage preparation is quite homogeneous with respect to adsorbability.

The adsorption rate constant, k, is the product of the probability of collision per unit of cell concentration per unit time and of the probability of permanent attachment per collision. The collision rate is proportional to the diffusion constant for the particles, which depends on the absolute temperature, T, and on the viscosity of the medium, η, according to the Einstein equation

$$D = \left(\frac{k}{6\pi r}\right)\left(\frac{T}{\eta}\right) , \qquad (2)$$

where k is the Boltzmann constant and r the radius of the phage particle. The dependence of the collision rate on temperature can therefore be calculated.

Accurate measurements of the adsorption rate constant of T2 have been carried out in the range 0 to 50°C. Data are presented in Fig. 2. Also shown in Fig. 2 are the same data after correction has been made for change of absolute temperature and change of viscosity with temperature (viscosity assumed equal to that of pure water). It is to be noted that the corrected values approach a constant value at higher temperatures but decrease at lower temperatures. Since these and numerous other experiments have demonstrated that the values are reproducible to within a few per cent, this residual change with temperature, even though small, must be considered as significant. It appears to reflect a change with temperature of the fraction of collisions that leads to attachment. The effect is apparently identical with, though smaller than, that found by Stent and Wollman with T4.[11] Puck did not find such an effect with T1.[13]

By using the measured diffusion constant of T4 and the average cell size, the collision rate per bacterium of T4 has been estimated at about 4.8×10^{-9} cm^3/min at 20°C, or about 7×10^{-9}

(10) M. Delbrück, *J. Gen. Physiol.* 23, 631-642 (1940).

(11) G. S. Stent and E. L. Wollman, *Biochim. et Biophys. Acta* 8, 260-269 (1952).

(12) T. T. Puck, A. Garen, and J. Cline, *J. Exptl. Med.* 93, 65-81 (1951).

(13) A. Garen and T. T. Puck, *J. Exptl. Med.* 94, 177-189 (1951).

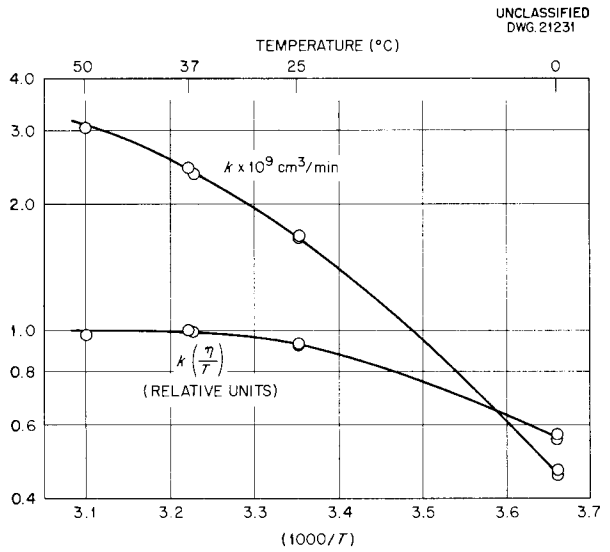

Fig. 2. Adsorption Rate Constant, k, as Function of Absolute Temperature, T.

cm^3/min at 37°C. T2, which is almost identical to T4 in size and shape, should behave similarly. The measured adsorption rate constant for T2 at 37°C is 2.4×10^{-9} cm^3/min, which suggests that about one-third of all collisions lead to attachment. The rather rapid increase with temperature of the fraction of successful collisions toward a limiting value close to the theoretical limit suggests the interplay of more than one reaction, at least one of which changes considerably with temperature. This possibility has been discussed by Stent and Wollman.[11] The present results add significance to their work.

Cyanide present in 0.01 *M* concentration has no effect whatever on the adsorption rate. Bacteria killed by heat (70°C for 15 min) also adsorb phage at the same rate as live cells. These results indicate that the metabolism of the cell plays no part in adsorption, which is in agreement with the views developed by Puck and co-workers.[12,13]

Infrared–X-Ray Studies on Chromatid Aberrations in *Tradescantia*

(Swanson)

It is well established that near-infrared radiation of approximately 10,000 Å is effective in increasing the aberration frequency in both *Drosophila* and *Tradescantia* when used in combination with X rays. How infrared acts on the chromosome to bring about this potentiating action is, however, unknown. In an effort to learn more about how infrared acts, an investigation was made of those factors which may possibly influence the magnitude of its effect.

Effectiveness of Infrared as a Function of Temperature During Infrared Exposure. It has been previously shown[14] that temperature had some effect on the action of infrared. Therefore a study of the effect of infrared, in combination with a given dose of X rays, on chromatid aberrations in *Tradescantia* was carried out, with the infrared given to cells that were maintained at different temperatures during pretreatment exposure. X radiation was given at room temperatures. The results are given in Table 5, and it seems quite clear that the temperature at which a maximum effect is achieved is in the neighborhood of 12°C. At temperatures higher than 20 to 22°C the infrared is no longer effective. What is not so obvious, however, is whether there is a single effect having an optimum at 12°C, or whether the curve obtained from plotting these data is the result of two opposing tendencies, one which increases and the

[14] C. P. Swanson, C. S. Rupert, and H. T. Yost, Jr., *Am. J. Botany* (in press).

TABLE 5. EFFECTIVENESS OF INFRARED–X-RAY COMBINATION TREATMENTS ON CHROMATID ABERRATIONS AS A FUNCTION OF THE TEMPERATURE DURING INFRARED EXPOSURE*

TEMPERATURE (°C)	PERCENTAGE INCREASE IN CHROMATID BREAKS**
2.0	50
7.0	77
12.5	127
15.0	79
15.0	86
19.0	28
21.0	10
23.0	0
26.0	5
30.0	0

*X-ray dose, 50 r.

**Exchanges scored as two breaks.

other which decreases with increasing temperature. The data do not permit a distinction to be made between these two possibilities at the present time.

Effectiveness of Solar Radiation in Inducing an Infrared Effect. Because the quality of solar radiation is such as to suggest that a good infrared effect on chromosomes could be obtained if the temperatures during exposure were lower than 20°C, such an experiment was done. The results are as were expected, namely, that a strong infrared effect on X-irradiated chromosomes was obtained. No attempt was made, however, to compare the laboratory source of infrared (infrared drying lamp) with solar radiation as to relative efficiency in this respect.

Saturation Effects with Infrared. It was assumed, in previous publications dealing with the effects of temperature on infrared-treated chromosomes, that if infrared is present, it induces an activated state of unknown nature in the chromosomes such that a subsequent dose of X rays is more effective in inducing chromosomal aberrations. This interpretation is based on the observations that a temperature shock of 48°C for 30 sec can wipe out a previously induced infrared effect. Also, the same temperature treatment given to chromosomes previously exposed to X rays can prevent a posttreatment of infrared from being effective. It would appear therefore that both infrared and X rays can induce what might provisionally be called "potential breaks" but that these are not realized until both types of radiation have acted upon the chromosomes. It became of interest to determine whether pre- and posttreatments of infrared given to the same chromosomes in conjunction with a single dose of X rays would be effective in increasing breakage. Table 6 indicates the results obtained.

From these data it would appear that a summation effect can be achieved. Precisely how these data are to be interpreted in terms of activated states or "potential breaks" remains uncertain except that they are not in conflict with the assumption that both types of radiation can induce such breaks, which, however, are only realized as visible breaks when the two types of radiation are used in combination.

Relationship Between Infrared and the Wave Length of the Ionizing Radiation. Initial experiments seem to indicate that the softer the ionizing radiation, the more effective is any given dose of infrared in increasing the frequency of aberrations. Thus a comparative study of cobalt gamma radiation, 250-kvp X rays with 4 mm of Cu plus 1 mm of Al filtration, and 100-kvp X rays with 1 mm of Al filtration – at a 50-r dosage – showed that the percentage increases were considerably greater with the soft X rays than with the gamma radiation and that the 250 kvp X rays were intermediate in this respect. The same relationships seem to hold when the infrared is given as a posttreatment, although the data are not so complete in this respect. On this basis it might be assumed that

TABLE 6. EFFECTIVENESS OF VARIOUS INFRARED–X-RAY COMBINATIONS ON CHROMATID ABERRATIONS IN *TRADESCANTIA*

TREATMENT	TOTAL CELLS	ABERRATIONS PER 100 CELLS			PERCENTAGE INCREASE IN BREAKS*
		Chromatid Deletions	Isochromatid Deletions	Exchanges	
50 r of X ray	800	27.0	15.5	9.4	
3 hr of infrared + 50 r of X ray	450	38.9	20.2	18.9	58.1
50 r of X ray + 3 hr of infrared	450	38.6	18.4	16.9	48.1
3 hr of infrared + 50 r of X ray + 3 hr of infrared	500	43.1	23.6	26.8	96.2
1½ hr of infrared + 50 r of X ray + 1½ hr of infrared	350	36.9	18.0	17.4	46.3

*Exchanges scored as two breaks.

an infrared-neutron combination would be quite effective, but it has not been possible to obtain any increases when such a combination is used.

Infrared Effect on Onion Root-Tip Chromosomes. The experiments described above were carried out with *Tradescantia* as the experimental material. The question, however, of whether the infrared effect can persist through a cell division cannot adequately be answered with the use of microspore material; therefore onion root tips were used. These root tips were X rayed at various time intervals after infrared had been given, and although there appeared to be a slight effect of the infrared when X radiation followed immediately after infrared exposure, no real increases were found when the period between infrared and X rays was lengthened.

Studies on the Mechanism of Action of Radiation in *Vicia faba*

(Wolff)

Vicia faba, with only six pairs of chromosomes, has long been a popular organism for cytological studies. In the past few years its popularity has increased since the organism lends itself so readily to studies on the mechanism of action of ionizing radiations. A paper now in press shows that British Anti-Lewisite (BAL) and, similarly, irradiation *in vacuo* are able to decrease the amounts of chromosomal aberrations caused by X rays. Some evidence is also presented that the BAL increases the restitution time of broken ends of the chromosomes. At present, further experiments are being performed on *Vicia* to determine exactly the average restitution time of chromosomes in the seed. Also, other reducing compounds such as cysteinamine are being checked for possible protective effects. This work is preliminary to further studies on the locus of the oxygen effect.

Localized Shattering of Chromosomes by High Doses of Ultraviolet Radiation of 2650 Å Wave Length

(Lovelace)

In a study of the modifying effects of pretreatment with ultraviolet radiation on gamma-ray-induced aberrations in *Tradescantia* pollen tube chromosomes, a peculiar shattering of portions of chromosomes was observed in tubes grown from dry pollen which had received 10^6 ergs/cm^2 of 2650-Å ultraviolet radiation just before sowing. Usually a portion of only one or two chromosomes was shattered (that is, broken into many small fragments), while the rest of the set contained few or no breaks. Swanson,[15,16,17,18] in his studies of the effects of ultraviolet on pollen tube chromosomes, found only terminal chromatid deletions produced when growing tubes were exposed to ultraviolet radiation. Since no shattering of chromosomes by ultraviolet has been reported, further studies on this effect were undertaken.

Monochromatic radiation was isolated, by means of a monochromator, from a water-cooled mercury capillary arc operating at 3 amp and 100 volts. Incident dosage was determined by means of galvanometer deflection with an Epply thermopile. Pollen was desiccated over Drierite for about 1 hr, spread in a monolayer, and sealed in a dry box with a clear, fused-quartz window for irradiation. It was planted on a colchicinized agar-lactose medium immediately after irradiation, fixed 20 hr later, stained with Feulgen reagents, and metaphase nuclei were scored for aberrations. Doses of 2650-Å radiation ranging from 10^4 to 5×10^6 ergs/cm^2 were delivered to the dry pollen grains. None of the metaphases contained shattered chromosome portions when the pollen had received less than 5×10^5 ergs/cm^2, although chromatid and isochromatid deletions occurred with low frequencies at lower doses. In one experiment, 1% of 300 metaphases contained shattered chromosome portions when the pollen had received 5×10^5 ergs/cm^2, while 25% of 450 metaphases were so affected following 10^6 ergs/cm^2. Above 10^6 ergs/cm^2, the frequency of cells containing shattered portions of chromosomes becomes greater with increased dosage. This effect has not been observed following the same doses of 3130- and 3650-Å ultraviolet.

Nothing is known of the transmission of ultraviolet by the wall of *Tradescantia* pollen grains, nor is the absorption by the material between the wall and the generative nucleus known. The location of the crescent-shaped nucleus in the pollen grain and the random orientation of the grains in

(15) C. P. Swanson, *Proc. Natl. Acad. Sci. U.S.* 26, 366-373 (1940).

(16) C. P. Swanson, *Genetics* 27, 491-503 (1942).

(17) C. P. Swanson, *Genetics* 29, 61-68 (1944).

(18) C. P. Swanson, *J. Gen. Physiol.* 26, 485-494 (1943).

the monolayer, together with lack of knowledge regarding transmission, make it impossible to estimate the amount of energy actually reaching the generative nuclei. Different nuclei, or different portions of the same nucleus, may have received very different amounts of energy. Localized shattering of portions of chromosomes by high doses of monochromatic 2650-Å ultraviolet radiation has been observed in six independent experiments separated by a period of as long as one year.

Effect of Certain Chemical Agents on Geotropism of Timothy Roots

(Brumfield)

Since cell division and elongation can be observed directly in primary roots of timothy seedlings in the living condition, these roots serve as material for the study of the factors controlling plant growth. It has already been shown that unilateral exposure to ultraviolet radiation (2537 Å) induces curvatures in the root,[19] thus indicating a modification of the rate of cell elongation on the exposed side. The hypothesis that these curvatures are mediated through the inactivation of auxin was tested by combining treatment with indole-3-acetic acid (IAA) and exposure to ultraviolet.[20] Although treatment with IAA modified slightly the curvatures induced by ultraviolet, no definite conclusions could be drawn from these experiments, since IAA depresses root growth and since curvatures in plants are the result of unequal rates of cell elongation on opposite sides of the organ. Thus the effect of the IAA treatment may have been due primarily to a lower rate of root growth.

The seedling root of timothy exhibits strong positive geotropism when turned horizontally. Although the mechanism is not completely understood, it is clear that geotropism results from unequal rates of cell elongation on the upper and lower sides of the root and it appears that auxin is in someway involved. The geotropic response affords a means of testing the relationship between root growth and curvature, thus making it possible to determine the effect of IAA alone on root curvature. Furthermore, the possibility exists that geotropic curvatures and the curvatures induced by ultraviolet are mediated through the same or related agents. With these considerations in mind, it was thought worth while to test the effect of certain compounds on growth, on the geotropic response, and on the curvatures induced by ultraviolet. IAA, 2,4,6-trichlorophenoxyacetic acid (2,4,6-TCPA), maleic acid hydrazide (MH), and riboflavin have been used in preliminary experiments. Although the work is still in progress, the studies of the effects of IAA and 2,4,6-TCPA on growth and geotropism are sufficiently complete to warrant a report on this phase of the research.

In the procedure followed, the root was treated with the compound under test and then put in a horizontal position for subsequent observation; because of variation in growth of different roots, it was necessary to determine the rate of growth of each root prior to treatment. The simple apparatus used for carrying out these operations with a minimum of disturbance to the growing root is shown in Fig. 3. A 17- by 38-mm rectangular opening was cut in the center of a circular piece of Lucite 57 mm in diameter and 3 mm in thickness. A thin, moist chamber was formed by affixing cover slips to opposite sides of the opening with a heavy, silicone, stopcock grease. A hole 1.5 mm in diameter was drilled from the edge of the circle to the rectangular chamber, and solutions could be added to or withdrawn from the chamber by means of a syringe with a blunt needle. Before the second cover slip was added, strips of moist filter paper and a seedling previously germinated on moist filter paper until the primary root was 4 to 7 mm long were placed in the chamber as shown in Fig. 3. A cradle to carry the Lucite circle was cut, in the pattern shown, from aluminum 5 mm thick. The semicircular cradle was grooved to receive the Lucite circle. When this apparatus is placed on the stage of a microscope in a horizontal position (stage vertical), the seedling root grows along the inner surface of the cover slip bearing it, and may be observed directly. The root may be turned to any angle in the plane of focus of the microscope objectives by rotating the Lucite circle. Since visible light apparently has no effect on the growth of these roots, the image of the root can be projected onto sensitized paper and the behavior of the root can be studied from the photographs at leisure. In the experiments described here, a 10X objective and a 5X ocular were used, the distance from the ocular to the paper holder being adjusted

[19] R. T. Brumfield, *Biol. Quar. Prog. Rep. Aug. 15, 1950*, ORNL-807, pp. 23-24.

[20] R. T. Brumfield, *Biol. Quar. Prog. Rep. Aug. 10, 1952*, ORNL-1393, p. 13.

to a magnification of 100X. A Bausch and Lomb ribbon-filament lamp with a water cell served as the microscope illuminator.

A device to keep track of time automatically was constructed in the following manner. A clock was mounted in one end of a box and two electric lamps to illuminate its face were mounted at the other. A lens, mounted in a tube inserted between the lamps and opposite the clock, and a prism (since the box was placed at right angle to the paper holder) projected the image of the clock onto one corner of the paper holder. By connecting the lamps in parallel with the microscope illuminator, the image of the root and the image of the clock were projected simultaneously, and the time at which the photograph was taken could be read directly.

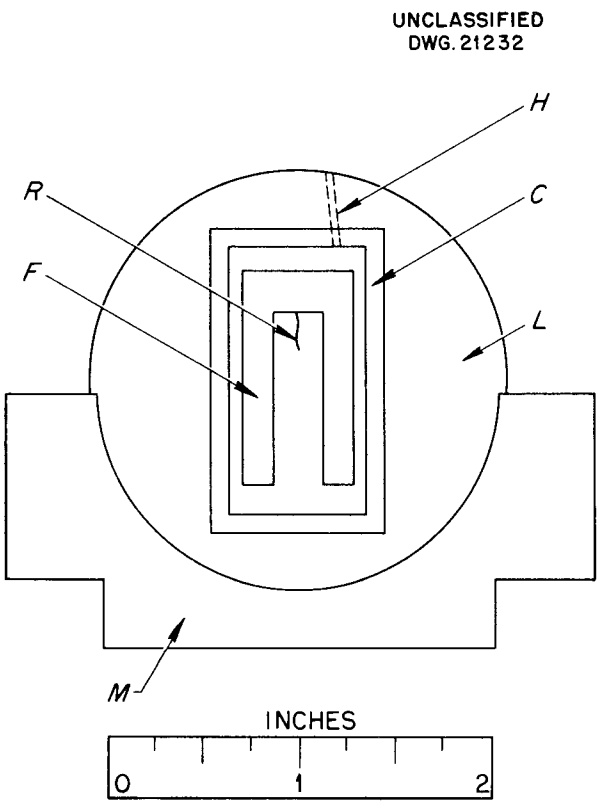

Fig. 3. Diagram of Apparatus Used for Testing the Effect of Chemical Compounds on Geotropism in Timothy Roots. The Lucite circle, *L*, is 3 mm thick with a rectangular opening covered on both sides with cover slips, *C*. One cover slip bears moist filter paper, *F*, and the seedling with its root, *R*. The hole, *H*, allows solutions to be introduced into or removed from the chamber by means of a blunt needle. A metal plate, *M*, which is 5 mm thick, fits the mechanical stage of a microscope and contains a grooved semicircular cradle in which the Lucite circle can be rotated.

One operator could handle four roots, in separate chambers, and at the same time could take photographs of each at 20-min intervals. In all experiments, four seedlings were mounted, one to a chamber (roots vertical), and 1 hr was allowed for the roots to become adjusted to the new environment. Photographs were then taken at 20-min intervals for 1 hr to determine the rate of growth prior to treatment. The chamber was then flushed with the solution by filling it to a level just below the root and then withdrawing the solution. Fresh solution was then added to cover the distal 3 to 4 mm of the root, the solution being withdrawn after 2 minutes. Control roots were handled the same way except that distilled water was used. After treatment, the Lucite circle was rotated to put the root in a horizontal position; the root was photographed immediately afterward and then at 20-min intervals for a period of 120 minutes. The experiments were carried out at room temperatures, 27 to 29.5°C.

Curvatures could be accurately measured with a protractor, since the linear rows of cells in the root serve as guide lines. Root length was measured with a pair of dividers and, with curved roots, the total length was taken as the sum of short distances measured along the center of the root.

The results are presented in Table 7 and are shown graphically in Fig. 4. Figure 4 shows the average increase in length of the four roots in each experiment after 20, 40, 60, 80, and 100 min, while the ordinates are the average angle of curvature of the four roots after the same intervals of time. This treatment of the data makes clear the relationship between growth and curvature.

No extended discussion is contemplated at this stage of the research; however, attention should be called to the following points.

1. IAA markedly affects root growth but it does not appreciably influence the amount of curvature per unit of root elongation.

2. 2,4,6-TCPA in concentrations of 5 to 20 mg/liter does not appreciably affect root growth but it markedly influences the amount of curvature per unit of elongation.

3. No definite conclusions can be drawn concerning the effect of the lower (5 and 10 mg/liter)

concentrations of 2,4,6-TCPA on root growth because, as shown in Table 7, there was too much variation in controls and experimentals prior to treatment.

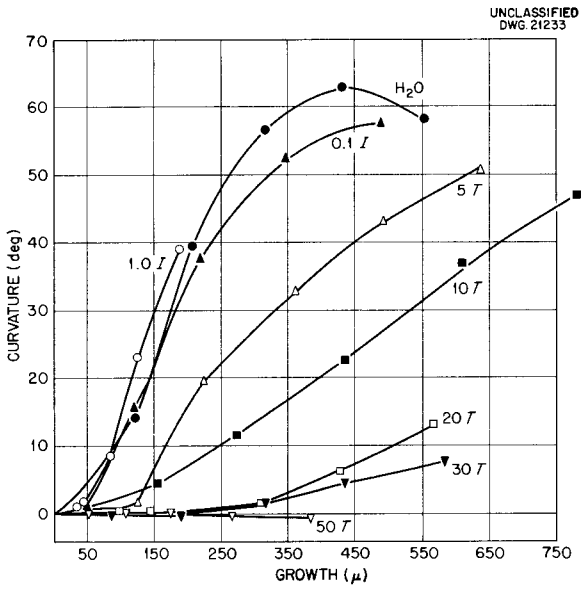

Fig. 4. Geotropic Curvature with Respect to Growth After Treatment with Indole-3 Acetic Acid, *I*, or 2,4,6-Trichlorophenoxyacetic Acid, *T*. Abscissas represent the growth 20, 40, 60, 80, and 100 min after treatment. Each point represents an average of four roots. Concentrations are shown in mg/liter.

In continuing the research it is intended to determine the effect of 2,4,6-TCPA on the rate of elongation of individual cells along the axis of the growing point; the effect of combining treatment with 2,4,6-TCPA and exposure to ultraviolet; and the effect of other compounds on growth, geotropism, and curvatures induced by ultraviolet.

Effect of X Rays on Chromatid Aberrations in Air and in Nitrogen

(Swanson, Schwartz)

Because of what are considered by the reporting authors to be discrepancies between experimental data and formulated hypothesis in the oxygen studies of Giles and his co-workers on X-ray-induced chromosomal aberrations in *Tradescantia*, a rather extensive program of research has been carried out to determine the effects of reduced oxygen tensions on chromatid rather than on chromosome aberrations. The chromatid aberrations were chosen because one of the types of induced aberrations – chromatid deletions – involves breakage only and thus should provide a critical test of the breakage hypothesis of Giles.[21] The four separate experiments carried out are discussed.

Breakage as a Function of Stage of Cell Division. During the winter, the cell cycle is such as to permit a study of chromatid aberrations over

[21] H. P. Riley, N. H. Giles, and A. V. Beatty, *Am. J. Botany* 39, 267-284 (1952).

TABLE 7. GROWTH AND GEOTROPIC CURVATURE OF TIMOTHY ROOTS AFTER VARIOUS TREATMENTS

GROWTH MADE IN 60 min PRIOR TO TREATMENT (μ)	CHEMICAL TREATMENT FOR 2 min		GROWTH MADE IN INDICATED TIME AFTER TREATMENT (μ)					CURVATURE OBSERVED IN INDICATED TIME AFTER TREATMENT (deg)				
	Agent*	Concentration (mg/liter)	20 min	40 min	60 min	80 min	100 min	20 min	40 min	60 min	80 min	100 min
381.5	H_2O		120.5	202.5	315.0	430.7	551.0	14	39.5	57.5	63.2	58.2
414.2	T	5	126.7	223.5	355.0	494.2	638.0	1.2	19.7	33.7	43.2	50.7
537.5	T	10	153.7	271.7	433.0	608.0	780.5	4.4	11.7	22.4	37.0	47.5
487.5	T	20	97.5	147.6	307.0	425.7	566.5	0	0	1.5	5.7	13.2
433.3	T	30	86.6	190.3	309.6	434.0	573.0	–0.7	–0.7	1.5	4.6	7.6
472.7	T	50	50.0	107.5	178.7	269.5	384.5	0	0	0	–0.5	–1.0
475.7	I	0.1	47.5	120.0	218.2	346.7	486.5	0.6	15.2	37.6	52.5	57.2
437.2	I	1.0	32.7	47.5	75.5	124.7	188.7	0.5	1.2	8.0	23.2	39.1

*T = 2,4,6-trichlorophenoxyacetic acid
I = Indole-3-acetic acid

a period of approximately 48 hr, at which time the chromosomal aberrations make their appearance. Slides were made from 8 to 64 hr after radiations, at intervals of 4 hours. From such a study, the frequency of aberrations induced in air and in nitrogen can be determined, and for the chromatid and isochromatid deletions, which show a linear relationship to dosage, the air/nitrogen ratios can be computed directly. The results showed that the chromatid deletions were much less reduced (the average air/nitrogen from 8 to 52 hr was 1.34) than were the isochromatid deletions (average air/nitrogen was 4.3) and exchanges. These last two types involve breakage and reunion, and it is therefore possible that recovery processes were involved in their reduction. There was also good indication that the air/nitrogen are not constant, as would be expected if the breakage hypothesis were interpreted quite strictly. It was therefore necessary to establish this point with certainty through dosage studies.

Breakage as a Function of Dose at Various Time Intervals after Irradiation. Dosage curves for the various chromatid aberrations were determined at 8, 12, and 24 hr after radiation. During this period the sensitivity of the chromosomes to radiation damage changes markedly, and it can consequently be determined with certainty whether the air/nitrogen ratios are in any sense a reflection of chromosome sensitivity or whether the ratios are a constant, as demanded by the breakage hypothesis. In air the doses ranged from 25 to 200 r, in nitrogen from 50 to 350 r, with the intensity at 50 r/min. At these three time intervals, the air/nitrogen ratios for chromatid deletions were approximately 1, indicating that the absolute number of this type of aberration was relatively unaffected at radiation of 100 kvp. For isochromatid deletions, ratios of 2.8, 2.6, and 2.3 were obtained for the 8-, 12-, and 24-hr periods, respectively; for chromatid exchanges, the ratios for the same periods were 2.9, 2.4, and 2.0. The different ratios for isochromatid deletions are significantly different from each other; those for exchanges are not, although the reality of the differences is indicated by their paralleling rather closely those for the isochromatid deletions. The lack of constancy in the air/nitrogen ratios indicates that the breakage hypothesis cannot account for the experimental facts.

Breakage as a Function of Wave Length. The data obtained from the time and dosage studies confirm and extend the findings of Riley, Giles, and Beatty,[21] although the reporting authors differ from them in interpreting the results. In the work being reported here, however, 100-kvp X rays with a filtration of 1 mm of Al were used, while the other authors[21] employed 250-kvp X rays filtered through 3 mm of Al. The air/nitrogen ratios determined by the reporting workers for chromatid and isochromatid deletions being lower and higher, respectively, than those obtained by Riley, Giles, and Beatty suggested that the aberration frequencies, and consequently the air/nitrogen ratios, may be a function of wave length. Therefore three different experiments have been done using 50-kvp X rays, 100-kvp X rays with 1 mm of Al, 250-kvp X rays with 4 mm of Cu, and gamma rays from the Co^{60} facility. The three experiments agree with each other quite well; the results of one are given in Table 8. It is quite apparent that the air/nitrogen ratios change with wave length, and it is interesting to note that the direction of change for chromatid deletions is quite the reverse of that for isochromatid deletions; that is, the longer the wave length, the smaller the ratios for chromatid deletions and the larger for isochromatid deletions. Three other points can be made from these data. First, at 50 and 100 kvp, the number of deletions in nitrogen actually exceeds that induced in air; second, the isochromatid deletions show no apparent wave length effect in nitrogen, while they clearly do in air; and third, the sum of the chromatid and isochromatid deletions in air is the same for all four wave lengths. It would appear, therefore, that the ratio of chromatid to isochromatid deletions in air is governed by wave length but that the absolute number of the combined deletions is not so governed. The data further suggest that as the wave length changes there is a shift of one type of deletion into another, for example, as the chromatid deletions diminish as the wave length lengthens, there is a compensatory increase in the isochromatid deletions. On an ion-density basis this shift is understandable. The increase in chromatid deletions in nitrogen as the wave lengths lengthen may be accounted for by assuming that the partial restitution of isochromatid deletions and chromatid exchanges transforms many of the two-hit aberrations into one-hit types. The lack of a wave-length effect on isochromatid deletions in nitrogen remains unexplained at present.

TABLE 8. EFFECT OF IONIZING RADIATIONS OF DIFFERENT WAVE LENGTHS ON CHROMATID ABERRATIONS IN AIR AND IN NITROGEN

(150 r at an Intensity of Approximately 9 r/min)

TYPE OF RADIATION	ABERRATIONS PER 100 CELLS					
	Air			Nitrogen		
	Chr. del.	Iso. del.	Exch.	Chr. del.	Iso. del.	Exch.
50-kvp X rays (unfiltered)	49.3	84.6	35.3	98.2	15	9.5
Air/N_2 ratio	0.50	5.6				
100-kvp X rays (1 mm Al)	66.0	62.5	30.5	79.3	14.0	7.3
Air/N_2 ratio	0.83	3.9				
250-kvp X rays (4 mm Cu)	78.0	44.0	28.0	63.3	16.6	6.0
Air/N_2 ratio	1.23	2.7				
Gamma rays	96.2	32.5	26	50.5	14.0	5.0
Air/N_2 ratio	1.9	2.3				

Induced Stickiness of Chromosomes in Air and in Nitrogen. It has long been known that radiation induces a "stickiness" of the chromosomes and that the duration, as well as the degree of stickiness, is a function of dosage and of stage of division. It was early apparent in the time study described above that there was less stickiness induced in nitrogen than in air. Consequently, a study was begun of this phenomenon. At 150 r in air, stickiness was not fully expressed until 1 hr had passed. At this time, 100% of the cells showed sticky metaphases and anaphases, and this condition continued until about 9 hr after irradiation. A gradual decline in stickiness then was evident, and at about 12 hr after irradiation no cells showed a sticky condition. In nitrogen, 150 r induced no stickiness, and to achieve the same degree of stickiness that is induced by 150 r in air, it was necessary to increase the dosage to approximately 600 r. With smaller doses in air and in nitrogen, there is a graded decrease in stickiness. These results immediately suggest that there is a possible relationship between stickiness and breakage, or between stickiness and restitution, and attempts are being made to approach the problem experimentally.

Conclusions. The experiments described do not support the breakage hypothesis proposed by Giles and his co-workers to explain the action of oxygen on the induction of chromosomal aberrations by ionizing radiations. They are, on the other hand, interpretable on the differential reunion hypothesis proposed by Schwartz.[22] The wave-length experiments also provide a suggestion as to how one type of aberration may be shifted into another type, depending upon the wave length and the atmosphere in which radiation exposures are made.

On the Mechanism of Chromosome Duplication and Recombination

(Schwartz)

The Belling hypothesis of crossing over has not been seriously considered by most geneticists because it fails to explain the occurrence of three- and four-strand double crossovers. However, evidence recently presented for sister-strand crossing over[23] made possible reconsideration of the Belling system or modifications thereof in an attempt to develop an hypothesis of crossing over which will adequately explain the mass of existing data.

The proposed hypothesis is as follows: Chromosomal replication occurs by the formation of a new chromatid on a template which is provided by the parent chromosome (the parental chromosome itself

[22] D. Schwartz, *Proc. Natl. Acad. Sci. U.S.* 38, 490-494 (1952).

[23] D. Schwartz, *Genetics* 38, 251-260 (1953).

acting as the template). Replication is initiated at a number of loci along the length of the chromosome. No correlation is needed between the loci where duplication is initiated in the homologous paired chromosomes. In the absence of crossing over, the chromosome duplicates until all the partial replicas laid down on one template meet and join to form a new chromatid. Crossing over occurs when the homologous parental chromosomes are closely paired, and union occurs between the end of a replica from one chromosome with the end of a replica from the other. The parental chromosomes acting as templates ensure that only equal crossing over will occur, that is, since *a* is next to *b* on the parental chromosome, a replica with *a* at its end can only unite with the *b* end of another replica.

On this hypothesis, crossing over occurs only between the two new chromatids. A three-strand double results from a double crossover where a sister-strand crossover occurred in one of the chromosomes between the nonsister crossovers. Similarly, a four-strand double results from a sister-strand crossover in each of the chromosomes.

This hypothesis for recombination was tested by studying somatic crossing over between attached-X chromosomes in *Drosophila melanogaster*. Since there is little or no sister-strand crossing over in somatic tissues of *Drosophila*, as shown by the very low frequency of dicentric rings arising from monocentric ring chromosomes, different results would be obtained from somatic crossing over, depending on whether the exchange involved only the newly formed chromatids or old and new chromatids, as well. As shown in Fig. 5, when an attached-X chromosome heterozygous for yellow (y) and singed (sn) is employed, crossing over between an old and a new chromatid will result in a twin spot (one spot being yellow and the other singed). Crossing over between the two newly formed chromatids will give rise only to wild-type tissue (neither yellow nor singed) since the new chromatids are attached to a common centromere and hence always move to the same pole in anaphase separation.

The results of this experiment strongly support the proposed mechanism for recombination. One thousand flies were examined for twin spots, and only one such spot was found. On the basis of Stern's studies[24] with unattached-X chromosomes using identical markers, it was estimated that, if crossing over occurred at random between the chromatids of the homologous chromosomes, at least 100 twin spots should have been found. The data further suggest that chromosome duplication does not occur by a lengthwise splitting of the chromosome to form two equal halves, that is, chromatids. If such were the case, either chromatid could be involved in a crossover. The limitation of crossing over to the newly formed chromatids suggests that recombination is a direct result of the process of chromosome replication.

[24] C. Stern, *Genetics* 21, 625-730 (1936).

Effect of Oxygen Concentration on Irradiated Onion Root Tips

(Schwartz, Phelps)

Hayden and Smith[25] studied the effect of oxygen concentration on the frequency of anaphase bridge

[25] B. Hayden and L. Smith, *Genetics* 34, 26-43 (1949).

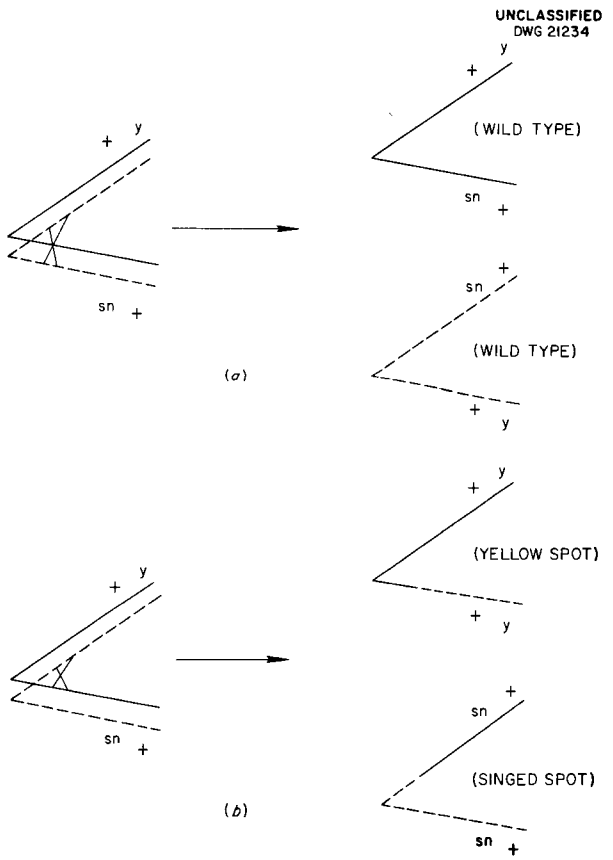

Fig. 5. Diagrammatic Representation of the Results of Somatic Crossing Over that Involves (*a*) an Old and New Chromatid and (*b*) Two New Chromatids. The broken lines represent the newly formed chromatids.

formation in irradiated barley root tips. They found a 4.7-fold difference between the frequency of bridges obtained from treatment in air and that obtained from treatment in vacuum. These studies have been repeated with the use of onion root tips in experiments designed to compare the magnitude of the oxygen effect on single and double anaphase bridges. The root tips were irradiated with 300 r of X rays in air and nitrogen and were fixed 48 hr after irradiation. Sax[26] has shown that chromosomes act as single strands 48 hr prior to anaphase and thus that only the chromosomal types of aberrations appear. The double bridges are formed by dicentric chromosomes (two-hit aberrations), while the single bridges result from terminal deletions followed by the fusion of the broken ends of sister chromatids.

The data are shown in Table 9. The results compare favorably with those reported for similar experiments in maize in which genetic markers were used.[27] The mosaic kernels in maize are to be compared with the single bridges in onion, since both result from terminal deletions followed by sister-strand fusion. The whole-loss kernels are a composite class, including both one-hit and two-hit aberrations. The protection values obtained for the maize mutant types are included in the table for comparison.

[26] K. Sax, *Genetics* 26, 418-425 (1941).

[27] D. Schwartz, *Proc. Natl. Acad. Sci. U.S.* 38, 490-494 (1952).

Homology Tests of *Neurospora* Lethal Mutations

(Atwood, Mukai)

During the past year, 100 X- and gamma-ray-induced recessive lethal mutations in *Neurospora* have been tested for interhomology in every possible two-by-two combination. The methods of obtaining and testing these mutants have been previously described.[28,29,30,31,32] It was expected that the results of these tests might permit an estimate to be made of the number of different loci involved in the induced mutations and of the frequency and size of induced chromosomal deficiencies. It is evident from the results given in Fig. 6 that the mutants can be divided into the two categories of high homology (HH) and low homology (LH); the HH stocks make up about 20% of the total. The five stocks showing the fewest homolgies with other stocks were found to have reverted to a nonlethal condition at some time during the course of the experiment. If these five are excluded from the data, it appears that the HH stocks are homologous with all or nearly all the other stocks in the series, whereas the LH stocks show very few instances of homology among themselves.

[28] K. C. Atwood, *Biol. Bull.* 97, 254 (1949).

[29] K. C. Atwood, *Biol. Bull.* 99, 332 (1950).

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

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

[32] K. C. Atwood and F. H. Mukai, *Biol. Quar. Prog. Rep. Nov. 10, 1952*, ORNL-1456, pp. 18-20.

TABLE 9. COMPARISON OF OXYGEN EFFECT ON ONE-HIT AND TWO-HIT ABERRATIONS IN ONION AND MAIZE

		TOTAL CELLS SCORED	NUMBER OF NORMAL CELLS	DOUBLE BRIDGES		SINGLE BRIDGES		TOTAL BRIDGES	
				Number	%	Number	%	Number	%
Onion	Air	2184	2070	56	2.56	58	2.66	114	5.22
	Nitrogen	2565	2521	7	0.27	37	1.44	44	1.64
	Air/Nitrogen			9.5		1.9		3.2	
Maize				Mosaics		Whole losses		Total mutants	
	Air/Nitrogen			9.9		2.7		3.2	

Before any conclusions can be drawn from the data, it is essential that the nature of the HH stocks be understood. The only explanation it has been possible to offer previous to the present report is that the HH stocks represent large chromosomal deficiencies which can be mapped by the extent of their homology with other mutants. The more complete data now available lead to the substantial modification of this hypothesis, for the following reasons.

1. If the hypothesis were correct, each HH stock would have to carry nuclei deficient for some 75 randomly chosen loci and these loci must be distributed among several chromosomes; yet all the mutants tested were obtained at relatively low doses at which, for the most part, they can be

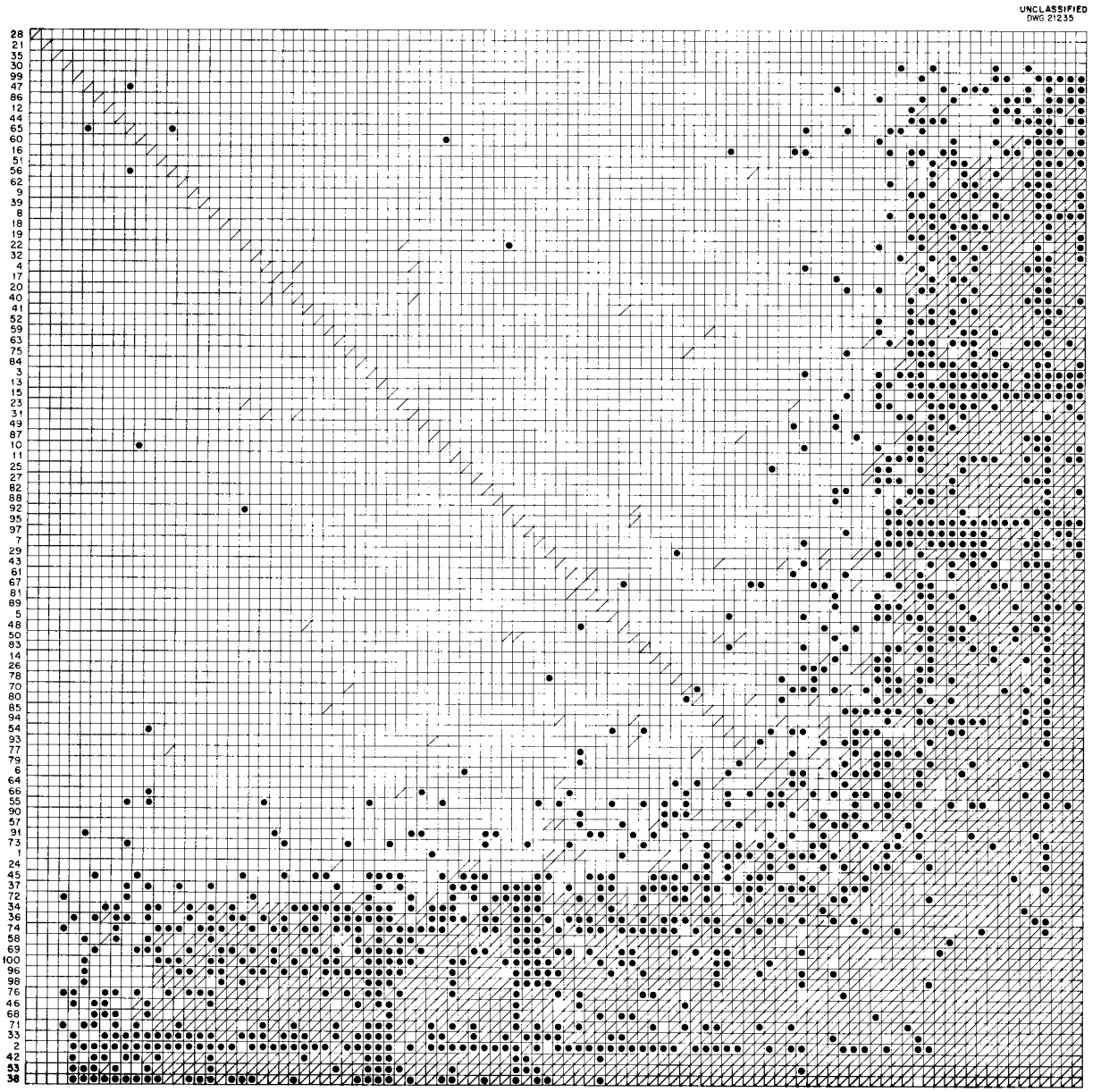

Fig. 6. Allelism Tests of Induced Heterokaryon Mutants. Hatched squares indicate homologous tests, open squares nonhomologous tests, and dotted squares equivocal tests. The pattern is reproduced in mirror image for convenience in recognizing alleles by comparison along a single axis.

ascribed to single events. It does not seem possible that simultaneous deficiencies for loci in several chromosomes could be produced as single-event phenomena.

2. If deficiencies were being recovered in appreciable numbers, a fairly continuous variation in the sizes of the deficiencies would be expected; yet the data show that deficiencies of intermediate sizes are very rarely recovered.

3. The high correlation between the incidences of homologous and equivocal tests suggests that the HH stocks are, in some way, predisposed to cause failure of the tests even when their nuclei are genetically complementary to those of the other contributing stocks.

4. If the HH stocks were deficiencies of the size indicated by their homology patterns, the amount of the genetic material lost would be sufficient to cause a drastic reduction in the size of the lethal nuclei relative to the size of the normal nuclei in the same heterokaryon. Cytological examination of the HH stocks (haploid nuclei in the conidia) failed to reveal nuclear dimorphism or any other abnormality.

On the basis of these considerations, an attempt was made to discover any differences between HH and LH stocks which would account for the consistent failure of the HH stocks to give nonhomologous tests even when they are genetically possible. The successful functioning of the test depends on the formation of a secondary heterokaryon from two primary heterokaryons, each of which carries a recessive lethal mutation in its amycelial-methionine component. If this heterokaryon is formed and if the nuclei of the contributing stocks are reproduced normally during the growth of the coenocyte, these nuclei are reshuffled and appear in all combinations among the conidia formed by the complex heterokaryon. In one of these combinations, the amycelial-methionine-lethal nuclei from both contributing stocks are together in the same conidia without other types of nuclei being present. These conidia can grow and produce amycelial colonies on methionine medium if, and only if, the lethals carried in the contributing stocks are not homologous. Thus the presence of the amycelial colonies signifies nonhomology of the lethals.

Whereas the nonhomologous tests are conclusive, there are many potential opportunities for spurious homologous tests. For instance, the complex heterokaryon may fail to form, or, if it does form, one of the nuclear types essential to the test may be at a selective disadvantage in the new heterokaryon and may be greatly reduced in numbers or may even be eliminated. In some cases, the nuclear ratio of one of the contributing stocks is so far in favor of the ornithine component that too few amycelial nuclei are contributed to form the required combination in appreciable frequency. The selective disadvantage of the amycelial-methionine-lethal nucleus might, in some cases, be reflected in a decreased growth rate of the contributing stock. Aberrant nuclear ratios can be detected through the occurrence of unusually large numbers of ornithine homokaryons when the stocks are plated on ornithine medium. Some of these aberrant ratios will be missed experimentally because of lethals in the ornithine component.

Accordingly, the growth rate of each of the 100 mutant stocks was determined by the growth-tube method.[33] The ratio of ornithine homokaryons to heterokaryons among the conidia of each stock was determined by plating on ornithine and minimal media. It is significant that, among the ten stocks having the lowest growth rate and the ten having the highest ornithine-to-heterokaryon ratio, no less than 13 HH stocks are found.

It may be concluded that the basis for an HH stock is an alteration which renders it incapable of functioning normally in the homology tests. If this alteration is a deficiency in the amycelial nucleus, it usually becomes impossible to map the deficiency by the extent of its homology with other deficiencies and point mutations. There are two indications that chromosomal deficiencies may fall into the HH group: HH stocks have been found only among induced lethals, not among spontaneous lethals;[34] the induced LH stocks can be characterized as point mutations or small deficiencies. Larger deficiencies must be produced by the radiation, and since they are not among the LH stocks, they must be among the HH stocks or among the cells which do not survive. It appears that genetic completeness of the coenocyte is not a sufficient condition for growth and nuclear division. A certain minimum degree of genetic completeness of a nucleus is also necessary for its normal functioning, irrespective of the presence of genetically complementary nuclei.

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[33] F. J. Ryan, G. W. Beadle, and E. L. Tatum, *Am. J. Botany* 30, 784-799 (1943).

[34] K. C. Atwood and F. H. Mukai, *Proc. Natl. Acad. Sci. U.S.* (in press).

INSECT CYTOLOGY AND GENETICS

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Effects of C^{14} Beta Radiation on Mitotic Rate

(Gaulden, Totter, Best)

Formate labeled with C^{14} was used in preliminary experiments[37] for determining whether X rays inhibit nucleic acid synthesis, and if so, whether there is a correlation between inhibition of nucleic acid synthesis and inhibition of mitosis. In order to evaluate the results of these experiments, it was necessary to determine whether the small amount of beta radiation emitted by the C^{14} in the formate had a mitotic effect.

Grasshopper embryos were separated from their membranes and yolk. Half the embryos were incubated at 38°C for 30 min in 2.1 ml of artificial culture medium containing 0.9 μmole of potassium formate labeled with C^{14} (7.6 μc) and half were incubated in the absence of labeled formate. (Formate is utilized by embryos in synthesis of nucleic acids.) Following incubation, the embryos were made into culture preparations, and the mitotic rate was determined as previously described.[38]

The results are shown in Fig. 7. An analysis of the variance test (by A. W. Kimball, Mathematics Panel) revealed that the mitotic rate of cells incubated with C^{14} did not differ significantly from the mitotic rate of cells incubated in the absence of C^{14}. Therefore since C^{14}-labeled formate does not induce mitotic effects, it can be used as a tool for determining the inhibiting effects of X rays on nucleic acid synthesis and on mitosis.

Effects of Hypo- and Hypertonicity on Living Mitotic Cells

(Gaulden)

A detailed study of the effects of abnormal tonicity on cell structures as observed in living neuroblasts of *Chortophaga viridifasciata* embryos has been completed. The embryos were separated from their membranes and yolk while in a culture medium that was isotonic to them; then they were made into culture preparations in hanging drops of isotonic medium. The appearance of the chromatin and cell structures in varying stages of mitosis was noted. The isotonic medium in the hanging drop was replaced immediately with a medium that was hypo- (0.65 × isotonic salt concentration) or hypertonic (1.65 × isotonic salt concentration) and the cells were re-examined.

Subjection to a hypotonic medium causes the cells to swell. The chromatin in all stages of mitosis becomes less distinct; in fact, it becomes invisible in late telophase through early prophase nuclei. Growth of the spindle during prometaphase is inhibited, and size of the mature spindle at metaphase is reduced to one-third its normal size – the mitotic progress of these two stages is consequently greatly retarded. Cytokinesis is often completely inhibited.

Subjection to hypertonic medium causes the cells to shrink. The chromatin of all mitotic stages becomes more distinct, and, as a consequence of this alteration, or coincident with it, the changes which normally occur in the chromosomes at telophase do not take place. The chromosomes of middle and late prophase nuclei become shorter and thicker so that they come to resemble the chromosomes of the succeeding stage in isotonic preparations. The chromosomes of prometaphase through late anaphase cells coalesce (Figs. 8 and

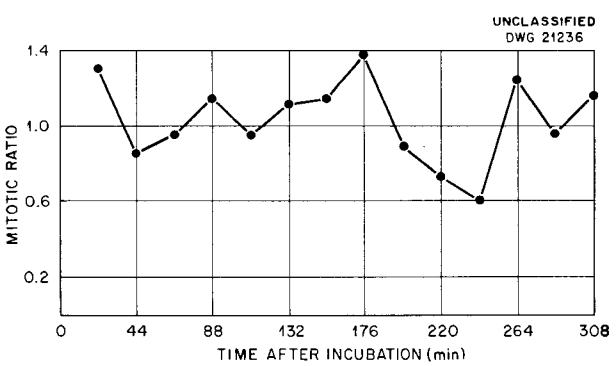

Fig. 7. Mitotic Rate in Embryos Following Incubation With and Without C^{14}-Labeled Formate (Eight Embryos per Treatment).

$$\text{Mitotic ratio} = \frac{\text{Number of mid-mitoses in embryos with } C^{14}}{\text{Number of mid-mitoses in embryos without } C^{14}}$$

(35) Consultant.

(36) Research Participant.

(37) J. Totter, M. E. Gaulden, and K. Loemker, *Biol. Quar. Prog. Rep. Nov. 10, 1952*, ORNL-1456, p. 26.

(38) M. E. Gaulden and M. L. Nix, *Biol. Quar. Prog. Rep. May 15, 1950*, ORNL-727, p. 41.

9) and resemble in all respects the "sticky" chromosomes produced by high doses of ionizing radiation. Chromosome fragments similar to those induced by radiation can be observed in some cells (Fig. 10). The spindle and cytokinesis are unaffected by exposure to hypertonic medium.

When cells are returned to isotonic medium, they recover completely. Recovery is particularly striking in the case of sticky metaphase and anaphase chromosomes in hypertonic medium – on return to isotonic medium, the chromosomes become distinct entities again and division proceeds normally.

Thus it is apparent that alterations in the water content of the cell cause pronounced changes in cell structures. Experiments are now in progress for determining whether altered tonicity of the cell modifies its response to radiation.

Effect of Hypertonicity on Mitotic Rate

(Gaulden, Loemker)

Previous work[39] revealed that exposure of neuroblasts to hypertonic medium causes chromosomes in prophase nuclei to shorten and thicken rapidly. This observation raises the question of the effects of hypertonic medium on mitotic rate of prophase cells. Since the duration of the mid-mitotic phases is unaffected by hypertonicity, counts of cells in these phases have been made to determine effects on mitotic rate.

Embryos were separated from their membranes and yolk while in isotonic culture medium. Half the embryos were made into culture preparations in hanging drops of isotonic medium and half in hanging drops of slightly hypertonic medium (1.07 × isotonic salt concentration). Mitotic counts were made as previously described.[37] The results are presented in Fig. 11. An analysis of variance test (A. W. Kimball, Mathematics Panel) revealed that the mitotic rate of cells in hypertonic medium is significantly higher than that in isotonic medium. The extremely high mitotic ratio found at 66 min suggests that the cells most susceptible to the "stimulating" effects of hypertonicity are those in early-middle prophase.

X-Ray-Induced Chromosome Stickiness

(Carlson, Harrington, Gaulden)

X-ray-induced stickiness is found in cells that are irradiated at very late prophase, prometaphase,

[39] See section on *Effects of Hypo- and Hypertonicity on Living Cells* of this report.

Fig. 8. Neuroblast in Metaphase. The cell has been subjected to hypertonic culture medium. Note that the chromosomes (23 or 24, depending on sex) have coalesced to form four groups.

Fig. 9. Neuroblast in Early Anaphase. Subjected to hypertonic medium. Note the extreme stickiness of the separating chromosomes.

Fig. 10. Neuroblast in Early Telophase. Note chromosome fragment induced by subjection to hypertonic culture medium at earlier phase.

metaphase, or anaphase and that are examined within an hour of treatment. With a series of doses ranging from 64 to 4096 r, it is found that successively increasing doses successively increase the degrees of response in the grasshopper neuroblast as follows: (1) delayed anaphase separation of a few of the sister chromatids in cells that were in very late prophase at the time of treatment and that were, therefore, the last cells to pass through mitosis before the interval of minimal mitotic activity, (2) a similar effect but with many sister chromatids involved, (3) stickiness involving different chromosomes, induced at very late prophase and evident in prometaphase, metaphase, and anaphase, (4) fusion of different chromosomes by treatment at the same stage in which it is observed, namely, prometaphase, metaphase, and anaphase, and (5) fusion of all the chromosomes into a single, irregular mass at prometaphase, metaphase, or anaphase as a result of treatment at the same stage (Table 10).

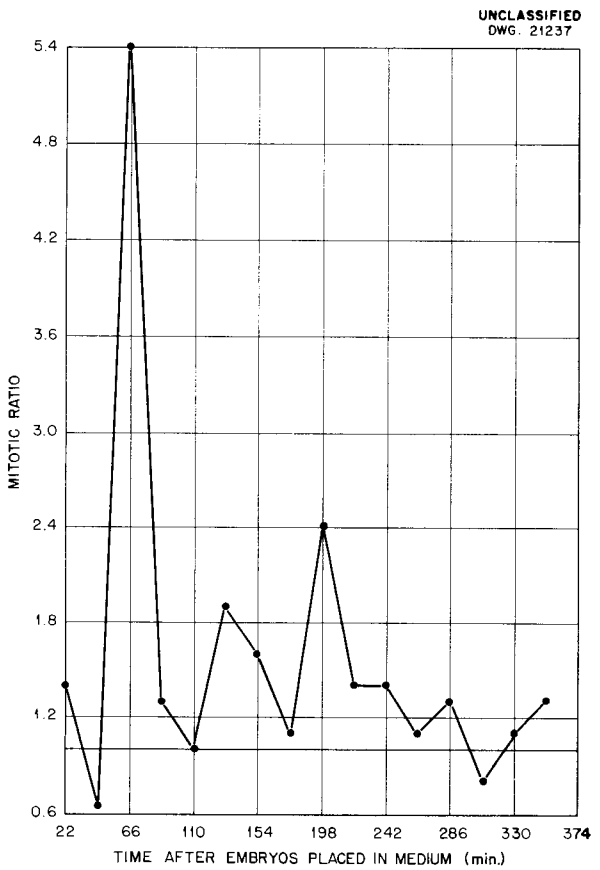

Fig. 11. Effects on Mitosis of Culture Medium Hypertonic to Cells (Eight Embryos per Treatment).

$$\text{Mitotic ratio} = \frac{\left(\text{Number of mid-mitoses in embryos in hypertonic medium}\right)}{\left(\text{Number of mid-mitoses in embryos in isotonic medium}\right)}$$

Effects of X Rays on the ATP Content of Grasshopper Embryos

(Tipton, St. Amand)

The effects of X rays on the adenosinetriphosphate (ATP) content of grasshopper embryos were determined by means of the firefly luminescent system. Eggs from several cases were divided into lots of ten so that each lot contained an equal number of eggs from a case. The eggs were irradiated in a polystyrene dish on moist filter paper. Immediately after irradiation, the embryos were removed from the eggs (both treated and control) and placed in 4 ml of culture medium at 38°C. In a few instances, embryos were irradiated in culture medium. At the end of the incubation period, the embryos were transferred to a small test tube of boiling distilled water. After 10 min of boiling, the embryos were homogenized, and the volume was brought to 5 ml with distilled water. The culture medium in which the embryos had been incubated was also boiled for assay. Preparations were assayed by using firefly enzyme extracted by the method of McElroy and Strehler.[40] One milliliter of the sample was added to 4 ml of buffer (2 ml each of $\frac{1}{15}$ M solutions of sodium arsenate and magnesium sulfate, pH 7.4) and 1 ml of firefly enzyme and water. The amount of firefly enzyme and water was varied according to the strength of the enzyme preparation. Thirty-second light measurements were made with a liquid-nitrogen-temperature photomultiplier that employed the quantum-counting technique,[41] and each preparation was assayed three times to determine the average amount of ATP present.

Media with and without glucose were used, and no difference in ATP content of the treated or control embryos or treated and control media was

[40] W. D. McElroy and B. L. Strehler, *Arch. Biochem.* 22, 420-433 (1949).

[41] B. L. Strehler, *Arch. Biochem. and Biophys.* 34, 239-248 (1951).

TABLE 10. RELATION OF DOSE, MITOTIC STAGE AT TREATMENT, AND MITOTIC STAGE AT DETECTION OF STICKINESS TO TYPE OF STICKINESS INDUCED*

STICKINESS OBSERVED AT →	PROMETAPHASE		METAPHASE			ANAPHASE			
NEUROBLAST IRRADIATED AT (r) →	Very Late Prophase	Prometa-phase	Very Late Prophase	Prometa-phase	Meta-phase	Very Late Prophase	Prometa-phase	Meta-phase	Anaphase
64	O	O	O	O	O	T	O	O	O
256	O	O	O	O	O	ST	T	T	O
512	O	O	S	O	O	ST	T	T	O
1024	S	O	S	O	O	ST	ST	ST	T
4096	S	O	S	S	S	ST	ST	ST	ST

*O, no detectable stickiness; S, adherence of different chromosomes; T, adherence of sister chromatids. Data based on continuous observation of selected cells *in vitro*.

observed when the media were isotonic. No reduction in ATP was found immediately after irradiation or after 4 hr of incubation following treatment with 1000 or 5000 r, but treatment with 10,000 r caused an immediate reduction of 25%. The ATP content in embryos receiving 10,000 r decreased with time at the same rate as that in the controls so that after 4 hr of incubation it was still 25% lower. The ATP in nonirradiated embryos reached an average of 0.019 μg of ATP per microgram of dry weight after 4 hr of incubation.

Effects of X Rays on the Respiration of Grasshopper Embryos

(Tipton, St. Amand)

Studies on the sensitivity of the respiration of grasshopper embryos to X radiation have continued. Embryos were dissected from the eggs, cleared of residual yolk, and placed in Warburg flasks (5-ml capacity) with culture medium containing glucose. Forty embryos were used per flask. X radiation was given through the flat bottom of the flasks. After treatment, the flasks were placed in the bath at 38°C and flushed with oxygen for 3 min during the 10-min equilibration period. Readings were made every 15 min. The results of the first hour are shown in Table 11. The smallest effective dose is apparently between 2000 and 3500 r.

Quantitative Studies on Certain Factors in Cell Division

(Roberts)

Phase-contrast cinematographic records have been made of dividing secondary spermatocytes of the beetle *Popiluis disjunctus* Illiger. A total of 1450 ft of film, at a rate of 8 frames/min, has thus far been recorded of control and irradiated materials. From these film records, measurements will be made of chromosome movement measured from the equator and poles of the spindle, spindle length, spindle volume, and cell volume. Evaluation of the data will be made during the coming academic year.

Records of divisions following X irradiation at 500, 1000, 2500, and 5000 r have been made. The work this summer has been largely exploratory, and the results obtained will guide future investigation.

Preliminary investigations with ultraviolet radiation have been made. The prominent residual "spindle" of the interphase secondary spermatocytes has been shown to react to ultraviolet in much the same manner as the metaphase and anaphase spindle. This suggests the continuation during interphase of oriental spindle material from the first maturation division. This investigation will be continued.

TABLE 11. THE EFFECT OF X RAYS ON THE RESPIRATION OF GRASSHOPPER EMBRYOS

DOSE (r)	PROBABILITY OF A HIGHER VALUE OF t[a]	MEAN DIFFERENCE, CONTROL MINUS TREATED (mm^3 of O_2 per hr per embryo)	DEGREES OF FREEDOM
2,000	0.20	−0.0214	5
3,500	0.05	+0.1050	4
10,000	0.02	+0.1210	4
20,000	0.01	+0.1833	5

[a]G. W. Snedecor, *Statistical Methods*, 4th ed., p. 43-5, The Iowa State College Press, Ames, 1946.

A start has been made in applying the method to additional materials. Work is progressing with *Chortophaga* embryonic cells, especially neuroblasts. Film records of controls have been made, and X-ray effects will be recorded in the near future. The feasibility of applying the method to *Tradescantia* staminate hair cells has been investigated, and film records will be made later.

Correction Factors for Flattening of Spheres and Oblate and Prolate Spheroids in Volume Calculations

(Kimball[42], Roberts)

In quantitative studies of dividing cells, a slight flattening of the cell against the cover slip enhances visibility of internal structures. Such flattening introduces an error into calculations of cell volume, and the error depends on the degree of flattening. Correction factors for the percentage of flattening have been calculated. In the calculations, three assumptions have been made.

1. The solid is oriented so that one of the three axes is perpendicular to the visible cross section (irrelevant if the solid is a sphere).
2. Flattening is uniform on the upper and lower surfaces of the solid and operates in planes parallel to the plane of the cross section.
3. The solid, if not truncated, would be one of the solids considered in the calculation.

The volume of a spheroid is

$$V = \frac{4\pi ab^2}{3},$$

where a and b are the semiaxes. If $a > b$, the solid is a prolate spheroid. If $a < b$, the solid is an oblate spheroid. If $a = b$, the solid is a sphere. If the a axis is shortened by an amount ϵ at both ends, the volume of the truncated solid is

$$V_\epsilon = \frac{2\pi b^2 (a - \epsilon)}{3a^2}\left[3a^2 - (a - \epsilon)^2\right] . \quad (1)$$

The ratio of the two volumes is

$$K = \frac{V_\epsilon}{V}$$

$$= \frac{1}{2a^3}\left\{(a - \epsilon)\left[3a^2 - (a - \epsilon)^2\right]\right\} . \quad (2)$$

Furthermore, if ϵ is expressed as a proportion p of a, that is, if $\epsilon = pa$, the volume ratio becomes

$$K = 1 - \frac{p^2}{2}(3 - p) . \quad (3)$$

On the other hand, if the b axis is shortened by an amount ϵ at both ends, the volume of the truncated solid is

$$V_\epsilon = \frac{2\pi a(b - \epsilon)}{3b}\left[2b^2 + 2b\epsilon - \epsilon^2\right] , \quad (4)$$

and the ratio is

$$K = \frac{b - \epsilon}{2b^3}(2b^2 + 2b\epsilon - \epsilon^2) . \quad (5)$$

Once again, if $\epsilon = pb$, the ratio reduces to Eq. 3.

Thus for any kind of spheroid in which flattening occurs perpendicular to any of the three axes, the corrections given in Fig. 12 are appropriate. Since

[42]Mathematics Panel.

a dumbbell-shaped solid may be approximated as two connected spheres, the correction factors should be appropriate also for cells in telophase.

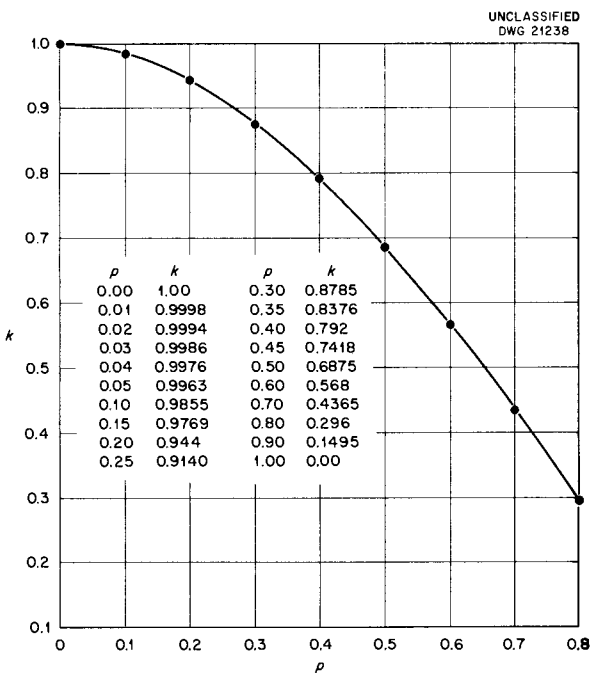

Fig. 12. Correction Factors for Flattening of a Sphere, Prolate or Oblate Spheroid, or Two Spheres.

DROSOPHILA GENETICS

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Induction by Fast Neutrons of Dominant Lethals in *Drosophila*

(Baker, Von Halle)

Males of the Oregon-R strain of *Drosophila melanogaster* were exposed to fast neutrons from the ORNL 86-in. cyclotron. The flies were irradiated in the small facility described by Sheppard and Darden,[45] and the dosage measurements were made by following procedures mentioned in their report. After exposure, the males were mated and the egg hatch was determined in accordance with the method described previously.[46]

No significant difference is noted between the frequency of dominant lethals induced in the first sperm batch ejaculated by the males and in at least one subsequent sperm batch, as is found after irradiation with X rays. The combined data from the sperm batches are presented in Fig. 13. In order to compare the neutron effect with that found with 250-kvp X rays, previously published data of Baker and Von Halle[46] are included in this figure.

Two significant facts are apparent from these data. In the first place, there is no "two-hit" component in the relationship between the percentage of eggs hatched and the neutron dose.

[43]Temporary Employee.

[44]Research Participant.

[45]C. W. Sheppard and E. B. Darden, *Physical Dosimetry in Typical Biological Experiments with Fast Neutrons from a Cyclotron Source*, ORNL-1559 (1953).

[46]W. K. Baker and E. S. Von Halle, *Proc. Natl. Acad. Sci. U.S.* 39, 153-161 (1953).

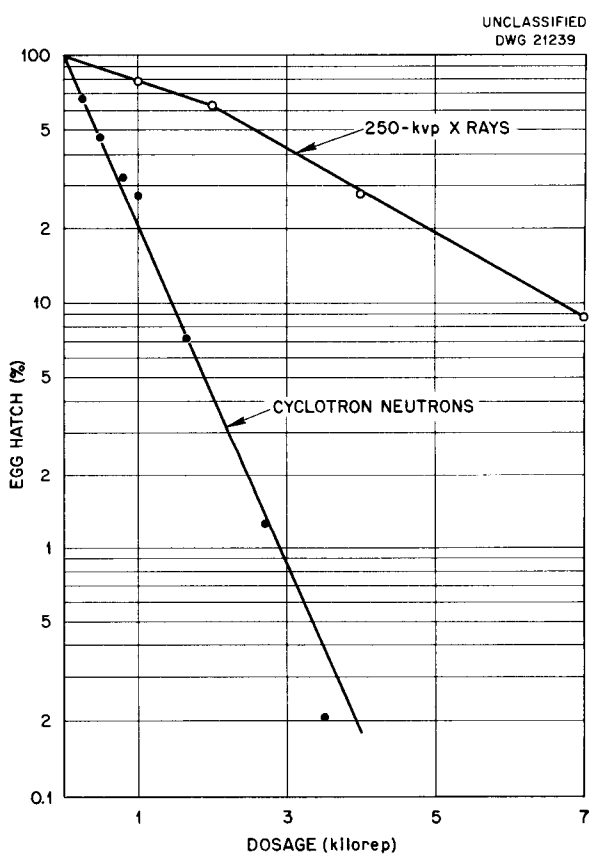

Fig. 13. Dominant Lethals Induced by Comparable Doses of Fast Neutrons and X Rays.

The relationship follows the equation

$$Y = 100e^{-0.161D} ,$$

where Y is the percentage of eggs which hatch and D is the dose in kiloreps. This means that a multihit dominant lethal is caused by one ionizing particle, as is the case with other chromosomal breakage effects. Also, it is evident that the relative biological effectiveness (RBE) of the neutrons as compared with 250-kvp X rays varies from 4.5 to 7.0 over the dosage range covered in these studies. These RBE's are minimum estimates, since there is about 10% gamma contamination in the cyclotron facility used. Still, these values are much higher than those reported previously in the literature for *Drosophila*. For example, Lea,[47] in summarizing the data on dominant lethals available at that time, estimated the RBE for fast neutrons to be about 1.5. The present results, however, are in line with the findings of Giles for chromosome aberrations in *Tradescantia*.[48]

Cytological Analysis of X-Ray-Induced Mutations at Specific Loci in *Drosophila melanogaster*

(Ward)

A cytological analysis of a group of X-ray-induced mutations in *Drosophila melanogaster* has been completed. Induced mutations at eight specific loci in the third chromosome, *roughoid, hairy, thread, scarlet, pink-peach, curled, stripe,* and *ebony-sooty*, were used in this investigation. These mutations were obtained from irradiated spermatogonia and mature sperm by M. L. Alexander.[49] The analysis was undertaken for the purpose of determining the proportion of point mutations to those mutations which are associated with various types of structural changes of the chromosome and comparing the occurrence and the frequency of such aberrations in irradiated sperm and spermatogonia. The viability of the various mutations has been tested by Alexander, and this information is correlated with the types of mutations.

The data are summarized in Table 12. Each mutation was included in one of the four categories on the basis of the cytological analysis. Those mutations which were associated with no cytologically detectable alteration of the salivary gland chromosome were classified as "point mutations." The "deletion" category includes all those mutations which were associated with a detectable loss of genetic material; both one- and two-hit deletions were included in this class. Only those inversions in which one break involved the known location of the mutated gene were included in the "inversion" class. Two types of translocations are represented in the last group: reciprocal translocations and heterobrachial shifts. In all heterobrachial shifts which were studied, the shifted portion of the chromosome was also inverted. All the translocations had one break at, or very close to, the location of the mutated gene.

Of the total of 64 mutations which were analyzed cytologically, 56 were induced in mature sperm and 8 in spermatogonia. In 30 of the 56, 53.6%, mutations from irradiated sperm, it was not possible to detect any chromosomal aberration in the salivary gland chromosomes; 13 of the 56, 23.2%, were found to be due to, or associated with, deletions of various sizes; 5 of the 56, 8.9%, were associated with inversions; and 8 of the 56, 14.3%, were associated with translocations. All mutations from irradiated spermatogonia were classified as point mutations. It is interesting to note that in the case of the point mutations and inversions approximately one-half the mutations in each class are viable and the remaining half are lethal or semilethal. All the deletions are lethal in the homozygous condition. It should be noted that not all the mutations associated with translocations were tested to determine their viability; this will account for the discrepancy in the totals in the table.

The chromosomal aberrations obtained in this investigation were used in locating the eight specific loci. Of the three classes of aberrations, the deletion is the most useful in locating the position of a gene. The evidence from this study confirms the locations of *roughoid, hairy, thread, pink-peach,* and *curled* given by Bridges,[50,51] and in some instances the present information has allowed a more exact location. However, three of the loci, *scarlet, stripe,* and *ebony-sooty*, have been given new locations on the basis of the new information.

[47] D. E. Lea, *Action of Radiations on Living Cells*, Macmillan, New York, 1947.

[48] N. H. Giles, *J. Cellular Comp. Physiol.* 35, Supp. 1, 83-88 (1950).

[49] M. L. Alexander, *Biol. Quar. Prog. Rep. May 10, 1952*, ORNL-1297, pp. 34-37.

[50] P. N. Bridges, *J. Hered.* 32, 64-65 (1941).

[51] P. N. Bridges, *J. Hered.* 32, 299-300 (1941).

The *scarlet* gene has been placed in the 73 A region on the basis of four different *scarlet* deletions. Three deletions and three translocations involving the 90 D segment of the chromosome are evidence that the *stripe* gene is located in that region. Two deletions indicate that the *ebony-sooty* gene is located in the 93 B region.

In these data, which represent a complete series of X-ray-induced mutations, approximately one-half the mutations are associated with no chromosomal aberrations, one-fourth with deletions, and the remaining fourth with inversions and translocations. In both the point mutation and inversion classes, approximately half the mutations are found to be viable in the homozygous condition and the remaining half are lethal or semilethal. Interesting comparisons can be made between these data and the data of other investigators who are analyzing mutations which have been induced by various types of radiations and by chemical mutagens.

TABLE 12. CYTOLOGICAL ANALYSIS OF X-RAY-INDUCED MUTATIONS

CYTOLOGICAL ANALYSIS	LOCI								TOTAL			
									Sperm		Spermatogonia	
	ru	*h*	*th*	*st*	p^p	*cu*	*sr*	e^s	Number	Per cent	Number	Per cent
Point mutation	1	(2)[a]	2	1	6[b]	7 (2)	3 (2)	10[c] (2)	30	53.6	8	100.0
Viable	1			1	3	5 (2)		6 (2)	16		4	
Semilethal		(1)			1	1			2		1	
Lethal		(1)	2		2	1	3 (2)	4	12		3	
Deletion	3	1	1[d]	4[d]			3	2	13	23.2		
Viable												
Semilethal												
Lethal	3	1	1[d]	4[d]			3	2	13			
Inversion		2			2			1	5	8.9		
Viable		1			2			1	4			
Semilethal		1							1			
Lethal												
Translocation		1	1[e]	2		1	3[e]		8	14.3		
Viable												
Semilethal												
Lethal			1				1		2			
Total tested	4	4 (2)	4[d]	7[d]	8	8 (2)	9 (2)	13 (2)	56		8	
Viable	1	1		1	5	5 (2)		7 (2)	20		4	
Semilethal		1 (1)			1	1			3		1	
Lethal	3	1 (1)	4[d]	4[d]	2	1	7 (2)	6	27		3	

[a] Indicate mutations from irradiated spermatogonia.

[b] One mutation is present with an inversion in the third chromosome but cannot be classified as a position effect.

[c] Two mutations are present with inversions in the third chromosome but cannot be classified as position effects.

[d] One deletion involving both the *th* and *st* loci.

[e] One mutation is associated with a shift; the shifted portion is also inverted.

This investigation also provides a comparison of the types of mutations obtained by the irradiation of two different stages in the process of spermatogenesis, the spermatogonium and the mature sperm. Although the sample size of mutations from irradiated spermatogonia is small, there is an indication that the frequency of mutations which are associated with chromosomal aberrations is reduced, if not entirely eliminated.

Comparison of Mutation Rates Produced by Fast Neutrons and by X Rays at Specific Loci in the Third Chromosome of *Drosophila melanogaster*

(Mickey, Yanders)

The degree of radiation damage to biological material can be measured in many different ways by using both cytological and genetic techniques. The latter technique includes studying the induction of dominant lethals, recoverable translocations, recessive sex-linked or autosomal lethals, dominant visible mutations, and recessive visible sex-linked or autosomal mutations. The present investigation utilized the method of determining mutation rates at specific autosomal gene loci. This method should offer one of the most precise means of estimating the relative effects of different types of radiation, as well as comparing the reactions of different genes to the same treatment.

The stocks used were the same as those studied by Alexander,[52] that is, Oregon-R wild type and the *res* stock, which is homozygous for the eight recessive genetic markers, *ru, h, th, st,* p^p, *cu, sr,* and e^s. Irradiated wild-type males, from two to four days old, were mated to virgin *res* females and were transferred to fresh food bottles three times before being discarded. The males remained with the females for a maximum of six days after treatment, thus assuring that only postmeiotic male germ cells were used in producing progeny. All F_1 flies that either partially or entirely exhibit any one or more of the mutant phenotypes were tested further in order to determine whether the variants should be classified as specific germinal mutations or as phenocopies, mimics, dominant mutations at other loci, or modifiers.

Table 13 summarizes results obtained from X-ray experiments and from weapons tests; also, it compares the present X-ray data with those obtained by Alexander. The average mutation rate per roentgen obtained in these X-ray experiments is reasonably close to that of Alexander, 3.24×10^{-8} vs. 5.72×10^{-8}. For the purpose of this table, all data from the weapons tests have been combined and calculated on the basis of rate per roentgen equivalent physical (rep) per locus $\times 10^{-8}$. These figures have been used to calculate the relative biological effectiveness (RBE) of fast neutrons as compared with X rays of 250 kvp, as shown in Table 14. The

[52] M. L. Alexander, *Biol. Quar. Prog. Rep. May 10, 1952*, ORNL-1297.

TABLE 13. MUTATION RATES AT SPECIFIC LOCI AND AVERAGE RATE FOR THE THIRD CHROMOSOME OF *DROSOPHILA MELANOGASTER*

	DOSE (r)	SAMPLE SIZE	RATES AT SPECIFIC LOCI PER ROENTGEN EQUIVALENT ($\times 10^{-8}$)								AVERAGE RATE PER ROENTGEN EQUIVALENT ($\times 10^{-8}$)
			ru	*h*	*th*	*st*	p^p	*cu*	*sr*	e^s	
X ray	3000	39,823	(3)*	(1)	(2)	(3)	(2)	(3)	(7)	(11)	(31)
			2.51	0.84	1.67	2.51	1.67	2.51	5.86	9.21	3.24
			[0.50 to 7.36]**	[0.08 to 4.68]	[0.167 to 6.03]	[0.50 to 7.36]	[0.167 to 6.03]	[0.50 to 7.36]	[2.34 to 12.05]	[4.52 to 16.49]	[2.19 to 4.60]
Alexander***	3000	49,521	(3)	(6)	(4)	(9)	(13)	(8)	(12)	(13)	(68)
			2.02	4.04	2.69	6.06	8.75	5.38	8.08	8.75	5.72
			[0.40 to 5.92]	[1.48 to 8.82]	[0.67 to 6.87]	[2.69 to 11.51]	[4.64 to 15.01]	[2.29 to 10.64]	[4.17 to 14.14]	[4.64 to 15.01]	[4.54 to 7.22]
Fast neutrons	Various	14,439	(9)	(3)	(4)	(7)	(10)	(2)	(5)	(2)	(42)
			32.49	10.83	14.44	25.28	36.11	7.22	18.05	7.22	18.05
			[14.44 to 61.75]	[2.17 to 31.05]	[3.61 to 36.83]	[10.11 to 51.99]	[16.97 to 66.44]	[0.722 to 25.99]	[5.77 to 42.25]	[0.722 to 25.99]	[12.91 to 24.59]

*The figures in parentheses indicate the actual number of mutants.

**The figures in brackets show the 95% confidence limits of the rates.

***Alexander's unpublished data and ORNL-1297.

RBE's range from less than 1 to more than 21 at different loci. The values for the four loci on the left arm of the chromosome may be considered in the same range; those for *cu* and *sr* are equivalent; but the value for p^p is quite high and that for e^s is surprisingly low. Whether the values represent a real difference in response of these loci to different radiations remains to be seen. The RBE for the average of all eight loci on the third chromosome is 5.6, which is in the middle range of RBE's found for dominant lethals.[53]

It should be emphasized that dosages in the weapons tests were less accurately known than those in the X-ray experiments and that although the best available estimates of reps at the various stations were used, the mutation rates and especially the RBE's at different loci must, nevertheless, be considered as very crude approximations. In order to obtain more accurate measurement of effects of fast neutrons, the ORNL 86-in. cyclotron is being used for experiments in which more reliable dosimetry is available.

TABLE 14. ESTIMATED RELATIVE BIOLOGICAL EFFECTIVENESS (RBE) OF FAST NEUTRONS AS COMPARED TO X RAYS OF 250 kvp APPLIED TO SPECIFIC LOCI

GENE LOCI	RBE
ru	12.9
h	12.9
th	8.6
st	10.0
p^p	21.6
cu	2.9
sr	3.1
e^s	0.8
Average rate	5.6

RADIATION PROTECTION AND RECOVERY

Alexander Hollaender — Ruth W. Whittle
G. E. Stapleton — C. W. Edington
Daniel Billen — Dorothy W. McKee
P. W. Rueff, Jr.[54]

Postirradiation Recovery of *Escherichia coli* B/r

(Stapleton, Edington, Rueff)

The general principles involved in the assay for postirradiation recovery of *Escherichia coli* have been described.[55] Recently, a simpler assay method has been devised which involves plate assay with incubation at 37°C. The basis for this method is shown in Fig. 14. A comparison of the relative survivals of irradiated bacteria plated on complete medium (yeast extract agar) and on synthetic medium (inorganic salts + 1% glucose) at a variety of temperatures suggests that plate assays at 37°C may be the most sensitive assay system available. These data may suggest that the assay system described is nothing more than a gross screening of radiation-induced biochemical

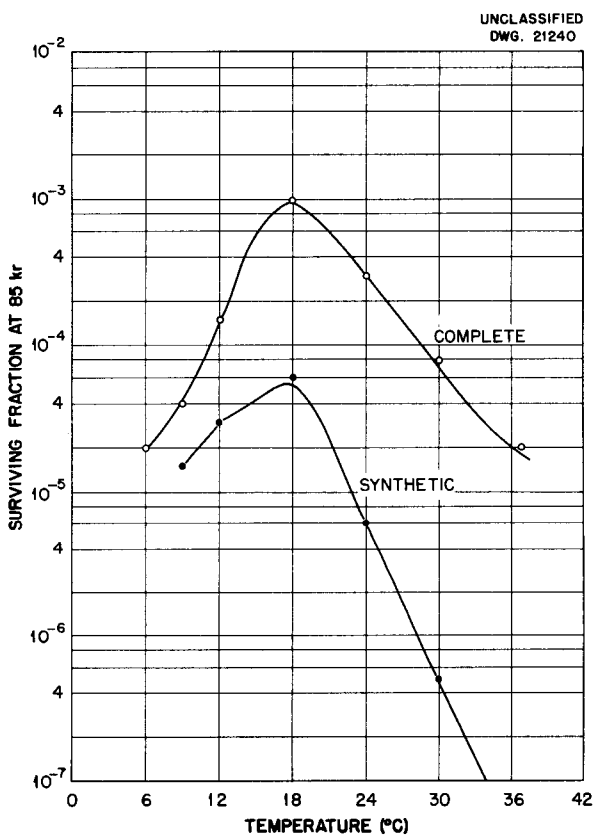

Fig. 14. Surviving Fraction at 85-kr Gamma Rays for *Escherichia coli* B/r Incubated at Various Temperatures on Synthetic Medium Compared with That Obtained on Complete Medium (Difco Nutrient Agar).

[53] W. K. Baker and E. S. Von Halle, *Induction by Fast Neutrons of Lethals in* Drosophilia, of this report.
[54] Temporary Employee.
[55] *Biol. Quar. Prog. Rep. Nov. 10, 1952*, ORNL-1456.

mutants; however, this possibility appears to be ruled out by two lines of evidence: (1) short-time incubation (about 15 min at 37°C) of irradiated cells with yeast extract, followed by washing of cells, results in their ability to survive equally well on complete or synthetic media, and (2) a large number of isolates of colonies of irradiated bacteria were made from complete media; subsequent plating on synthetic media gave no indication of stable induced factor-requiring mutants. Therefore it seemed that some factor in the complete medium may reverse or prevent a process which would result in cell inactivation on a limiting medium. On this basis, it appeared justifiable to attempt an isolation of the factor or factors responsible for reversing or overcoming the radiation-induced damage.

Various source materials, including homogenates of mammalian tissues, were examined for the presence of the factor responsible for reversing or overcoming radiation-induced damage. Figure 15 shows the results of the assay of several tissues of the adult rabbit. Although these data indicate that the factor is generally distributed, the spleen contains relatively more activity per unit of dry weight than do other tissues of the rabbit. As a result of this finding, much effort has been expended during the past period in an attempt to isolate the spleen factor. A pilot experiment on isolation or purification has resulted in a somewhat

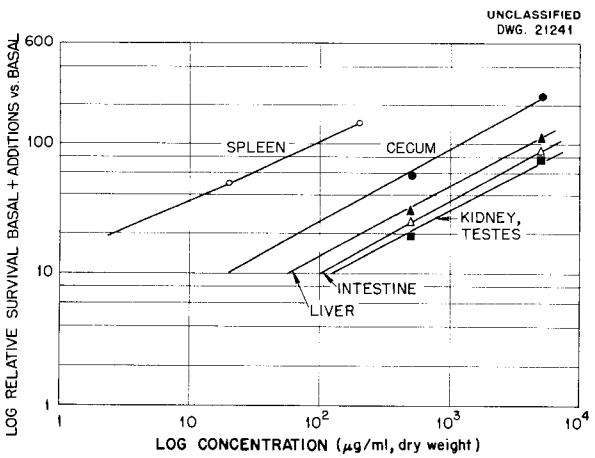

Fig. 15. Relative Activity of Various Rabbit Tissue Homogenates in Increasing Viable Cell Count of Gamma-Irradiated *Escherichia coli* with 37°C Incubations.

purified preparation (approximately 500-fold purified with respect to factor) that is still too crude, however, to permit identification. The quantity of this material obtained indicated that a very low yield (>5%) was being obtained. This suggests the possibility of a multiple requirement, which would explain the low yields since the purification technique used is designed essentially for fractionation with respect to a single factor. Future assays of fractions should and will include combinations of separated fractions, which may demonstrate a multiple requirement and give the necessary information for later identification.

Effects of Postirradiation Temperature Treatment on Induced Mutations in *Escherichia coli*

(Billen, Whittle)

The earlier reported observations[56] on the influence of postirradiation temperature treatment in bringing about an alteration in the survival of X-ray-exposed *Escherichia coli* have led to an investigation of the effects of similar temperature treatment on the induction of mutations in several *E. coli* auxotrophs. The mutations observed in each case were from a particular growth-factor requirement to the nonrequiring or prototrophic state. The several strains studied included strain P82/r (purineless), strain P62 (prolineless), strain T83-8 (tyrosineless), and strain A45-25 (arginineless).

The bacteria were grown for 17½ hr in aerated nutrient broth and then harvested, washed, and resuspended in *M*/15 phosphate buffer (pH 6.8). The cells were then exposed to 40,000 r at 2°C in an atmosphere of oxygen.[57] To determine the number of induced mutations, 0.1 ml of the exposed cell suspensions (approximately 1.5×10^9 cells/ml) was plated on *M*/10 agar plates prewarmed to the temperature at which they were to be incubated for 48 hours. Experimentation[58] established this period of incubation, at any of the temperatures studied, to be sufficient to allow maximal effect of such temperature treatments. Following the incubation period, all plates were placed in the 37°C

[56] G. E. Stapleton, B. Grayson, and A. Hollaender, *Biol. Quar. Prog. Rep. Nov. 10, 1951*, ORNL-1167, pp. 48-51.

[57] For details of the procedure see E. H. Anderson, *Proc. Natl. Acad. Sci. U.S.* 37, 340-349 (1951).

[58] E. H. Anderson, unpublished data.

incubator for a total of 5 days (with the exception of strain T83-8 which was held for 8 to 10 days). For the determination of the surviving cells, the appropriate dilutions of an exposed suspension were made and plated on M/10 agar to which a sufficient concentration of the required nutrient to allow maximum growth was added. These plates were then treated in the same manner as those used in the reversion studies. Unexposed cells were given the same treatment to obtain the "spontaneous" reversion rate. In all cases the data represent the results of at least five duplicate experiments.[59]

The effects of postirradiation temperature treatment on the induced mutation rate per 10^8 viable organisms were found to vary with the strain studied. Strains P62 and P82/r showed a maximum induced mutation rate (Fig. 16) in the temperature range in which minimal survival of the exposed cells was obtained (Fig. 18). (Because of limited space, only the data pertaining to strains P62 and T83-8 will be given.) With strain T83-8, the induced mutation rate increased with increasing incubation temperature up to 18°C, beyond which further temperature increase had no effect (Fig. 17). With this strain there appears to be no simple relationship between the influence of postirradiation temperature treatment on survival and the influence on the induced mutation rate. The fourth strain, A45-25, showed a third type of response in that the maximum induced mutation rate was obtained in the same general temperature range (18 to 24°C) as that at which maximum survival is reached. In all strains studied, with the exception of A45-25, the "spontaneous" rate showed a temperature response pattern that is different from that of the induced rate (Figs. 16 and 17). As a result of incubation at the higher temperatures, strain P62 showed an increase in "spontaneous" rate as compared with a decrease in the induced rate over the same temperature range (Fig. 16).

From the survival data given in Fig. 18, it is seen that the temperature treatment greatly influences survival of these strains and thus has an important influence on the induced mutation rates which are calculated on the basis of viable organisms present following a given temperature treatment. (Similar temperature treatment following plating had no effect on the viability of unexposed cells.) The influence of the temperature treatment is well illustrated by a comparison of the absolute number of revertants and the mutation rate which is based both on the number of viable cells and on the number of mutants. In the four strains studied, it was found that the number of induced mutants per plate (the inoculum size being the same on all plates of a given experiment) was always maximal at 18 to 24°C (Tables 15 and 16). With the unexposed controls, there was no such specific effect on the temperature treatment on the absolute number of revertants since each strain was observed to exhibit a different response to incubation at the various temperatures.

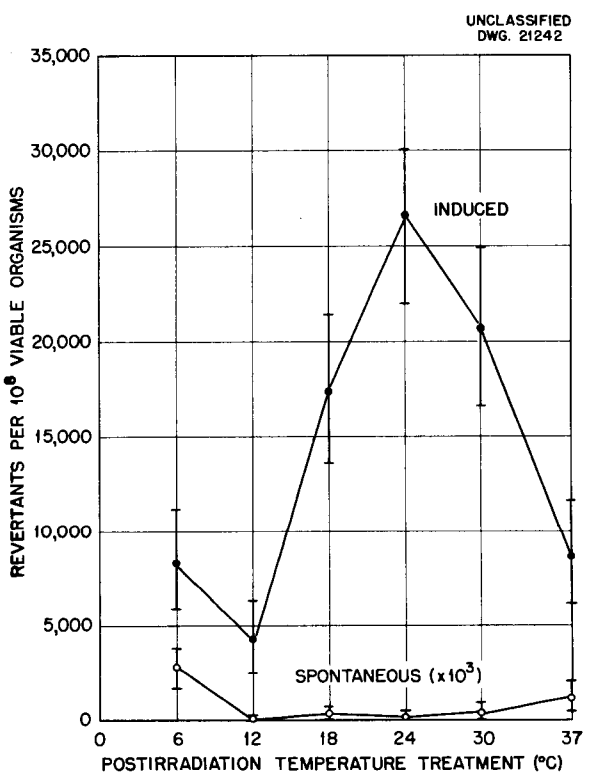

Fig. 16. Effect of Postirradiation Temperature Treatment on the Induced Mutation Rate of *Escherichia coli* Strain P62. The limits shown in the graph and Table 15 are 95% confidence intervals. The number of revertants obtained per plate at the various temperatures of incubation is given in the table.

[59] With the exception of the "spontaneous" reversion data of strain A45-25, the differences that resulted from the 48-hr temperature treatment were found to be statistically significant at the 1% level.

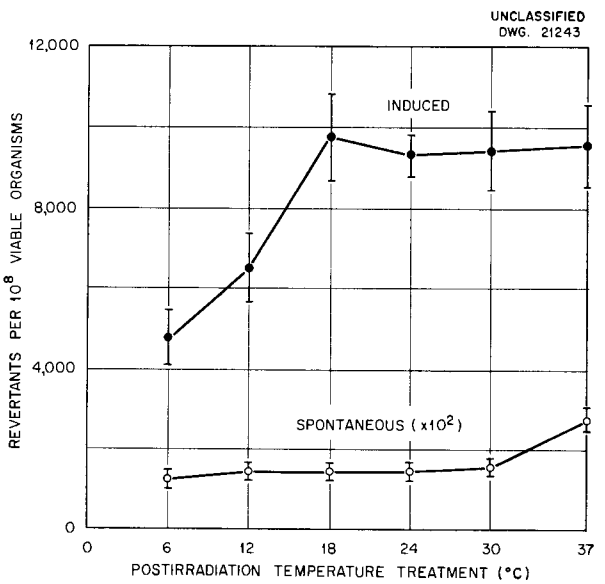

Fig. 17. Effect of Postirradiation Temperature Treatment on the Induced Mutation Rate of *Escherichia coli* Strain T83-8. The limits shown in the graph and in Table 16 are 95% confidence intervals.

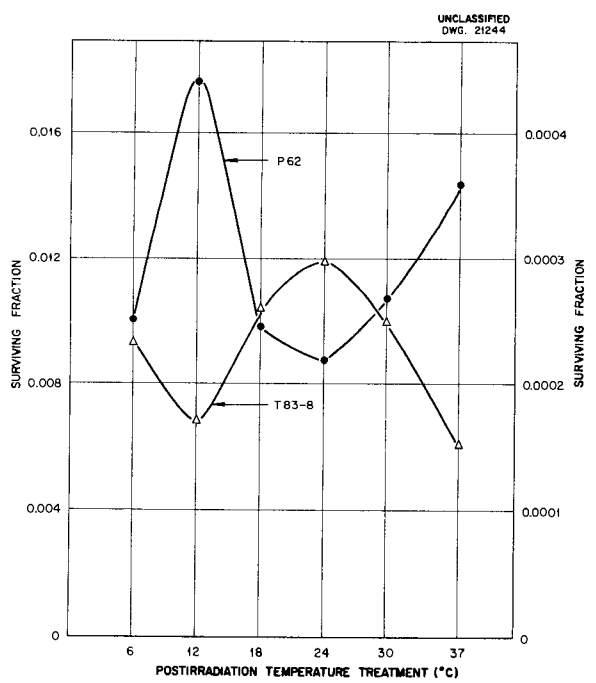

Fig. 18. Effect of Postirradiation Temperature Treatment on Survival. The values plotted for strain T83-8 refer to the left scale, while those for strain P62 refer to the right scale.

ENZYMOLOGY AND PHOTOSYNTHESIS

J. R. Totter
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Audrey N. Best
Janet V. Passonneau[60]

Metabolism of Formate by Rabbit Bone Marrow *in vitro*

(Totter, Best)

A study of the action of an antifolic acid compound (aminopterin) on the incorporation of formate-C^{14} by rabbit bone marrow has indicated the complexity of the reactions involved.

Table 17 gives the representative data that were obtained. The kinetic interpretation of these and additional similar data suggests that there are three routes of fixation of formate carbon which are, in all probability, independent. One route involves reactions which are not inhibited by aminopterin, as is indicated by the failure of the values in the table to approach zero with increasing inhibitor concentration. A second route is illustrated by that formate which was fixed into the acid soluble fraction. In this case, the behavior is that of simple Michaelis-Menten competitive inhibition kinetics. The third route is that of formate fixation into the deoxyribonucleic acid (DNA). In this case, the major portion of the radioactivity has been found to be incorporated into the methyl group of the thymine. The kinetics of this series of reactions indicate that the inhibition is more closely related to the square of the inhibitor concentration. There are several possible interpretations of this result, one of which is that the inhibited enzyme may form a substrate complex containing two molecules of substrate per complex. On the other hand, the inhibition may occur at more than one site in a chain of reactions.

Further dissection of these chemical reactions is necessary to help in interpretation of the biochemical effects of radiation in the formate metabolism of marrow. Irradiation in moderate dosage (500 to 10,000 r) has been found to affect only the series of reactions involving the fixation of formate into DNA, that is, at stages later than the reactions involved in the formation of the acid-soluble compounds.

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[60]Consultant.

TABLE 15. EFFECT OF POSTIRRADIATION TREATMENT ON THE ABSOLUTE NUMBER OF INDUCED REVERTANTS IN *ESCHERICHIA COLI*, STRAIN P62

48-hr INCUBATION TEMPERATURE (°C)	NUMBER OF REVERTANTS PER PLATE*					
	"Spontaneous" Reversions			Induced Reversions		
	Upper limit	Mean	Lower limit	Upper limit	Mean	Lower limit
6	9.60	6.50	4.00	6.01	4.54	3.28
12	0.30	0	0	5.64	4.22	3.01
18	1.80	0.61	0.05	13.25	11.02	9.01
24	1.40	0.39	0.01	16.46	13.97	11.69
30	2.30	0.91	0.20	14.67	12.32	10.18
37	5.40	3.16	1.50	8.82	7.02	5.43

*Total number of cells per plate = $\sim 1-2 \times 10^8$ and for any given experiment was constant for all six temperatures.

TABLE 16. EFFECT OF POSTIRRADIATION TREATMENT ON THE ABSOLUTE NUMBER OF INDUCED REVERTANTS IN *ESCHERICHIA COLI*, STRAIN T83-8

48-hr INCUBATION TEMPERATURE (°C)	NUMBER OF REVERTANTS PER PLATE*					
	"Spontaneous" Reversions			Induced Reversions		
	Upper limit	Mean	Lower limit	Upper limit	Mean	Lower limit
6	23.4	20.1	16.9	77.2	68.1	59.5
12	25.9	22.5	19.2	90.9	81.0	71.7
18	27.1	23.4	20.0	176.3	162.4	149.1
24	27.1	23.5	20.1	172.9	159.1	145.9
30	28.4	24.7	21.2	162.8	149.5	136.7
37	48.9	44.0	39.3	106.0	95.3	85.1

*Total number of cells per plate = $\sim 1-2 \times 10^8$ and for any given experiment was constant for all six temperatures.

Evidence that Molybdenum is a Nondialyzable Component of Xanthine Oxidase

(Totter, Burnett, Monroe,[61] Whitney,[61] Comar[61])

The isolation and purification of xanthine oxidase from the milk of cows which have received Mo^{99} have provided evidence that molybdenum is an integral part of the xanthine oxidase of milk and, presumably, of other biological materials. This finding is in accord with the evidence given by Westerfeld and Richert[62] for the existence of a nutritional factor that is essential for the production and maintenance of rat intestinal xanthine oxidase, and is in agreement with the identification of this factor as molybdenum by De Renzo *et al.*[63]

Labeled sodium molybdate was injected intravenously into a dairy cow, and xanthine oxidase

[61] UT–AEC Agricultural Research Program.

[62] W. W. Westerfeld and D. A. Richert, *J. Biol. Chem.* 129, 35 (1951).

[63] E. C. De Renzo, E. Kaleita, P. Heytler, J. J. Oleson, B. L. Hutchings, and J. H. Williams, *J. Am. Chem. Soc.* 75, 753 (1953).

TABLE 17. EFFECT OF AMINOPTERIN ON ACID-SOLUBLE SUBSTANCES AND DNA

AMINOPTERIN CONCENTRATION (μg/ml)	RELATIVE ACTIVITY (percentage of control)	
	Acid-Soluble Substances	DNA
0	100	100
0.24	91	91
0.48	85	87
0.95	79	84
1.43	68	59
1.9	62	55
2.4	66	58
2.9	62	62
3.3	63	59
3.6	61	34
3.8	61	48
4.3	62	36
4.8	59	33
9.5	55	33
15.2	53	24

TABLE 18. RATIO OF RADIOMOLYBDENUM TO XANTHINE ACTIVITY AT VARIOUS STEPS DURING THE ISOLATION OF XANTHINE OXIDASE FROM THE MILK OF A COW

	COUNTS/SEC OF Mo^{99} PER UNIT OF XANTHINE OXIDASE
Milk	1095
Cream	71
Na_2HPO_4 extract	67
Na_2HPO_4 extract after $CaCl_2$ treatment	88
Xanthine oxidase after 1st $(NH_4)_2SO_4$ precipitation	12.1
Xanthine oxidase after 2nd $(NH_4)_2SO_4$ precipitation	12.6
Xanthine oxidase after 20-hr dialysis	10.9
Xanthine oxidase after 40-hr dialysis	10.6
Average for 4 different purified samples (7 milkings)	10.61 (range 10.4 to 10.78)

was isolated by the method of Ball[64] from milk collected one to several days thereafter. Two weeks later, this procedure was repeated with the same cow. In all cases after the initial removal of excess molybdenum by one or two treatments with 25 to 40% ammonium sulfate, the ratio of Mo^{99} to xanthine oxidase activity remained essentially constant despite rigorous purification procedures. This is indicated in Table 18 for a typical sample. Xanthine oxidase was estimated by Kalckar's method[65] and Mo^{99} was estimated by radioassay procedures.

The Mo^{99} was not removed from, and probably did not enter, the xanthine oxidase merely by exchange. This was demonstrated by the addition of 2 g of ammonium molybdate to a sample of milk from the treated cow and the subsequent isolation of the enzyme. This treatment with inert molybdenum did not change the ratio of Mo^{99} to xanthine oxidase. In another experiment, nonradioactive milk (126 units of xanthine oxidase) was mixed with an equal amount of radioactive milk (46 counts/sec per unit of xanthine oxidase; 10.6 counts/sec per unit of xanthine oxidase after purification; 91 units of xanthine oxidase). After isolation of the enzyme and dialysis against water for 40 hr, the xanthine oxidase had a value of 4.23 counts/sec per unit, which is in good agreement with the value of 4.43 that would be expected if there were no exchange of the excess Mo^{99} with the inert molybdenum of the nonradioactive xanthine oxidase (Table 18).

Spectrophotometric data suggest a molar ratio of flavin to molybdenum of 2:1.

It has long been recognized that molybdenum is an essential nutrient for plants, in which it serves metabolic functions involved in the utilization of nitrogen. It would now seem that molybdenum also plays an essential role in animals, at least to the extent that xanthine oxidase is concerned with necessary metabolic processes. In addition, the toxic behavior of excess molybdenum in the copper-molybdenum imbalance may possibly be explained in terms of oxidase activity.

[64] E. G. Ball, *J. Biol. Chem.* 128, 51 (1939).

[65] H. M. Kalckar, *J. Biol. Chem.* 167, 461 (1947).

Photosynthesis

W. A. Arnold Eleanor S. Meek
J. M. Siegel[66]

Further Studies of Delayed Emission Spectrum in Green Plants and Purple Bacteria (Arnold, Meek). A delayed light that is emitted by green plants and that lasts for some seconds after the plants have been irradiated has been described.[67] The determination of the emission spectrum of the delayed light has been completed and has been found to be the same as the spectrum of the fluorescent light of chlorophyll in the living plant. This result justifies the assumption that the delayed light represents the reversal of some of the early steps in the process of photosynthesis.

It has been shown that the purple bacteria also have a delayed light emission after irradiation and that the intensity of the emission is of the order of a hundred times smaller than that of the green plant. It has been possible to show that the action spectrum for the delayed light from purple bacteria has a peak between 8000 to 9000 Å, which conforms to the peak in the action spectrum of their photosynthesis. Because of the very low intensity of the light emitted, it has been impossible to determine the emission spectrum of the delayed light for the bacteria, except to show that the wave length is longer than 8000 Å.

Lately, the investigation of the green plants has been resumed in order to gain a better understanding of the relation between the delayed light and photosynthesis. No results are available as yet, except for the finding that when the plants are illuminated with flashes of light shorter than a few hundredths of a second, the intensity of the delayed light depends only on the product of intensity and the duration of the flash.

Quantum Yields in Bacterial Photosynthesis (Siegel, Arnold). Preliminary studies have been made to determine the quantum yield for the conversion of acetone to acetoacetate by *Rhodopseudomonas gelatinosa:*

$$\text{Acetone} + CO_2 \longrightarrow \text{Acetoacetate} \qquad \Delta F = +10\,\text{kcal}$$

The reaction has been shown to be the first step in the metabolism of acetone by this bacterium, and, under the conditions of low light intensity, it appears to be the major photochemical reaction.[68] The reaction is particularly significant in that it accomplishes, in a single step, the fixation of carbon dioxide into a molecule that has the same oxidation level as carbohydrate and that is approximately equivalent in free energy content.

As the first step in the investigation, manometric techniques were employed to determine the rates of photosynthesis with acetone and acetate as a function of light intensity. The saturation value for acetate was found to be about five times the value for acetone. The ratio of the slopes (which are proportional to the quantum yields) indicated that the quantum yield for acetate is about 1.5 to 1.7 times that of acetone on a molar basis, that is, moles of substrate metabolized per quantum. It was noted, however, that the initial portion of the acetate curve deviated from linearity and indicated a net production of acid in the absence of light.

The correspondence between acetate utilization and the manometric data was investigated by means of acetate-$2C^{14}$, and the results are presented in Fig. 19, in which it can be seen that the uptake of C^{14} (triangles), that is, the actual utilization of acetate, corresponded very closely with the manometric data (circles) at all light intensities.

When succinate-$2C^{14}$ was substituted for acetate, the C^{14} uptake at low light intensities did not coincide with the manometric data but diverged only slightly from an extrapolation of the curve at high light intensities.

These findings can be interpreted as indicating that acetate is produced from endogenous substances by a nonphotosynthetic process which can be reversed by light. The reversal, however, must involve at least two light-dependent reactions with different quantum yields in order to account for the change in slope.

The observation that acetic acid is produced by a fermentative process that is "inhibited" by light indicated the possibility of an explanation for the previous observations regarding the accumulation of acetate from acetone. Therefore light intensity curves for acetone were redetermined by a manometric technique that permitted the simultaneous measurement of acetate produced and acetone metabolized. The results, presented in Fig. 20,

[66] Research Participant.

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

[68] J. M. Siegel, *Federation Proc.* 11, 287 (1952).

indicated that the accumulation of acetate continues at light intensities far greater than the intensity required to completely suppress the production of acetate by the nonphotosynthetic process.

At present the data are insufficient for an explanation to be made of the accumulation of acetic acid during the photometabolism of acetone. However, the original observations have been reaffirmed, and it has been demonstrated that the nonphotosynthetic production of acetate cannot account for the acetic acid accumulation.

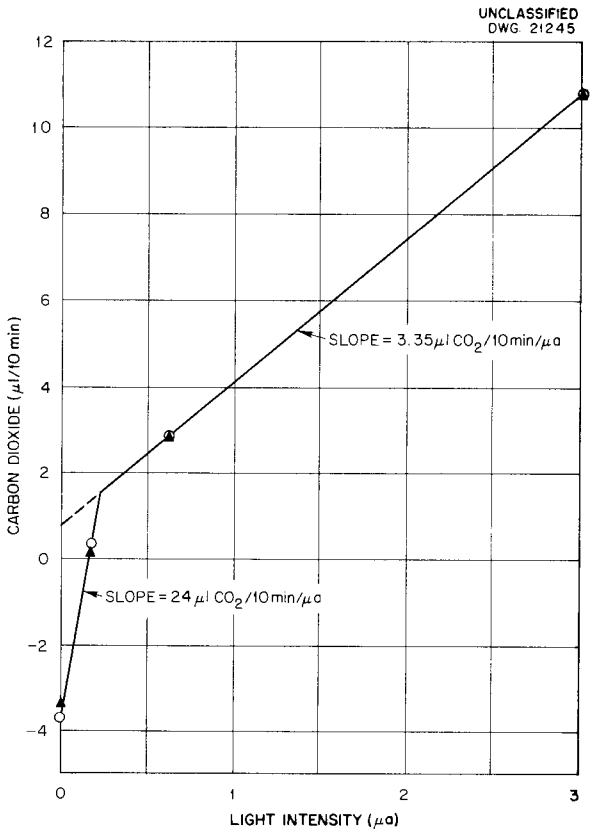

Fig. 19. Rate of Metabolism of Acetate-2C^{14} as a Function of Light Intensity. Each pair of points represents data obtained from a single Warburg vessel. The circles represent the rate of uptake or evolution of carbon dioxide and the triangles represent the rate of incorporation of C^{14}. Each vessel contained 6.0 ml of a bacterial suspension in 0.0025 *M* $NaHCO_3$ and 0.6 ml of 0.1 *M* sodium acetate-2C^{14}. The final cell density was 21 mm^3/ml. Light intensities on individual Warburg vessels were adjusted by means of screens.

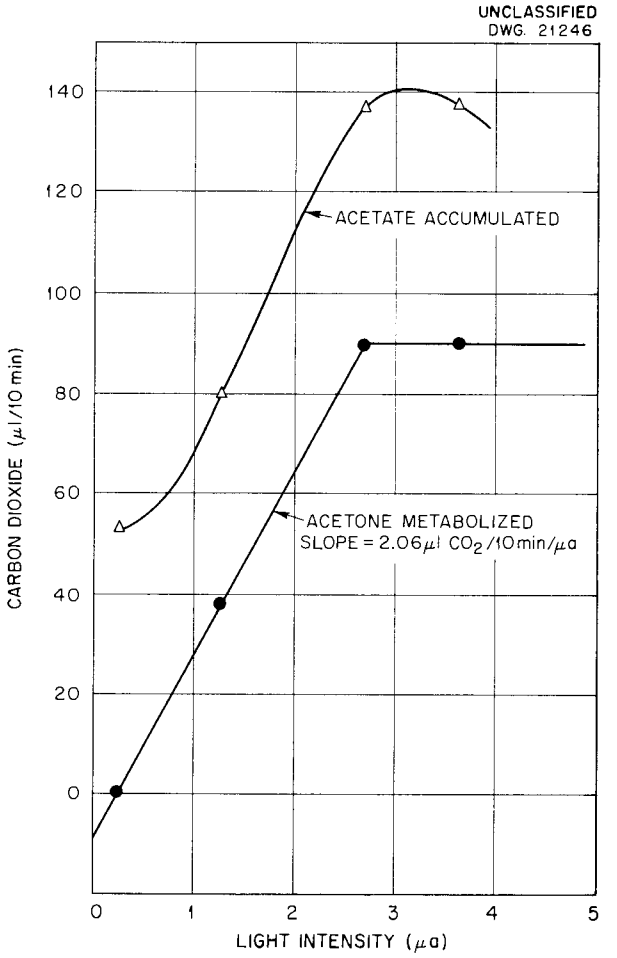

Fig. 20. Simultaneous Measurement of the Rate of Acetone Metabolism and Acetate Accumulation as a Function of Light Intensity. The pair of points at each light intensity represents data obtained from a single Warburg vessel. The circles represent the net disappearance of carbon dioxide, and the triangles represent the liberation of carbon dioxide from the buffer. Each vessel contained 6.0 ml of a bacterial suspension in 0.0033 *M* $NaHCO_3$ and 0.3 ml of 0.2 *M* acetone. The final cell density was 17.5 mm^3/ml. Light intensities on individual Warburg vessels were adjusted by means of screens.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell – Section Chief

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Radiation-Induced Mutations in the Mouse

(W. L. Russell, Oakberg, Gower, Bangham, Crowell, Kelly, Major, Millemann, Spear)

Additional data from the 1000-r X-ray experiment on specific loci strengthen the evidence that the mutation rate at the 1000-r dose is lower than expected, on the hypothesis of linearity, from the rate obtained at 600 r. This finding suggested the possibility that the rates for doses lower than 600 r may be higher than expected from the 600-r rate. Therefore, as was emphasized in the latest report,[1] it appeared important, for the estimation of human hazards, to determine the mutation rate for at least one lower dose. A new experiment, using 300 r, was accordingly started.

The preliminary data from the 300-r experiment show a total of 14 presumed mutations, at the seven specific loci tested, in a total of 23,034 offspring examined. This rate is slightly, but not significantly, higher than expected on the hypothesis of linear increase between 0 and 600 r. Final conclusions on the shape of the curve cannot be made until the mutants have been tested and more data have been collected.

The 300-r data already collected are adequate in one respect. They support, at this dose level, the most important conclusion reached from the 600-r data, namely, that the induced mutation rate in the mouse is approximately one order of magnitude higher than the rates obtained from similar experiments on *Drosophila*.

Neutron-Induced Dominant Lethals in the Mouse

(Russell, Russell, Gower)

During the past few months, an intensive study has been made of the rate of induction by neutrons of dominant lethal genetic changes in the mouse. The work has included a laboratory experiment and a field test of a nuclear weapon. The field test will be reported elsewhere. A summary of the laboratory experiment is given here. The information obtained was not only important in itself, in view of the lack of knowledge of genetic effects of neutrons in mammals, but also was required for efficient planning of the field test. The laboratory experiment had to be made somewhat hurriedly, and the only suitable facility immediately available was the ORNL 86-in. cyclotron. The authors are greatly indebted to C. W. Sheppard and E. B. Darden of the Biophysics Section for designing and testing an exposure chamber and for resolving the difficulties of physical dosimetry. Details of exposure and dosimetry are given in a report by Sheppard and Darden.[2]

Four young adult 101×C3H hybrid male mice were exposed at each of the doses listed in Table 19, with the exception that only two mice were irradiated at the 305- and 369-rep doses. On the day following exposure, each male was placed with four nonirradiated females of the same strain. The uteri of females that were pregnant from matings made from two to six days after irradiation of the male were removed at a late stage in pregnancy and the uterine contents examined. At the same time, the number of corpora lutea in each of these females was recorded. The results are given in Table 19.

There are various possible ways of reducing the results to a simpler form for dosage-effect comparisons. The method chosen here is to express survival in terms of the number of ovulated eggs as measured by the number of corpora lutea. Since it is apparent that, as with X rays, death from induced dominant lethal aberrations occurs before or shortly after implantation, "survivors" have been counted as those embryos which live through day 10½ of gestation. Survival measured in this way is plotted against dose in Fig. 21. A weighted least-squares fit of the data on the assumption that log survival is linearly related to dose is also given in Fig. 21.

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[1] W. L. Russell *et al.*, *Biol. Quar. Prog. Rep. Feb. 10, 1953*, ORNL-1497, p. 26.

[2] C. W. Sheppard and E. B. Darden, *Physical Dosimetry in Typical Biological Experiments with Fast Neutrons from a Cyclotron Source*, ORNL-1559 (1953).

TABLE 19. DOMINANT LETHALITY IN OFFSPRING OF MALE MICE EXPOSED IN CYCLOTRON

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
DOSE RECEIVED BY MALE (rep)	NUMBER OF PREGNANCIES	NUMBER OF CORPORA LUTEA	PERCENTAGE OF CORPORA LUTEA REPRESENTED BY					WEIGHTED LEAST-SQUARES FIT* OF COLUMN 8
			Living Fetuses	Embryos or Fetuses Dying After Day 10½	Embryos Dying Between Implantation and Day 10½	Embryos Dying Before Implantation (100 minus sum of columns 4, 5, and 6)	"Survivors" (sum of columns 4 and 5)	
0	20	192	79.69	2.60	6.77	10.94	82.29	80.64
47	10	92	53.26	1.09	20.65	25.00	54.35	58.37
56	13	125	48.80	0.80	25.60	24.80	49.60	54.87
82	12	125	44.00	0.00	19.20	36.80	44.00	45.89
99	10	92	40.22	0.00	27.17	32.61	40.22	40.83
116	8	77	36.36	0.00	28.57	35.06	36.36	36.33
141	5	45	22.22	0.00	40.00	37.78	22.22	30.60
160	10	93.7**	25.61	2.13	33.08	39.17	27.75	26.85
194	6	66	27.27	1.52	21.21	50.00	28.79	21.26
209	5	52.7**	22.77	0.00	28.46	48.77	22.77	19.18
252	4	38.7**	10.34	0.00	25.84	63.82	10.34	14.27
257	2	20	10.00	5.00	45.00	40.00	15.00	13.79
305	1	9	11.11	0.00	22.22	66.67	11.11	9.92
311	1	9.7**	0.00	0.00	30.93	69.07	0.00	9.52
369	0							

*Calculated by A. W. Kimball of the Mathematics Panel. Weights employed were reciprocal variances of log proportion of survivors, that is, $\frac{1}{V(\log p)} = \frac{pn}{q}$.

**Includes one pregnancy in which, because of early death of all embryos, there were no corpora lutea. In this case the number of ovulated eggs was taken as 9.7, which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

Data collected earlier by Kile on the same strain of mice in an X-ray experiment in which there were 50 pregnancies from males exposed to 800 r and 47 pregnancies from control males show a survival proportion of 0.38 for the 800-r dose. Comparing this with the cyclotron data, and assuming that 10% of the dose in the cyclotron figures was due to gamma rays, it appears that, for dominant lethals in the mouse at this level of effect, the RBE of neutrons compared with X rays is about 8.

Many details not mentioned in this summary will be presented in the final report. Perhaps the most important of these is that the dominant lethal effect is much greater in matings made at a later time, namely, 19 to 26 days, after exposure.

Effects of Prenatal X Irradiation on the Vertebral Column and Thorax of Newborn Mice

(L. B. Russell)

In continuation of the work dealing with the morphological effects of prenatal irradiation, abnormalities of the vertebral column and thorax have been studied in detail. Four hundred and thirteen newborn mice, irradiated with 250-kvp X rays during uterine life, were examined in cleared, stained, skeletal preparations. Treated stages differed by 24-hr intervals and ranged from day ½ to day 13½ after fertilization. The dosages employed were 200 r (100 r in a few cases) for a survey of all stages and 300 and 400 r for dosage comparisons at the last five stages. The irradiated embryos and controls were hybrids between two pure strains (C57BL female × NB male). All the irradiated animals and most of the controls came from second litters.

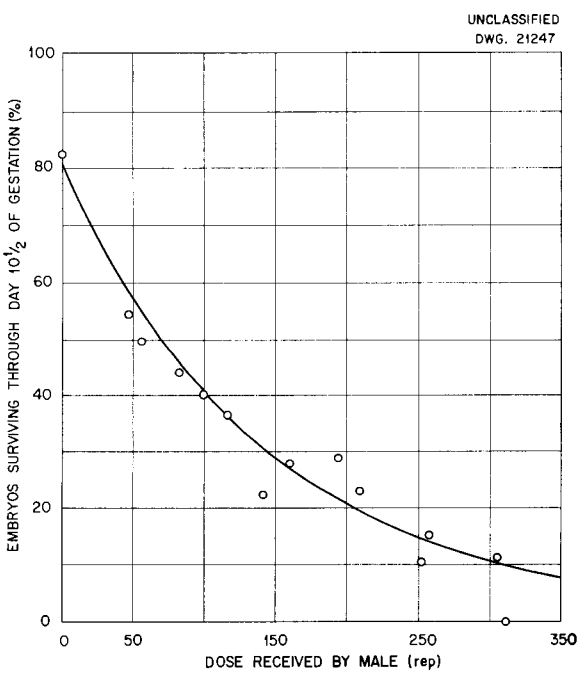

Fig. 21. Dominant Lethality in Offspring of Male Mice Exposed in Cyclotron. The data are from Table 19. The weighted least-squares fit is based on the assumption that log survival is linearly related to dose.

The following is a summary of the results:

1. As had been the case with anomalies involving external and gross visceral characters,[3] each skeletal abnormality in the vertebral column and thorax was, in general, characteristic for irradiation during only a short period in prenatal development and was usually obtained with high incidence following treatment at that stage. The developmental interval during which radiation must be applied to produce a given abnormality at some specified stage of observation (birth, in this experiment) is here referred to as the "critical period" for that abnormality.

2. Survivors from irradiation during preimplantation stages, which greatly increases prenatal mortality,[4,5] are almost free of abnormalities. The critical periods of characters studied fall between days 6½ and 13½ postconception, when irradiation produces little or no prenatal mortality.[5]

3. Since as many as 50 types of abnormalities of the vertebral column and thorax have been listed and described, only a few major syndromes can be summarized below. All critical periods are expressed in days postconception.

(a) Intersegmental jumbling (which often entails loss of vertebrahalves) in the cervical and thoracic regions, with correlated changes in ribs, costal cartilages, and sternum, has its peak critical period on day 7½.

(*b*) The critical peak for posterior shift at the thoracolumbar border, highly correlated with the occurrence of an extra costosternal junction and the

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

[4] L. B. Russell, W. L. Russell, and M. Henderson, *Biol. Quar. Prog. Rep. Nov. 10, 1950*, ORNL-889, p. 27.

[5] L. B. Russell, W. L. Russell, and M. H. Major, *Biol. Quar. Prog. Rep. Aug. 10, 1952*, ORNL-1393, p. 30.

ossification of an extra sternebra, is day $8\frac{1}{2}$; day $7\frac{1}{2}$ is also highly sensitive.

(c) Depression, by various means, of the lumbosacral profile, often associated with spina bifida aperta, can be obtained by irradiation on day $9\frac{1}{2}$ only.

(*d*) A high threshold syndrome produced by irradiation on days $9\frac{1}{2}$ or $10\frac{1}{2}$ consists of reduction in number and broad fusions of sternal elements, (leading to a shortened sternum, which – in the extreme case – may consist of only one bone), irregular mesh formation of costal cartilages, a decrease in sternocostal junctions, and rib fusions.

(e) Arch-centrum fusions have as their peak stage day $11\frac{1}{2}$. When these fusions occur in the thoracic region, they are highly correlated with angulation of ribs and reduction of the last rib(s).

(*f*) Abnormalities of the arcus anterior of the atlas are producible at two different periods: in correlation with malformations of the lateral masses – day $7\frac{1}{2}$ peak; and in correlation with splitting of cervical centra – day $10\frac{1}{2}$ peak.

(*g*) Peak sensitivity to the splitting of both lumbar and thoracic centra occurs on day $9\frac{1}{2}$. Arch anomalies, which are of various types having different critical periods, probably occur independently of centrum splitting.

(*h*) Reduction in the average number of ossified tail vertebrae parallels externally observed tail shortening.[3] In some of the high-dose groups (peak day $11\frac{1}{2}$) the vertebral column ends with, or even within, the pelvic girdle.

4. Although comparison of radiation-induced abnormalities with the effects of genes that act on the axial skeleton must not be used to draw conclusions about the manner of primary gene action, it may lead to closer timing of some of the major gene effect. A few examples of parallelism follow:

(*a*) One set of radiation-induced symptoms (critical day $9\frac{1}{2}$) – tail abnormalities, urogenital and anal defects, spina bifida – copies part of the action of a group of phenotypically similar genes (*T, Fu, Ki, Sd, ur, Ts, Lp, Sp, pt*); there are other parallelisms with these genes but there are also disparities.

(*b*) Another group of abnormalities produced by irradiation on day $9\frac{1}{2}$ parallels most of the manifestations of the *screw-tail* gene.

(c) The jumbling syndrome copies the action of the gene *stub* but is less extreme.

5. The developmental action of irradiation at any one stage does not copy the widespread effects of such genes as *undulated* and *short-ear*, whose primary action is probably on an entire tissue. In the case of radiation injury, the pattern of differential distribution of the initial cellular damage may be determined by some common attribute (for example, high rate of activity) of a variety of morphologically unrelated precursor processes.

6. The critical periods for environmental factors that normally produce homeotic variations in the axial skeleton have been determined. Thus, the thoracolumbar border is sensitive to posterior shift on day $8\frac{1}{2}$ and, less so, on day $7\frac{1}{2}$. It is sensitive to anterior shift on day $11\frac{1}{2}$.

7. The correlation of a given change in development with embryological events that take place at the stage demonstrated to be critical for that change indicates that, in the case of a number of abnormalities, sensitivity is apparently linked with a high degree of activity in the primordia themselves. There are, however, several other abnormalities for which the sensitive stage would not have been predictable on the basis of visible events. In some cases, the sensitive stage involves an extremely early precursor of the character studied, while in other cases, the effective radiation damage may occur comparatively late.

Postirradiation Destruction of Spermatogenic Cells Following Exposure to X Rays

(Oakberg, Crowell)

In some recent reports on X-ray damage to the mammalian testis, the disappearance of spermatogenic elements has been attributed to inhibition of mitosis of spermatogonia.[6,7] This conclusion is based on failure to find degenerating cells rather than on demonstration of a reduced mitotic rate of surviving cells. However, reports by LeBlond and Clermont[8] on normal spermatogenesis in the rat, make it plausible to assume that even a moderate amount of necrosis of spermatogonia could explain a marked depletion of germinal elements after radiation. Mitoses among the few surviving spermatogonia would

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

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

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

be difficult to detect. The low number of spermatogonia observed at four days after irradiation suggests that cell death has occurred but that the time intervals chosen for study have not been suitable for demonstrating degenerating cells. Cell death may have an important bearing on the transmission of mutations induced in spermatogonia,[9] and an attempt has been made to determine the amount of cell degeneration which occurs in the testes of mice following exposure to X rays.

The influence of time after irradiation on degeneration following exposure to 600 r of X rays was chosen for initial study. 101×C3H hybrid males twelve weeks old were used in order to have material comparable to that used in genetic experiments being conducted in this laboratory. Both whole and partial body exposures were made; in the partial body exposures, approximately the anterior two-thirds of the animals were shielded with lead. Two mice were killed for each type of exposure (whole or partial body) at intervals of 1, 2, 3, 6, 12, and 18 hr, and at 1, 2, 3, 4, 5, 7, 10, 15, 21, and 28 days. One testis was fixed in B_{15}, and the second testis was fixed in Zenker-formol. Serial, 10-μ sections of the B_{15} material were stained in hematoxylin and eosin and were examined with a 44X objective and 10X oculars. A random sample of tubules was obtained by moving the sections back and forth across the field in the vertical plane, the only criterion of selection being that the tubular cross-sectional area be smaller than the microscope field. Lateral movement of the stage was made in only one direction in order to avoid previously sampled areas. A sufficient number of tubules were scored in each mouse to obtain 30 with degenerating cells; in some animals, one section was adequate but in other cases several sections had to be examined. No attempt was made to reduce variation due to the fact that cross-sections occur at random through the tubules, and, therefore, include different stages of the spermatogenic wave. Some selection of particular stages of development occurs automatically because degeneration is concentrated at specific times in the spermatogenic process. Since there was no striking difference between mice given whole and partial body radiation, the data were pooled to give a mean value for the four animals killed at each time interval. Numbers of degenerating cells per tubule given in Table 20 are based only on tubules showing that particular type of cell.

An early rise in number of degenerating spermatogonia is evident (Table 20), with a peak at 12 hr, in both number of necrotic cells per tubule and number of tubules affected. The mean for 18 hr is about half the 12-hr figure, and a rapid decline continues until subnormal values are reached on the fourth day. The frequently expressed opinion that there is only inconsequential degeneration of spermatogonia after X-ray exposure[6,7] is not supported by these data, for 70% of all tubules show a mean of 6.4 dead spermatogonia at one time interval (12 hr). Even this high rate may not represent the peak in degeneration, for each interval is a static segment of a dynamic process in which elimination of necrotic cells is rapid. If dead cells were not removed quickly, the large drop from 12 to 18 hr could hardly occur. Therefore all the data given in Table 20 can lead only to an underestimate of total destruction of cells. Much of the debate over radiation damage to spermatogonia undoubtedly arises from disagreement over classification of primitive cell types in the normal mammalian testis. The problem of classification becomes even more difficult when it is complicated by degenerative changes.

Most of the cells scored as degenerating spermatocytes have a highly acidophilic cytoplasm, with division arrested at metaphase or anaphase of the first meiotic division. Some cells also degenerate in pachytene. The amount of necrosis shows considerable variation during the 12-hr to ten-day interval, but there appears to be a regular increase in number of degenerating primary spermatocytes from 12 hr to seven days. Again, it is difficult to estimate total destruction, for it occurs in different tubules at different times and is spread over a ten-day period. Rapid lysis of necrotic cells occurs, and, as with spermatogonia, an underestimate of total destruction is certain to result. Degenerating primary spermatocytes disappear between the tenth and fifteenth day. Consequently, both the initial damage and the subsequent degeneration could occur during the 10½-day interval which has been estimated as the duration of the primary spermatocyte stage in the rat.[8] However, species differences and the effect of X rays on rate of maturation must be determined before this statement can be conclusive.

[9] W. L. Russell, *Biol. Quar. Prog. Rep. Feb. 10, 1953*, ORNL-1497, p. 26.

TABLE 20. DEGENERATION OF SPERMATOGENIC ELEMENTS AT VARIOUS INTERVALS AFTER 600 r OF X RAYS

TIME AFTER IRRADIATION	TOTAL TUBULES	PERCENTAGE OF TUBULES WITH DEGENERATING SPERMATOGONIA	PERCENTAGE OF TUBULES WITH DEGENERATING SPERMATOCYTES	PERCENTAGE OF TUBULES WITH DEGENERATING CELLS (all types)	MEAN NUMBER OF DEGENERATING SPERMATOGENIA PER TUBULE	MEAN NUMBER OF DEGENERATING SPERMATOCYTES PER TUBULE
Control	3,212	7.1[a]	6.5	13.1	1.7	2.1
1 hr	873	8.4[b]	8.3	14.8	1.7	2.2
2 hr	654	11.5	9.6	19.7	1.8	2.1
3 hr	595	12.3	11.3	21.8	1.9	3.2
6 hr	318	32.2	14.2	40.1	3.3	4.5
12 hr	153	69.8	33.4	79.3	6.4	7.6
18 hr	269	37.0	24.5	49.7	3.4	9.4
1 day	299	26.9	19.4	40.4	1.9	7.9
2 days	594	10.2	12.5	20.4	1.6	9.3
3 days	488	7.3	23.4	29.1	1.7	9.0
4 days	790	0.8	16.0	16.4	0.5	10.5
5 days	630	0.8	19.8	20.1	1.5	12.7
7 days	362	0.6	33.5	33.5	0.7	13.0
10 days	456	0.4	28.0	28.4	0.5	8.4
15 days	985	0.0	12.5	12.5		2.0
21 days	845	2.5	12.4	14.4	1.5	1.9
28 days	615	6.1	14.4	19.8	2.1	1.9
	12,138					

[a]Control values based on 14 males.

[b]Each figure represents mean of 4 males.

The degenerating spermatogonia and spermatocytes characteristic of the 6-hr to 12-day period are absent at 15 days. All tubules show evidence of severe cellular depletion. The degeneration occurring involves giant cells, many of them multinuclear.

The 21- and 28-day intervals are characterized by regeneration of the germinal epithelium. At 21 days, this repopulation of cells is limited primarily to spermatogonia, but by 28 days some cells are entering late primary spermatocyte stages. Some giant cells persist and show degenerative changes, but at these intervals most cell death occurs relative to repopulation of the tubules. Many primitive germinal cells apparently sustain injuries which do not become manifest during mitosis, but prevent the cell from completing meiosis.

Sufficient degeneration of spermatogonia has been demonstrated to account for the depletion of the germinal epithelium after exposure to 600 r of X rays. Also, a considerable number of primary spermatocytes receive damage which results in degeneration during the first meiotic division. Detection of necrotic cells is dependent upon selection of proper time intervals for study, which are about 12 to 18 hr after irradiation for spermatogonia and from 12 hr to ten days for primary spermatocytes. Spermatids and sperm show less evidence of degenerative changes. In view of the observed degeneration of spermatogonia, it appears unnecessary to invoke inhibition of mitosis as an explanation of the depletion of germinal elements following radiation.

PATHOLOGY AND PHYSIOLOGY

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Cataract Induction by Fast Neutrons

(Upton, Christenberry, Hurst,[5] Farbstein, Ledford)

Studies are in progress for determining the relative biological effectiveness, (RBE) in cataract induction, of neutrons of 14-Mev energy(Cockcroft-Walton generator[6]), neutrons of approximately 2.5-Mev energy (polonium-boron[7]), gamma rays (Co^{60}[8]), and 250-kvp X rays. The parameters investigated include the comparison of the effects of single exposures of mice, rats, and rabbits and the determination of the threshold cataract-inducing dose of fast neutrons in mice. Neutron and gamma-ray exposures have been fractionated at a single dose level so that the RBE of fast neutrons under conditions of remittent irradiation can be studied.

Preliminary observations indicate that the threshold opacity-inducing dose in mice is below 10^8 neutrons/cm^2, as mice exposed to this dose of 2.5-Mev neutrons have mild opacities (grade I) within eight months postirradiation. The radiosensitivity of the lens of the rat and of the rabbit is much lower; mild (grade I) opacities appear in rats eight months postirradiation only after 10^{10} neutrons/cm^2 and rabbits remain negative during the same interval after this dose. Also in contrast to mice, rats and rabbits have failed to develop opacities during the first year after exposure to 333 r of gamma radiation. Observations to date indicate that no appreciable decline of cataract-inducing effectiveness results from the fractionation of neutron radiation at weekly or subweekly intervals.

Cooperative Projects with ANP for the Air Force

(Nelson, Anderson)

So that the relative biological effectiveness (RBE) of neutrons and X rays could be determined, mice, guinea pigs, and rats were exposed to cyclotron neutrons and to 250-kvp X rays. The parameters studied thus far include LD_{50}/30 days, cataract induction, and bone marrow activity as indicated by Fe^{59} uptake in red blood cells and by white cell counts. The preliminary LD_{50} studies have been nearly completed; the others are in progress. The tentative neutron-to-X-ray ratios for acute lethality are as follows: In mice, 1 rep of neutrons equals about 3.4 rep of X rays; in rats, 1 rep of neutrons equals about 3.0 rep of X rays; and in guinea pigs, 1 rep of neutrons equals 2.3 rep of X rays.

A technique for quantitatively measuring erythropoietic depression in irradiated mice by the use of tracer quantities of Fe^{59} has been devised and tested.

Late Effects of Single Exposure to Atomic Detonation (Operation Greenhouse)

(Upton, Ledford)

Data on longevity and pituitary-tumor induction by a single massive exposure to gamma rays and neutrons are shown in Tables 21 to 23. These data indicate that pituitary tumors can be induced in mice by a single exposure to ionizing irradiation. The greatest incidence occurred among mice exposed to gamma-ray doses somewhat below the

(1) Consultant.
(2) U.S. Air Force.
(3) Temporary Employee.
(4) Research Participant.
(5) Health Physics Division.
(6) The Cockcroft-Walton generator was made available by J. L. Meem and R. G. Cochran, Physics Division.
(7) Source provided by K. Z. Morgan, Heolth Physics Division.
(8) Source provided by C. W. Sheppard and E. B. Darden, Jr., Biology Division.

TABLE 21. LONGEVITY AND INCIDENCE OF PITUITARY TUMORS IN MICE EXPOSED TO ATOMIC DETONATION

DOSE (r)[a]	NUMBER EXPOSED,[b] MALES AND FEMALES	FEMALE MICE SURVIVING FOR[d]						PITUITARY TUMOR		MALE MICE SURVIVING FOR[a]						PITUITARY TUMOR	
		30 days		1 year		25 months				30 days		1 year		25 months			
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
812 to 932	880	25	6	15	3	0	0	0	0	39	9	25	6	0	0	0	0
759 to 785	440	87	40	57	26	0	0	0	0	85	39	58	26	4	2	3	5
711 to 733	440	157	71	118	53	0	0	14	12	153	70	130	59	13	6	2	2
631 to 687	440	195	89	146	66	9	4	17	12	204	93	176	80	37	17	8	4
491 to 556	440	215	98	179	81	43	19	22	12	208	95	185	84	75	34	4	2
367 to 424	440	78[c]		66		23		7	11	108[c]		102		43		0	0
287 to 318	440	213	97	195	89	58	26	10	5	213	97	202	92	112	51	2	1
192	220	107	97	102	93	45	41	4	4	107	97	101	92	62	56	0	0
0	620	301	97	300	97	189	61	1	0.3	310	100	294	95	198	64	0	0

[a]Predominantly (over 90%) gamma radiation; the ratio of gamma rays to neutrons being somewhat greater in the lower dose range.

[b]Half were males, half were females.

[c]These figures are incomplete because of loss of animals during transportation from field laboratories.

[d]Postirradiation. The mice were irradiated at 6 to 12 weeks of age. This tabulation was prepared approximately 26 months postirradiation.

TABLE 22. INCIDENCE OF PITUITARY TUMORS IN MICE EXPOSED TO ATOMIC DETONATION

MONTHS POSTIRRADIATION	FEMALE			MALE		
	Number Dying	Pituitary Tumor		Number Dying	Pituitary Tumor	
		Number	Per cent		Number	Per cent
1 to 10	139	0	0	115	0	0
11 to 13	83	3	4	56	0	0
14 to 16	148	3	2	81	0	0
17 to 19	244	22	9	178	6	3
20 to 22	231	21	9	241	7	3
23 to 25	215	26	12	226	4	2

LD_{50}, and the tumor was about four times more frequent among females than among males. Neutrons may be more efficient than gamma rays in inducing these neoplasms, and the data suggest that doses as low as 40 to 70 rem may induce such tumors. The latency period of the neoplasms is very long, the maximum incidence being at 17 to 25 months postirradiation.

Essential Progressive Iris Atrophy in Mice

(Benedict, Christenberry)

The hybrid mice, designated LAF_1, that were used in atomic-detonation studies were found to show a hereditary abioatrophy of the iris. The atrophy made its appearance when the animal was approximately 9 to 11 months of age. In animals exposed to ionizing irradiation, the iris atrophy was more complete. The degree of iris atrophy could be divided into four grades (I to IV). Only occasionally did the unirradiated iris advance to an early grade III. Table 24 shows the degree of iris atrophy in relation to the dose of irradiation in X-rayed mice. The mice in this group were all exposed when six to nine weeks of age; at present they are 22 to 23 months of age. The higher the dose of X ray, the greater was the iris atrophy. Exposure to fast neutrons and gamma rays produced similar iris changes.

TABLE 23. INCIDENCE OF PITUITARY TUMORS IN FEMALE MICE EXPOSED TO NEUTRON RADIATION FROM ATOMIC DETONATION

DOSE (rem)*	NUMBER EXPOSED	MICE SURVIVING FOR				PITUITARY TUMOR	
		30 days		1 year	25 months		
		Number	Per cent	Number	Number	Number	Per cent
450	30	27	90	20	4	2	10
180	30	28	93	22	3	3	14
140	20	20	100	18	2	3	17
70	30	30	100	29	14	2	7
40	30	27	90	24	13	1	4
0	310	301	97	300	189	1	0.3

*The rem values were calculated on the basis of thymic weight loss following 230-kvp X radiation given by R. E. Carter and associates.

TABLE 24. IRIS ATROPHY IN MICE IN RELATION TO X-RAY DOSE*

X-RAY DOSE (r)	NUMBER OF ANIMALS	
	Grade I	Grade II
0	8	99
25	16	2
50	12	5
100	10	10
200	10	8
300	6	10
400	4	14
500	1	13
Totals	67	161

*Mice were irradiated at 6 to 9 weeks of age and examined 21 to 22 months postirradiation. The controls were 29 months old.

ACTH-Secreting Transplantable Pituitary Tumors

(Gadsden, B. Anderson, Gurney)

Two pituitary tumors that occurred in mice exposed to ionizing irradiation proved to be readily transplantable and caused, in the recipients, obesity with thymic atrophy, hyperglycemia, polyuria, sometimes with glycosuria, severe lymphocytopenia, and hypertrophy of the adrenal cortex. On the basis of these findings, it is concluded that the tumors secrete ACTH. There is no evidence of stimulation of any endocrine organ other than the adrenal.

Morphologic Changes Associated with Thyrotrophin-Secreting Pituitary Tumors

(Gude, Gadsden)

A study of the morphologic changes of I^{131}-induced pituitary tumors of different types has been concluded and the results have been submitted for publication. Morphological characteristics of dependent and autonomous transplanted pituitary tumors, which were originally induced by radiothyroidectomy, and the secondary changes caused by these tumors are described. Dependent tumors are composed of chromophobe cells that are fairly uniform in size and shape; they lack the features of cancer cells. Autonomous tumors exhibit features of anaplasia that are characteristic of a malignant neoplasm. Retrogressive changes (necrosis, fibrosis, hemorrhage, and hemosiderosis) are more often encountered and are more marked in autonomous growths. Factors to explain the latter are discussed in the monograph to be published.

Chromophobe cells are capable of secreting thyrotrophins, and secretion of thyrotrophins by these tumors invariably occurs. Secretion of thyrotrophin is correlated with morphologic evidence of stimulation of the thyroid gland and with formation of adenomata in normal hosts that bear autonomous

tumors. Gonadal stimulation is usually present in radiothyroidectomized hosts that bear large tumors, and this may be due to secretion of minute quantities of gonadotrophins by some of the tumor strains studied or may result from some other factors.

The morphologic changes in hosts that bear these tumors suggest a lack of secretion of pituitary hormones other than thyrotrophins, with the possible occasional exception of traces of gonadotrophins. Cystic dilatation of the biliary tract occurs only in radiothyroidectomized mice, and, with very rare exceptions, it is associated with gonadal stimulation.

The following alterations seen in mice that bear pituitary tumors remain to be explained: retrogressive changes in the reticular zone of the adrenal; hyperplasia with cystic dilatation of the extrahepatic biliary tracts; extensive ductal hyperplasia of the mammary gland of radiothyroidectomized female mice; cleft-like spaces in pancreatic cells; pathogenesis of sudden death with signs suggestive of thyrotoxicosis in mice that bear intrapulmonary thyroid grafts. The secondary changes in relation to pituitary tumors induced by I^{131} in different hosts are shown in Table 25. The monograph to be published is illustrated by 77 photographs and photomicrographs.

Leukemogenesis by Ionizing Irradiation

(Furth, Upton)

The results of the study of leukemogenesis by ionizing radiation were presented at the International Radiological Congress in Copenhagen, Denmark and will appear in the *Acta Scandinavica Radiologica*. The essential findings of recent research on leukemogenesis by ionizing irradiation are surveyed. All types of ionizing radiations are leukemogenic, and the induction rate varies with the total dose, dose rate, and interval of fractionation. The leukemogenic action of irradiation is markedly influenced by partial shielding and by genetic and physiologic factors.

Three sets of new experiments in mice are summarized:

1. The leukemogenic effect of instantaneous, massive, whole-body irradiation from nuclear detonation on a large population of genetically uniform adult mice is shown in Table 26. The induction of lymphomas within 16 months is high (14 to 16%) after doses above the LD_{50}; it drops rapidly with smaller doses, and it is negligible below 400 rep (Table 26).

2. The leukemia-inducing action of thermal-neutron–gamma radiation was essentially identical

TABLE 25. SECONDARY CHANGES TO I^{131}-INDUCED PITUITARY TUMORS IN DIFFERENT HOSTS

	THYROID	OVARIES AND UTERI	BILIARY-TRACT ECTASIA	ADRENAL	THYMUS
Primary tumor	Absent	0	0	0	Normal or atrophic
D tumor Host I^{131}-treated	Absent	0 to +++*	0 to +++	Degenerative changes in reticularis	Atrophic
A tumor Host normal	+ to +++ enlargement	0	0 to +	Degenerative changes	Normal or atrophic
Host I^{131}-treated	Absent	0 to ++	0 to ++	Degenerative changes	Atrophic

*+ = degree of specific hormonal stimulation.

TABLE 26. INCIDENCE OF LYMPHOMAS IN MICE 18 MONTHS AFTER EXPOSURE TO NUCLEAR DETONATION

DOSE RANGE (rep)*	PERCENTAGE SURVIVING 4 MONTHS	TOTAL NUMBER SURVIVING 4 MONTHS**	PERCENTAGE OF LYMPHOMA		
			In Males	In Females	Total
812 to 841	6.8 to 17.8	54	17	11	14.8
759 to 785	32.7 to 41.8	164	16	13	14.6
711 to 733	63.6 to 75.9	307	8	4	6.2
631 to 687	86.8 to 92.7	395	7	10	8.4
491 to 556	94.5	416	3	5	3.8
192 to 424	96.3 to 97.7	833	0	0.5	0.2
0	98.5	611	0	0	0

*Approximate.

**Approximately half were males, half were females.

to that of X radiation and included the induction of myeloid, as well as lymphoid, leukemias.

3. Cortisone appeared to reduce the incidence of lymphomas and myeloid leukemia in mice when it was given with a certain time relation to irradiation. There are marked differences in the reduction with respect to sex.

Platlet Life-Span Studies

(Odell, Gamble)

It was previously reported that the platelet life span in the rat was believed to be four to five days. This estimate was based on determinations of the activity of platelets at intervals of 12 hr to ten days following the injection of formate-C^{14}. These data are subject to different interpretations, and other workers have suggested a life span of one or two days.

Experiments on the transfusion of platelets labeled with methionine-S^{35} *in vivo* into nonlabeled rats are intended for testing the validity of the four-to-five day life span, and data are now being collected. Present information demonstrates that some of the transfused platelets circulate for four days in the recipient rat, thus supporting the earlier interpretation of the results of the C^{14} experiments.

A comparison of the results of injecting two different levels of methionine-S^{35} into rats showed that the degree of labeling of platelets and plasma was in direct proportion, within the limits tested, to the amount of radioactivity injected. The platelets and plasmas of rats injected with 200 μc of methionine-S^{35} and sampled three days postinjection were four times more radioactive than those of rats injected with 50 μc.

It has been found that prebleeding enhances the platelet yield in rats and has the added advantage of providing platelets of a more uniform age. (After bleeding, the blood volume was restored with plasma.) The platelet count was found to be about 20% higher than is normal four days after a bleeding of approximately 30% of the rat's blood volume. Two bleedings at 24-hr intervals, each about 30% of the calculated blood volume, raised the platelet count somewhat more than did one bleeding.

The method of processing the C^{14} samples by wet oxidation, collection of the radioactive carbon dioxide in an ionization chamber, and counting on a vibrating-reed electrometer proved to be sensitive and dependable. It is, however, very time consuming and has limited the progress of the work. Therefore a gas flow counter has been adopted and it appears to be equally sensitive, allows more rapid determinations, and is adaptable to both C^{14} and S^{35} counting.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson

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M. I. Dolin Susanne Berger
J. M. Adams[1]
E. A. Delwiche[2]

Oxidation and Peroxidation of $DPNH_2$[3] in Extracts of *Streptococcus faecalis*, 10C1

(Dolin)

Among the streptococci, which contain no cytochrome components or catalase, even though terminal respiration passes through flavin carriers, it does not invariably yield peroxide. However, various workers[4,5] have found that cell suspensions of certain strains of streptococci carry out peroxidase-like, apparently nonhematin-dependent reactions in the presence of some organic substrates. Recent work in this laboratory on $DPNH_2$ oxidation in *Streptococcus faecalis*, strain 10C1, has produced further information.

When cell-free extracts of *S. faecalis*, strain 10C1, are prepared from organisms grown under conditions in which they carry out a lactic acid fermentation, they nevertheless contain a potent $DPNH_2$ oxidizing system (80 μl of oxygen uptake per hour per milligram of protein, in crude extracts).[6] Although these organisms contain no demonstrable cytochrome components, added cytochrome c acts as an electron acceptor and causes $DPNH_2$ to be oxidized about four times as rapidly as it would be in air. There is no cytochrome c oxidase or peroxidase activity. In the absence of cytochrome c, the oxidation of $DPNH_2$ occurs with the uptake of $\frac{1}{2}$ mole of oxygen per mole of $DPNH_2$ utilized and no peroxide accumulates, although the extracts are completely devoid of catalase activity. The oxidation has been shown to involve an intermediate peroxidation as follows:

$$DPNH_2 + O_2 \longrightarrow DPN + H_2O_2$$

$$DPNH_2 + H_2O_2 \longrightarrow DPN + 2H_2O$$

$$\text{Sum:}\quad 2DPNH_2 + O_2 \longrightarrow 2DPN + 2H_2O$$

Peroxidation can be made to be the limiting step by diluting the enzyme (but not by dialysis), and peroxide accumulates under these conditions. Peroxidation can be demonstrated spectrophotometrically by following $DPNH_2$ disappearance at 340 mμ under anaerobic conditions in specially constructed cuvets that can be evacuated. The reaction rate thus determined is consistent with the belief that peroxidation is an intermediate step in the over-all reaction.

The material balances are shown in Table 27. Peroxide was determined by a micromethod.[7] Control experiments show that the peroxidation is not due to the breakdown of H_2O_2 by the isolated components of the system or by ultraviolet light. Monometric experiments, in which 20 times as much $DPNH_2$ and H_2O_2 were used and in which peroxide was determined with catalase, gave entirely analogous results. Oxidized or peroxidized $DPNH_2$ can be reduced again with alcohol dehydrogenase in the presence of ethanol.

It is not yet established whether the $DPNH_2$–cytochrome c reductase is identical with the oxidase; however, the apoenzyme(s) has (have) been prepared by the method of Warburg and Christian[8] and can be reactivated with FAD but not with flavin mononucleotide. The cytochrome c reductase of *Escherichia coli*[9] is also FAD specific.

The enzyme responsible for the peroxidation has been completely separated from the oxidase by ammonium sulfate fractionation so that, aerobically, there is no reaction in the absence of added peroxide; however, there is as yet no evidence for the

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(1) ORINS Predoctoral Fellow.

(2) Temporary Employee.

(3) The following abbreviations are used: diphosphopyridine nucleotide, DPN; dihydrodiphosphopyridine nucleotide, $DPNH_2$; and flavin adenine dinucleotide, FAD.

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

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

(6) Enzymically reduced $DPNH_2$ that was prepared with alcohol dehydrogenase according to the method of N. O. Kaplan was used in all experiments.

(7) W. A. Patrick and H. B. Wagner, *Anal. Chem.* **21**, 1279 (1949).

(8) O. Warburg and W. Christian, *Biochem. Z.* **298**, 150 (1938).

(9) A. F. Brodie, *J. Biol. Chem.* **199**, 835 (1952).

TABLE 27. OXIDATION AND PEROXIDATION OF $DPNH_2$ IN EXTRACTS OF *STREPTOCOCCUS FAECALIS*

TYPE OF EXPERIMENT	ADDITIONS	Δ(μmoles)		
		O_2	$DPNH_2$	H_2O_2
Aerobic*	4.3 μmoles of $DPNH_2$	−2.1	−4.27	+0.05
Anaerobic**	1.14 μmoles of $DPNH_2$		−0.005	
	+0.17 μmoles of H_2O_2		−0.19	−0.17
	+0.34 μmoles of H_2O_2		−0.36	−0.34
	+0.34 μmoles of H_2O_2, without $DPNH_2$			0
	+0.34 μmoles of H_2O_2, without enzyme		0	0

*Conditions: Potassium phosphate, pH 6.0, 200 μmoles; $MnSO_4$, 2 μmoles; enzyme, 0.4 ml; $DPNH_2$ as shown; H_2O to 2 ml; incubation at 37°C for 20 min.

**Conditions: Conditions the same as aerobic experiment, except for 0.2 ml of enzyme; $DPNH_2$, and H_2O_2 concentrations as shown; H_2O to 2.7 ml; incubation at 22°C for 8 min.

nature of the prosthetic group. There is no inhibition by 0.01 M cyanide or 0.05 M azide. Present evidence rules out the intermediate reduction by $DPNH_2$ of the disulfide linkage in cystine, oxidized glutathione, or lipoic acid. The pH optimum of the peroxidation as catalyzed by the partially purified (65-fold) enzyme lies between 5 and 5.7.

The peroxidation demonstrated here represents a departure from the classical peroxidases and offers a mechanism for the peroxidation of organic compounds whose oxidation is DPN-coupled.

Succinic Acid Decarboxylation by Propionic Acid Bacteria

(Carson, Delwiche, Phares, Long, Berger)

Previous reports have described the ATP-CoA-dependent succinate activation by cell-free extracts of *Propionibacterium*, the nonstoichiometric production of propionate and carbon dioxide, and the reversible reaction in which propionate is incorporated, in preference to carbon dioxide, into succinate. These observations were interpretable in terms of a "C_1" compound (not carbon dioxide) that was derived from succinate and which could reversibly combine with propionate to produce succinate.

In an attempt to account for the remainder of the C_1 which did not combine with propionate or appear as carbon dioxide, experiments were carried out to determine any possible interrelationships between a presumed direct exchange of a C_1 compound with succinate and exchange via the better known mechanisms of carbon dioxide fixation. By utilizing a malonate block for the succinic-dehydrogenase system, C^{14}-labeled succinate (carboxyl-to-methylene specific activity ratio of 9:1) was incubated with cell extract in the presence of carbon dioxide, fumarate, malate, and a small amount of propionate. The resulting acids were isolated, purified, and degraded (Table 28). Radioactivity analyses showed that, in the case of the higher malonate concentration, the ratio of specific activities of the carboxyl carbons to noncarboxyl carbons was experimentally of the same order in the final succinate as in the final propionate, namely, 7:1, whereas that of the malate was 34:1. Less divergence in the ratios resulted when less malonate was used. The activities obtained in fumarate correctly placed it between succinate and malate. These results demonstrate that propionate is produced directly from succinate and not through the malate-pyruvate sequence of reactions or through other more remote reactions. The activity unaccounted for in the carbon dioxide (which contained less than 10% as much activity as did the propionate) was found to be present in the carboxyl groups of malate and fumarate. Indeed, the total

TABLE 28. MALONATE EFFECT

CONDITIONS	CARBOXYL-TO-NONCARBOXYL SPECIFIC ACTIVITY RATIOS			
	Propionate	Succinate	Fumarate	Malate
Initial		8.8		
No malonate	9.8	10.0	9.5	9.0
0.1 *M* Malonate	7.9	6.8	17.5	25
0.2 *M* Malonate	7.1	6.4		34

activity found in the carboxyl groups of malate and fumarate was greater than that predicted. This observation is explainable by considering that the radioactive C_1 fragment can exchange reversibly into the large malate-fumarate pool and thus yield a C_1 fragment of lower specific activity; the pool in this case acts as a sump for the radioactivity. However, the propionate, which has a very small pool, recombines with the diluted C_1 to reform succinate and thus causes a reduction in the propionate pool. It was shown previously in an exchange experiment that an appreciable percentage of labeled propionate is fixed into succinate under such conditions in spite of the unfavorable equilibrium. A dilution of the C_1 fragment, compared with that of the propionate, is also indicated in the carboxyl-to-noncarboxyl activity ratios of the propionate and of the succinate. Other experiments have shown that increasing malonate concentration increases propionate production. This observation, along with the ratio data, demonstrates the effectiveness of the malonate block in succinate-malate exchange and the absence of any effect in the succinate-decarboxylation system. The activity of the succinate-decarboxylation system seems actually to be enhanced by this type of metabolic isolation.

In order to determine the relationship of the C_1 fragment to the activated succinate, an experiment was conducted that employed succinyl-CoA labeled with C^{14} in the γ carboxyl as the substrate, together with succinate, carbon dioxide, and propionate. Incubation with a cell extract that had been previously treated with the anion-exchange resin, Dowex-1, in a 10% carbon dioxide–90% helium atmosphere resulted in the production of radioactive carbon dioxide and propionate in a ratio of 1:1.5. A control vessel in which symmetrically labeled succinyl-CoA was employed gave a ratio of 1:10. Thus radioactivity from the γ carboxyl of the asymmetrically labeled succinyl-CoA appeared in the carbon dioxide in a sevenfold ratio as compared with the activity in the propionate and indicated that the origin of the C_1 fragment is the unsubstituted carboxyl of the monosubstituted succinyl-CoA. Since propionate and carbon dioxide are nonstoichiometrically produced (as indicated by the control vessel), the reaction was studied with the anaerobic micrococcus, *Veilonella gazogenes*,[10] which causes a conversion of succinate to equimolar amounts of propionate and carbon dioxide and does so at a rate sufficient to make manometric techniques feasible. As observed by Whitely, cell-free extracts from *Veilonella gazogenes* displayed an ATP-dependent, CoA-independent activation mechanism (hydroxamic acid formation). Whitely also observed that the production of carbon dioxide from succinate requires ATP and CoA; both ATP and CoA play a catalytic role.

Experiments with these extracts, in which succinyl-CoA is used in catalytic amounts in the presence of succinate and in the absence of ATP, indicated that succinyl-CoA was replacing ATP, as well as acting as a "sparker." Carbon dioxide was produced in great excess over the amount of succinyl-CoA present in the Warburg vessel; hence a catalytic role was ascribed to succinyl-CoA in the decarboxylation of succinate. An identical effect was also observed with propionyl-CoA. Deacylase activity, as measured by the reappearance of sulfhydryl groups and the disappearance of hydroxylamine-reacting substrate, caused the eventual loss of catalytic activity in the evolution of carbon dioxide. The decarboxylating activity could be subsequently restored by the addition of

[10] H. R. Whitely, Jr., *J. Am. Chem. Soc.* **75**, 15-18 (1953).

either succinyl-CoA or propionyl-CoA (Fig. 22). When Dowex-1-treated extracts were incubated with (1) succinyl-CoA and carboxyl-labeled succinate and (2) with symmetrically labeled succinyl CoA and succinate, the resultant labeling in the products (carbon dioxide and propionate) showed that equal distributions of radioactivity took place in both cases. The equal activity between the two cases demonstrates that extremely rapid equilibration between succinate and succinyl-CoA is occurring. This is additional evidence for propionyl-CoA-succinate transphoryase activity.

Asymmetrically labeled succinyl-CoA that was incubated for 20 sec with the Dowex-1-treated

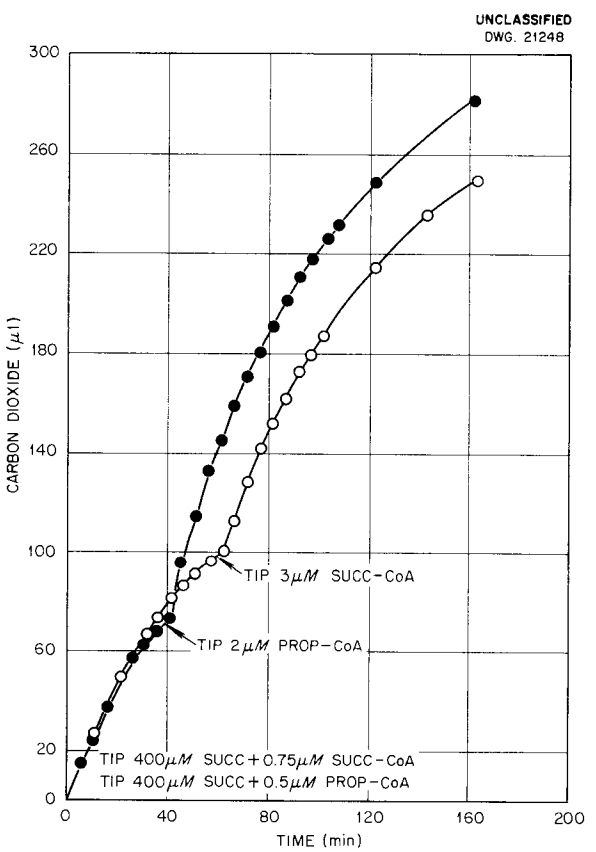

Fig. 22. Catalytic Effect of Succinyl-CoA and Propionyl-CoA in Succinate Decarboxylation.

Veilonella extract without added succinate produced radioactive carbon dioxide and propionate in a ratio of 4:1, whereas the control vessel with symmetrically labeled succinyl-CoA gave equal amounts of these products. These results substantiate the results from the previous experiments with *Propionibacterium* extracts and their activity on succinyl-CoA and are interpreted to mean that the carbon dioxide comes from the γ carboxyl with the resultant production of propionyl-CoA. These experiments, along with the manometric data concerning the catalytic effect of propionyl-CoA, indicate that the high-energy bond of succinyl-CoA is being conserved. It is visualized that transphoration of the propionyl-CoA with succinate results in the formation of succinyl-CoA and thus completes a catalytic cycle. Further evidence for a propionyl-succinyl-CoA transphoryase system was obtained by the incubation of succinyl-CoA and propionate with a Dowex-1-treated extract. After a 20-min incubation period, hydroxylamine was added to trap acyl-CoA compounds. Approximately equal amounts of the hydroxamic acids of both succinate and propionate were found by paper chromatographic methods.

Propionyl phosphate, as well as acetyl phosphate, showed a strong CoA-dependent catalytic effect in the production of carbon dioxide from succinate. These results indicate transacylation between propionyl-CoA and propionyl phosphate, and they also indicate transacetylation between acetyl-CoA and acetylphosphate with subsequent transphoration between acetyl-CoA and/or propionate and succinate.

A dialized preparation, when incubated with succinyl-CoA in the presence of arsenate, showed a strong arsenolytic decomposition of the succinyl-CoA as measured by hydroxamate formation. Coupled with the earlier observation of ATP dependence and CoA independence in succinate activation, these arsenolysis data are interpreted as strong evidence for a trans-succinylase mechanism.

These data are schematically presented in Fig. 23.

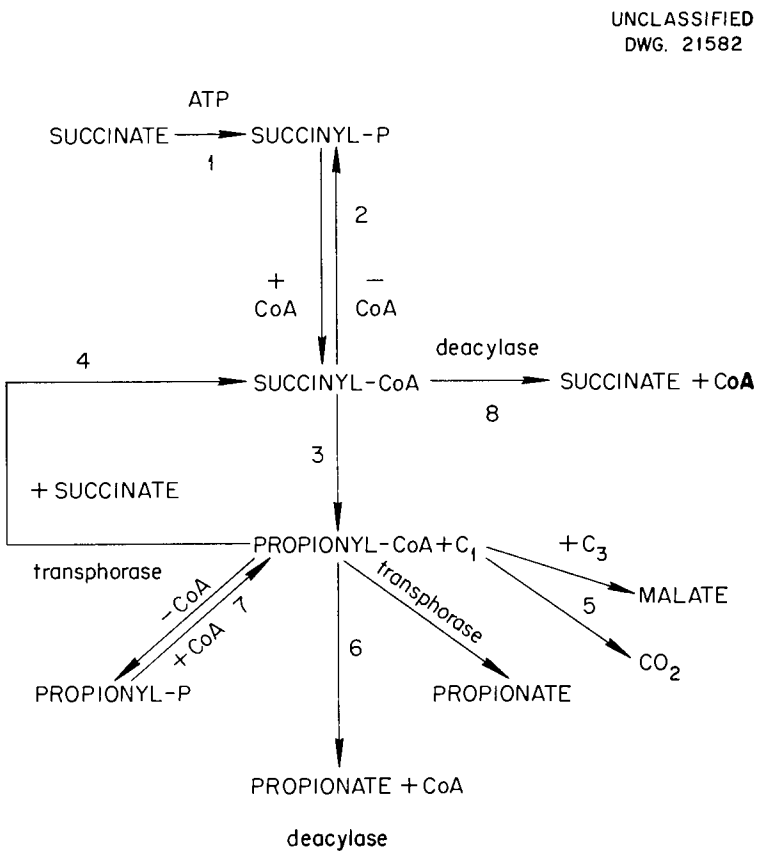

Fig. 23. ***Veilonella and Propionibacteria.***

BIOCHEMISTRY[1]

ERRATA

In the preparation of the previous progress report ORNL-1497 "seconds" was changed to "minutes." In lines 1, 2, 4, 9, and 17 (excluding the display lines) on page 46, the expressions should read: "10 and 31 seconds," "90 seconds," "2000 seconds," "30 seconds," and "30-second."

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

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Nucleic Acid Structure

(Cohn, Khym, Ottinger)

In the identification of the isomeric adenylic acids *a* and *b* as adenosine-2′- and 3′-phosphates, respectively, by degradation to the respective ribose phosphates,[2] there were several problems that were related to the chemical properties of the adenosine-3′-phosphate. Among these problems were: (1) the separation of inorganic phosphate from the ribose phosphates, (2) the crystallization of pure salts of the ribose phosphates for characterization by conventional physical properties, (3) the determination of the stability of the ribose phosphates in alkaline solutions, and (4) the establishment of the production of ribose-4-phosphate from ribose-3-phosphate by acid isomerization.

The first two problems were solved by the use of magnesia mixture, which precipitated magnesium ammonium phosphate free of ribose phosphates. The ribose phosphates were precipitated with barium acetate. This left in solution the excess salts from the previous phosphate precipitation. The barium salts of the ribose phosphates have thus been prepared and are being analyzed.

The relative stability of the ribose phosphates to alkali is involved in the ion-exchange separations, which are carried out at pH values around 9, as well as in the inorganic phosphate removal step. The need for ascertaining the degree of decomposition in alkaline solutions was first indicated by consistently low yields and the inability to free the products of inorganic phosphate. Ribose-2, -3, and -5-phosphates were allowed to stand at room temperature at pH 12 for several days, during which the appearance of inorganic phosphate and the disappearance of reducing power was followed. Ribose-2-phosphate retained its reducing power (aldehyde group) and 90% of its organic phosphate over a seven-day period. Ribose-3-phosphate retains only 10% of its reducing power and its organic phosphate after seven days at pH 12, the 50% mark being reached in 50 hours. Thus the product of decomposition is not ribose or another reducing sugar; however, ribose itself is unstable under these conditions in that it loses reducing power at about the same rate. Ribose-5-phosphate loses over half its reducing power and its organic phosphate in 20 hours.

Ribose-4-phosphate, which is believed to be the substance that accumulates to some degree on prolonged acid treatment of ribose-2 or -3-phosphate, has been prepared in small amounts but has not yet been positively identified. The circumstances of its production and its distinction from ribose-1, -2, -3, and -5-phosphates offer the chief means of identification at present.

Further attempts to identify and characterize ribose-4-phosphate are planned. Characterization of the other esters and a formal presentation of the work will conclude this phase of RNA investigation, which began with the discovery of the isomeric adenylic acids in this laboratory four years ago.[3]

Nucleic Acid Enzymology

(Volkin, Jones)

An investigation has been undertaken to study the enzymatic hydrolysis products of bacteriophage DNA. The purpose of the study has been to obtain

[1] Abbreviations used in the *Biochemistry* section are:
RNA – ribonucleic acid
DNA – deoxyribonucleic acid
DNAse – deoxyribonuclease
RNAse – ribonuclease
LP – lusate protein
KCF – kidney cortex factor
FMN – flavin mononucleotide

[2] W. E. Cohn, *Biol. Quar. Prog. Rep. Feb. 10, 1953*, ORNL-1497, p. 46.

[3] C. E. Carter and W. E. Cohn, *Biol. Quar. Prog. Rep. Nov. 30, 1948*, ORNL-220, pp. 25-41.

information on the biochemical processes that ensue upon bacteriophage infection and on the subsequent phage production. Recent work has indicated a close relationship between these processes and the bacterial nucleases.

From a biochemical standpoint, the phage cycle may be summarized in the following manner. Shortly after the phage is adsorbed, most of the DNA of the phage, but essentially none of its protein, penetrates the interior of the bacterial cell. The phage DNA is then partially degraded; moreover, the bacterial chromatinic material (DNA) appears to undergo degradation and rearrangement. The synthesis of new phage DNA starts very early in the latent period and reaches a high level before the first intact phage particle is produced. (Synthesis of phage protein apparently precedes phage DNA synthesis, however.) The DNA synthesis is carried out almost exclusively at the expense of DNA-degradation products from the host until this supply is exhausted; then, components from the medium are utilized to furnish the DNA requirements for phage growth. Experiments with labeled infecting phage reveal that as much as 35% of the parental DNA may be found in the progeny; however, the next progeny contains no more than 35% of its parental DNA; thus it is shown that there is no transmission of a chemically intact structure through successive generations. Generally, it has been found difficult to reconcile the various chemical data with the rather rigid genetic behavior associated with bacteriophage growth.

The presence of DNAse activity, which is absent in uninfected cultures, can be detected rather early after phage infection. A study of the products of the action of this enzyme on phage DNA is now under way. Likewise, a bacterial RNAse which may play a part in the initial phase of the infection process is being studied.

Preparation of Bacteriophage DNA. Two preparations of $T4r^+$ bacteriophage and one of T1 bacteriophage were prepared from 15 liters of lysate. One preparation of $T4r^+$ was carried out by initial Sharples centrifugation, which was followed by differential low- and high-speed centrifugation. The other preparations of $T4r^+$ and T1 were prepared by initial precipitation of the virus (and considerable lysate protein) at pH 4.0, which was followed by solution at neutral pH and then by purification by differential centrifugation. The phage DNA's were prepared by successive extractions with chloroform, octyl alcohol out of 2 *M* guanidine hydrochloride solution, then purified by alcohol precipitations. All preparations were typically highly viscous.

Preparation of Calf Thymus DNA. Thymus DNA, prepared as a substrate for bacterial DNAse assay, was purified by the detergent method of Dounce.

Preparation of Bacterial DNAse. One crude preparation of DNAse-containing protein was obtained by precipitating the filtrate of the Sharples-centrifuged $T4r^+$ preparation with ammonium sulfate. In the preparation, essentially all the lysate protein (labeled LP) was precipitated by ammonium sulfate that was between 0.3 and 0.8 saturated.

In phage preparations obtained by precipitation at pH 4.0, it was found that the DNAse activity was in the high-speed supernatant fraction obtained after neutralization and solution of the pH-4.0 precipitate. Initially, however, this fraction was found to be inactive because of the presence of a high concentration of bacterial RNA. After the RNA was digested with crystalline pancreatic RNAse and the products were removed by dialysis, the supernatant fraction was highly active. The DNAse was then purified by collecting the supernatant that remained after precipitation at pH 4.0. An additional purification is achieved by collecting the fraction that is precipitable by ammonium sulfate that is between 0.4 and 0.8 of saturation. This preparation, unlike preparation LP, is essentially free of bacterial phosphomonoesterase and deaminases, and it has a much higher specific activity than preparation LP. It has not been established whether this nucleolytic action is due to the presence of more than one enzyme.

Hydrolysis of $T4r^+$ DNA and Calf Thymus DNA with the Bacterial DNAse (LP). About 9 mg of $T4r^+$ DNA and 14 mg of calf thymus DNA were digested at pH 7.5 with preparation LP until the reaction was complete as measured by the liberation of titratable groups. The digest was then made 0.1 *M* with ammonium hydroxide and adsorbed on a Dowex-1 anion-exchange column. The column was eluted by increasing concentrations of chloride ion in a manner analogous to that used for the analysis of ribonuclease-hydrolyzed RNA. Essentially 100% recovery was obtained in both cases. The results of these analyses are shown in Table 29, wherein the yields of the various products are given in terms of micromoles and percentages of the four bases.

TABLE 29. PRODUCTS OF HYDROLYSIS OF $T4r^+$ DNA AND CALF THYMUS DNA WITH BACTERIAL DNAse (LP)

	BASE	NUCLEOSIDE		MONONUCLEOTIDE		OLIGONUCLEOTIDES*	
		μmoles	%	μmoles	%	μmoles	%
$T4r^+$ DNA	Adenylic	2.45	33	4.2	54	1.05	14
	Guanylic	2.2	47	1.0	21	1.5	32
	Thymidylic	3.3	38	2.6	30	2.9	32
	5-hydroxy-methyl cytidylic	0	0	2.5**	72	1.0**	28
Calf-thymus DNA	Adenylic	4.1	40	5.0	49	1.2	11
	Guanylic	4.9	48	3.7	36	1.6	16
	Thymidylic	3.5	31	1.85	16	5.9	53
	Cytidylic	0	0	2.9	31	6.3	60

*Calculated by difference of total minus nucleoside and nucleotide.

**Estimated; extinction coefficient unknown.

It is apparent that a significant amount of oligonucleotides remains after completion of the enzymatic action. The presence of large quantities of nucleosides indicates the presence of a phosphomonoesterase, and the action of a purine deoxyriboside deaminase is indicated by the isolation of some deoxyinosine and deoxyxanthosine, which are removed by preparing the DNAse from the pH-4.0 precipitable material. Further work is in progress to better define the action of the more highly purified preparation. Subsequently, the bacterial DNAse products of DNA from related and unrelated bacteriophages will be compared with those from phages inactivated by irradiation. A relationship between such products and the biologic specificity can be established only by more rigorous investigation.

Preliminary work is in progress on the specificity and possible connection with bacteriophage infection of an RNAse associated with bacterial membranes.

Enzyme Substrate Binding Studies

(Doherty, Purdy, Thomas)

The study of complex formation by α-chymotrypsin with synthetic substrates has been extended to the previously reported glyconyl peptides.[4] The glyconyl peptides are useful in that a wide variety of asymmetric configurations is potentially available. Through the extension of classical carbohydrate procedures and the development of new methods, several new glyconyl peptides have been synthesized to yield eight pairs of optical isomers (that is, D and L gluconyl derivatives) for kinetic enzyme studies. The following aldonic acids have been prepared and converted to glyconyl peptide amides: L-gluconic acid, L-mannonic acid, L-galactonic acid, D-gulonic acid, L-gulonic acid, D-lyxonic acid, D-xylonic acid, L-threonic acid, D-erythronic acid, and L-erythronic acid.

These substrates contain the four necessary structures for chymotrypsin activity, namely, (1) a carbonyl group at the sensitive bond, (2) an α-acylamino group, (3) an α-*p*-hydroxybenzyl group, and (4) an L configuration on the α-carbon. In addition, they have widely varying configurations in the α-acylamino group at position 2. The effects of this configurational change on the rate of hydrolysis of the glyconyl-L-tyrosine amides were determined in the conventional way, and the Michaelis constant K_s and the K_3 for seven pairs of glyconyl peptides were determined (for example, D-gluconyl-L-tyrosine amide and L-gluconyl-L-tyrosine amide). In addition, the hydrolysis constants of the simplest glyconyl derivatives, the

[4] D. G. Doherty, *J. Biol. Chem.* 201, 857 (1953).

D-glyceroyl and L-glyceroyl-L-tyrosine amides, were determined.

Four conclusions can be drawn from the values found for K_s and K_3. (1) As with the true peptides, an L configuration of the α-carbon of the aldonic acid is necessary for maximum activity, although this requirement is not absolute. (2) This evidence is indirect enzymatic proof that an L configuration in the carbohydrate series is equivalent to an L configuration in the amino acids. (3) The rate of hydrolysis is faster if both α- and β-carbons have the L configuration, thus indicating that these two carbon atoms are probably bound on the enzyme surface. (4) The configuration of the aldonic acid as a whole (that is, D or L series) is not important in the five- and six-carbon aldonic acid. Anomalous results were obtained with the glyceroyl derivatives in which the racemic compound, DL-glyceroyl-L-tyrosine amide, was hydrolyzed at a rate appreciably greater than that of either the D-glyceroyl-L-tyrosine amide or the L-glyceroly-L-tyrosine amide. This unusual behavior is under investigation.

Energy-Transfer Systems

(Strehler, Cormier)

Bacterial Extract Luminescence. The effect of various environmental factors and chemical agents on the luminescence of cell- and particle-free extracts of *Achromobacter fischeri* has been investigated in some detail. Briefly, the following has been found.

The first factor which becomes rate limiting for the luminescence of this extract is diphosphopyridine nucleotide, and the addition of this component or its reduced analog to an undialyzed preparation of the bacterial extract results in an emission of light. Dialyzed extracts retain their ability to respond to reduced DPN but are unable to respond to oxidized DPN in the absence of added substrates such as malate.

The enzyme is extremely sensitive to dilution; a tenfold dilution of a 2% extract produces complete inactivation within a few minutes. The temperature response of the system and the color of the light emitted correspond to the properties of the intact bacterial system. The pH dependence of the reaction exhibits two maximums: one at approximately pH 6 and the other at approximately pH 8.5. Various salts increase the luminescence over a concentration range and then, at higher concentrations, they inhibit the luminescence (maximum at ~1%). A number of inhibitors have been tested on the system.

It has been possible to demonstrate an absolute requirement of the reaction for a diffusible factor other than DPNH. Appreciable increases in luminescence have been obtained by adding a heat-precipitable fraction from boiled bacterial extracts or by adding a factor obtained from hog kidney cortex (KCF). Other factors, such as coenzyme A, FMN, and lipoic acid, have been shown, under certain conditions, to influence the amount of luminescence obtained.

Comparison of the Response of Various Strains of Luminous Bacteria. Through the courtesy of C. B. van Niel, ten different strains of luminous bacteria have been obtained, and their response to the factors required by *A. fischeri* has been tested. All the strains except two were similar to the *A. fischeri* in their biochemical response. One of the remaining strains gave no light at all in the absence of KCF, and it rapidly destroyed this factor. The other did not respond to KCF addition, but it luminesced brightly when $DPNH_2$ was added. A further systematic inquiry into the properties of these various extracts is in progress.

Isolation, Characterization, and Identification of KCF. The factor responsible for the increased luminescence caused by kidney cortex extracts has been purified by various partition and precipitation procedures. It has been found to give a fivefold stimulation in concentrations of 5×10^{-7} g/ml. The crude and purified samples gave a strong positive Fuchsin aldehyde test, and increasing purification produced low (~0.5%) nitrogen and phosphorus contents. A crystalline 2,4-dinitrophenylhydrazine derivative of the KCF that was prepared melted sharply at 104 to 105.2°C. Heptaldehyde was tested on the luminescent system and was found to be about one thirtieth as effective on a weight basis as the purified KCF. A further rigorous proof of the identity of the component is now in progress.

Plant Biochemistry

N. E. Tolbert L. P. Zill
Betty Ann Gwin

Carbon Dioxide Reduction by Cell-Free Systems from *Chara* and *Nitella*. A plant sap or cell-free leaf macerate has never been prepared which could utilize light energy for the reduction of carbon

dioxide to a carbohydrate level. Such a system, which represents the whole photosynthesis process of the plant, has often been sought so that it could be subjected to a detailed biochemical investigation. Failure to obtain an active cell-free leaf extract has been attributed to pH changes during grinding, changes in spatial arrangements between enzymes, or dilution of the protoplasm by vacuolar sap. However, the algae *Nitella* and *Chara* are large enough to permit careful physical separation of the individual cells into protoplasm, intracellular sap, and cell wall, with a minimal amount of disturbance or mixing. By using these algae, fixation of $C^{14}O_2$ in the light has been demonstrated for both cut cells and cell-free protoplasm.

The algae that have been used are *Nitella clavata*, *Nitella flexilis*, and a noncorticated *Chara*. *Nitella clavata* was grown in aquariums in the laboratory, and the others were obtained from nearby lakes. A measurement has been made of the fixation of $C^{14}O_2$ by whole cells serving as controls, as well as by cells with both ends cut off so that no whole cells were present and by cell-free protoplasm squeezed away from the cell wall.

Experiments were run in the light and in the dark, for comparison, and they were stopped by adding strong acid and boiling. Excess $C^{14}O_2$ was thus eliminated, although labile compounds and volatile products would have been lost before the subsequent counting procedure. In a typical experiment with the protoplasm, 0.005 ml of $NaHC^{14}O_3$ (about 10,000 counts/sec) and 0.01 ml of 0.1 *M* K_2HPO_4 were dried on a reaction dish; the protoplasm from three algae cells was squeezed onto the dish and allowed to stand without stirring for the allotted time; then acid was added and the dish was heated.

The $NaHC^{14}O_3$ fixed by the cells has been used as a measure of the photosynthesis rate. For the whole cells of *Chara*, photosynthesis proceeds at a constant rate (curve A, Fig. 24). An approximately equal amount of cut cells also fixed $NaHC^{14}O_3$ at the same rate as the whole cells, if fresh cells were used in the experiments (curve B). Control experiments that were run in the dark (curve D) fixed a small but measurable amount of C^{14}, and the amount was approximately the same for whole and cut cells.

Fixation of $NaHC^{14}O_3$ in the light and in the dark by the protoplasm from *Chara* cells is shown in Fig. 25. Two different experiments which were run about one month apart and which indicate the reproducibility of the results are reported. Phosphate buffer was needed to prevent loss of $C^{14}O_2$ from the somewhat acid protoplasm, although the phosphate was shown to reduce the rate of $NaHC^{14}O_3$ fixation. Carbowax 4000 as a suspending medium for the protoplasm resulted in an increased fixation rate of $NaHC^{14}O_3$.

In order to be representative of the process in the whole plant, these cell-free systems should be able to reduce the carbon dioxide to a carbohydrate level. Consequently, the products formed from the $NaHC^{14}O_3$ by the cells or protoplasm were investigated by means of paper chromatography and autoradiography. The main products formed from $NaHC^{14}O_3$ by whole cells in the light during 10 min of photosynthesis are the monosaccharides, sucrose, phosphate esters of sugars, alanine, glyceric acid, and aspartic acid. Similar products were formed by the cut cells. These products are

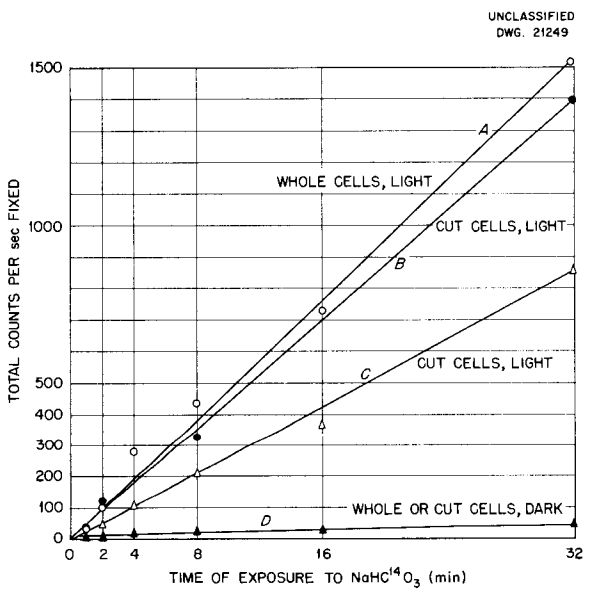

Fig. 24. Rate of $C^{14}O_2$ Fixation in the Light by Whole or Cut Intermode Cells of *Nitella*. Ten cells were placed in a test tube with 0.005 ml of $NaHC^{14}O_3$ solution (~6000 counts/sec), 0.025 ml of 0.1 *M* K_2HPO_4, and 12 ml of water, and exposed to about 1500 ft-c of light from a 200-watt photoflood lamp for various lengths of time. Curves *A*, *B*, and *D* were run approximately 2 hr after harvesting the algae from the lake; curve C was run 18 hr afterward.

characteristic of those formed by $C^{14}O_2$ photosynthesis in plants. In the dark, both whole and cut cells produced C^{14}-labeled aspartic acid, malic acid, fumaric acid, and glutamic acid, which are fixation products of $C^{14}O_2$ in the dark.

The protoplasm from all three algae fixed the $NaHC^{14}O_3$ into products that are associated with

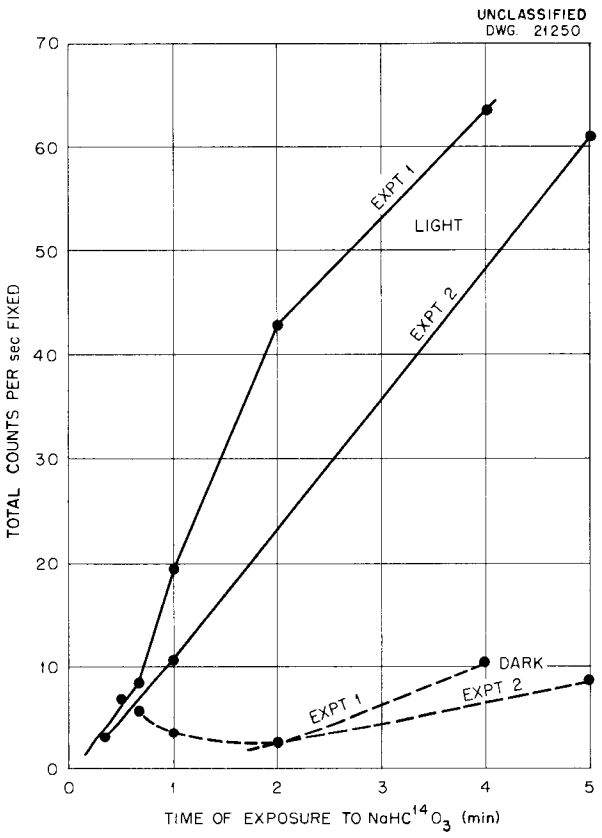

Fig. 25. Fixation of $C^{14}O_2$ in Light and Dark by the Protoplasm From *Chara* Cells. Protoplasm from three *Chara* cells was squeezed into a dish containing 0.005 ml of $NaHC^{14}O_3$ (~6000 counts/sec) and 0.01 ml of 0.1 *M* K_2HPO_4 previously reduced to dryness. The light intensity was about 1500 ft-c from a 200-watt photoflood lamp with a water filter for removing heat. Reaction was stopped by the addition of 0.1 mole of 1 *N* HCl and the plate was dried in hot air before counting.

the path of carbon in photosynthesis. The C^{14} appeared in as reduced a state as that of free fructose and sucrose. Thus a cell-free photosynthesis system has been obtained which should be applicable to further physiological and biochemical investigations.

Biosynthesis and Isolation of Sedoheptulose. It has been impossible to use biochemistry to explain all physiological effects on the basis of the known anaerobic glucose metabolism scheme. Results of research within the past several years have shown the importance of a second pathway of metabolism in the living cell. The new system involves different intermediary compounds such as ribulose, ribose, and sedoheptulose. In animals and plants, it appears to function as an alternate path in respiration, and, in addition, in plants the reverse of the system appears to be an important pathway for carbon dioxide reduction to carbohydrate.

The plant, *Sedum spectabile*, contains about 1% (on a wet weight basis) of sedoheptulose. However, sedoheptulose will not crystallize and only its dianhydride, sedoheptulosan, has been isolated. The established procedures for the isolation of the dianhydride have been modified for the isolation of the free sedoheptulose sugar labeled with C^{14}. Labeling was done by biosynthesis techniques. Final isolation of the pure compound in milligram amounts was accomplished by thick paper chromatograms regulated by a thin paper wick. The procedure results in the complete separation of a mixture of sugars. Approximately 100 mg of a sugar mixture can be separated per chromatogram. The sedoheptulose thus isolated had a specific activity of 3.7 $\mu c/mg$ and was approximately uniformly labeled.

During the biosynthesis procedure, other compounds became labeled with C^{14} and were isolated by the chromatographic procedures. These compounds included fructose, glucose, sucrose, malic acid, glyceric acid, and citric acids. An unknown compound that was present in considerable quantity could be hydrolyzed in acid to fructose, thus indicating that it was probably a fructosan.

It was also observed that the *Sedum* plant translocated sedoheptulose-C^{14} within its system along with labeled glucose, fructose, and sucrose.

BIOPHYSICS

GENERAL PHYSIOLOGY

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Radiological Physics

(Darden, Sheppard, Randolph)

A program which, it is hoped, will ultimately lead to a clearer understanding of the significance of the energy distribution of beta rays inside an irradiated tissue in the determination of energy-dependent biological effects has been largely completed. The first part of the work, a depth-dose study of P^{32} beta rays from a thick source in various light plastics, has been completed and the results are available.[2] Theoretical studies that began with the current theories of multiple scattering have been carried out on the spectral distribution of beta rays in an absorber in contact with a thick source. A comparison of the results with experimental determinations made with a scintillation crystal spectrometer is now under way. A paper that describes the work is almost complete.

A limited program devoted to the study of fast-neutron dosimetry in the ORNL 86-in. cyclotron was begun some time ago. The study is largely based on the so-called "Berkeley System," and involves the use of a standard Victoreen X-ray dosimeter. The program rapidly assumed major importance when the ORNL Biology Division was brought into the biomedical program connected with recent nuclear weapons tests. The reasons for the choice of this admittedly semiquantitative method, with its associated problems, of measuring fast-neutron dosages have been previously reported.[3] The information gained from advance biological experiments in the cyclotron that were made possible by these studies was of considerable value in arriving at preliminary estimates of the biological effects of fast neutrons in the field tests.

A few weeks prior to the initial field test, the responsibility for providing a major portion of the physical dosimetry for the biological samples to be exposed was placed on the Biology Division. Top priority was accorded this problem and, with the excellent cooperation of the plant facilities involved, 19 fast-neutron dosimeters were completed in time for the test. These instruments were small air-filled ion chambers with polyethylene electrodes. Based on the Sievert condenser principle, the chambers were similar in design to an instrument used in some previous work. Subsequently, nine additional chambers were built to measure gamma radiation in a fast-neutron field. These chambers were designed along similar lines and contained lead electrodes. The use and limitations of these instruments in the field are described in a classified report.[4]

The Cockcroft-Walton 250-kv accelerator that is intended for the generation of neutrons has been put in operation in a temporary installation, and a stable beam current of 0.5 ma at 250 kv has been obtained. A remote control station behind a boron-enriched water wall has been established, and it includes high-voltage controls, Selsyn-operated beam-focus and gas-pressure controls, target-current and ionization-gauge pressure meters, and two complete electronic circuits that are capable of simultaneous pulse-height analyses from counters located at known geometrics near the target. Various neutron-detection systems are complete: alpha proportional counter, fission counter, long counter, and ionization chamber. Calibration experiments are under way. Preliminary experiments on the counting of protons from the $d(d,p)$ reaction associated with the $d(d,n)$ reaction indicate that a fast-neutron calibration by the associated-particle method is possible with further improvement of the resolution of the counting system.

Erythrocytes

(Mathias, Sheppard)

It was observed during earlier work in this laboratory that X rays produce an acceleration of the

[1] Research Participant.

[2] E. B. Darden, Jr. and C. W. Sheppard, *J. Tenn. Acad. Sci.* **XXVII**, 210 (1952).

[3] C. W. Sheppard and E. B. Darden, *Physical Dosimetry in Typical Biological Experiments with Fast Neutrons from a Cyclotron Source*, ORNL-1559 (1953).

[4] C. W. Sheppard and E. B. Darden, Jr., ORNL CF-53-7-168 (1953) (classified).

rate of outgo of potassium from human erythrocytes.[5] The normal exchange process in control cells is disturbed by radiation, and a slow mass transfer from cells to plasma occurs. This disturbance of potassium equilibrium is typical of processes which have been observed in other types of tissue cells, such as anoxic nerve, and therefore seems to be of a fundamental nature. It has been shown that exposure of erythrocytes to eserine, which is a specific cholinesterase inhibitor, produces marked disturbances of the potassium-transport mechanism.[6] Taylor and Weller[7] showed that eserine depressed the entrance of potassium into the cells, whereas substances that inhibit choline acetylase, the enzyme which makes acetylcholine, produce an effect similar to the effect with X rays. Results of preliminary experiments in this laboratory suggested that the irradiation of red cells produced a depression of acetylcholine content. These results were not reported since it was suspected that they were due to an artifact, and a more careful investigation of the questionable acetylcholine content of red cells and of their possible acetylcholine production was begun. It was soon confirmed that the effect was a small one and that the proof of its existence or nonexistence would require an extensive investigation of the validity of the sensitive bioassay methods for detecting acetylcholine. As a result, it was found that human red cells do not contain enough choline ester to be detectable with certainty by bioassay. Furthermore, if the enzyme which hydrolyzes acetylcholine (cholinesterase) is blocked with eserine, the expected buildup of acetylcholine in red cells and in red cell preparations is not observed. The limit of detection of the bioassay method is approximately 0.1 μg of acetylcholine per cubic centimeter of red cells and 0.2 μg/cc/hr of synthesis. A number of attempts have been made to lower this limit of detection. Because of the numerous artifacts which appear, increased sensitivity is obtained only at the expense of certain identification of the ester. A number of reasons can be advanced for the very low level of acetylcholine turnover, if it exists at all. The most likely explanation would seem to be that the level of activity of the choline acetylase system is low. Whereas extracts from acetone-dried powders of rat brain readily catalyze the production of acetylcholine in a synthetic system, the synthesis by blood powder is normally at or below the level of detection. When every effort is made to achieve the optimum level of acetylating power, traces of some substance are produced by extracts of blood powders, and the possibility that acetylcholine synthesis has occurred under these exceptional conditions is suggested, but not conclusively proved. It is believed that the low level of acetylcholine turnover in erythrocytes is highly significant because it suggests that the cholinesterase in red cells is not present for the purpose of breaking down acetylcholine. Since the importance of the enzyme in electrolyte accumulation seems to be established, it is perhaps not too much to suggest that it plays a hitherto unsuspected dual role.

[5] C. W. Sheppard and G. E. Beyl, *J. Gen. Physiol.* 34, 691 (1951).

[6] M. E. Greig and W. C. Holland, *Arch. Biochem. and Biophys.* 23, 370 (1949).

[7] I. M. Taylor and J. M. Weller, *Biol. Bull.* 99, 311 (1950).

Autoradiographic Detection of Lithium

(Dent, Sheppard)

A refinement of a technique described by Ficq, in which the action of thermal neutrons on Li^6 is utilized to detect the concentration of lithium in tissue sections,[8] is being undertaken. Microscope slides that were partly covered by a film of gelatin containing 2% of lithium chloride have been fastened to 1- by 3-in. plates covered with Eastman 25-mμ NTA emulsion. These slides and plates were exposed in the thermal column of the ORNL graphite reactor for various periods that ranged from 1 to 9 minutes. After photographic development, the plates showed alpha-triton tracks in those regions which had been adjacent to the lithium-containing gelatin, but the tracks were obscured by background fog when exposures longer than 1 min were used. However, the satisfactory exposure time was increased from 1 to 3 min by shielding the preparations with 1 in. of pure lead during exposure. It is desirable to make the exposure time as long as possible since the sensitivity of the technique increases with the number of neutrons striking the tissues. Because it will be desirable to coat tissue sections with a thin layer of celloidin to prevent their adhering to the photographic emulsion, celloidin-coated gelatin films containing 2% of lithium

[8] A. Ficq, *Compt. rend.* 233, 1684-1685 (1951).

chloride were also exposed. The celloidin did not prevent alpha-triton track formation on the NTA plates. More dilute solutions of lithium and lithium-enriched tissues will be tested next.

Demonstration of Diiodotyrosine in Pigmented Tissue

(Dent, Morris[9])

It has been shown that I^{131} administered to tadpoles becomes localized not only in the thyroid gland but also in certain other regions, notably in the pigmented layer of the eye, the tapetum nigrum.[10] Since, as is well known, tyrosine is the basic constituent of melanin, it is reasonable to suppose that diiodotyrosine, and possibly thyroxin, is formed in this pigmented tissue as well as in the thyroid. A paper chromatographic separation of the components of the tapetum nigrum is being attempted to test this hypothesis. Preliminary results indicate that diiodotyrosine is present.

Physical State of Deoxyribonucleic Acid in Various Rat Tissues

(Anderson, Anderson)

Studies on the physical properties of homogenates of various rat tissues made 1 *M* with respect to sodium chloride have been continued. Very low doses of X rays (50 to 100 r) have been found to cause a transient increase in the viscosity of such preparations as are studied in Ostwald-Cannon-Fenske viscometers at 25°C. The previously reported characteristic drop in viscosity is then followed. This effect is of considerable interest because of its possible relationship to the stickiness of chromosomes that is often observed after low doses of ionizing radiations.

In order to make more complete study of the physical properties of deoxyribonucleic acid in fresh preparations, it has been necessary to design and construct a Couette viscometer that is suitable for the simultaneous study of viscosity and of birefringence of flow.

[9] Pathology and Physiology Section.

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

Effects of Various Normal Cell Constituents on Embryonic Development

(Horn, Anderson, Anderson)

During the past two months, a project has been initiated to study the effects of a number of constituents of normal cells on embryonic development. Proteins that are characteristically found in the cell nucleus and several other substances of wide vertebrate distribution were prepared from various sources and have been injected via the yolk and albumin along with preparations made from both adult and newly hatched chick tissues. To date, 13 substances have been tested in approximately 1700 eggs that were incubated to various stages prior to hatching. Gross morphological examination and staging of all incubated embryos have been made where feasible, and embryonic tissues, both normal and abnormal, have been preserved for histological study. An analysis of the results will be presented in a subsequent report.

Tradescantia Studies

(Kirby-Smith, Craig, Swanson[11])

In collaboration with C. P. Swanson, an investigation has been made of chromosome breakage in *Tradescantia* microspores from inflorescences that were exposed at Shot Annie, Operation Upshot of the recent Nevada tests. Before and after the field tests, the ORNL 86-in. cyclotron was used to calibrate the material for both chromosome and chromatid aberrations in terms of its response to fast neutrons. Observed aberration frequencies were in close agreement with those found by Conger and Giles for fission neutrons. A complete report of the field-test results on *Tradescantia* is in preparation.

A comprehensive series of experiments has shown definitely that the modifying effects of infrared radiation on chromosome breakage observed in *Tradescantia* microspores are totally lacking in the case of dry pollen. These final investigations included some studies in which pollen was obtained from plants that were maintained in total darkness for as long as eight days, irradiated, and successfully cultured.

[11] Cytogenetics Section.

Paramagnetic Resonance

(Kirby-Smith, Craig)

Apparatus for the observation of paramagnetic resonance absorption by free radicals has been assembled and put into operation. The present sensitivity of the equipment allows approximately 10^{-7} mole of some organic free radicals to be observed. Improvements that are now being incorporated into the detecting system are expected to increase the sensitivity sufficiently to allow radiation-induced radicals to be detected and to permit a study of free-radical mechanisms to be made in some organic reactions.