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BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

for Period Ending August 15, 1955

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director
Edited by E. J. Slaughter

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ORNL-244	Period Ending May 15, 1949
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ORNL-644	Period Ending February 15, 1950
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ORNL-1693	Period Ending February 15, 1954
ORNL-1776	Period Ending August 15, 1954
ORNL-1863	Period Ending February 15, 1955

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CONTENTS

PUBLICATIONS AND LECTURES 1
Publications 1
Scientific Society Lectures and Traveling Seminars 3
Visiting Lecturers 10
Education 12
Speakers at Professional Meetings, Fall 1955 12

CYTOLOGY AND GENETICS 13
Cytogenetic Effects of Radiation 13
Effect of postirradiation temperature on mutation induction in *Paramecium aurelia* 13
Effect of X rays on nuclear exchange at conjugation 14
Studies on cobalt transport in *Tetrahymena* 15
Effect of oxygen on primary breakage and fusion in *Tradescantia* chromosome aberrations 18
Incompleteness of exchanges 18
Ratio of terminal deletions to exchanges 18
Ascites tumor production by single-cell inocula 19
Production of two types of X-ray-induced chromosome breaks 20
Further studies on the biochemistry of chromosome rejoining 22
Dominance relations in *Neurospora* heterokaryons 23
Relation between the lethal effects of X rays on microconidia and ascospores 25
Radiation-induced mutations in maize 25
Effects of chemical treatments and radiations on the growth of timothy roots 26
Effects of alpha-particle irradiation 27
Insect Cytology and Genetics 27
X-ray-induced dominant lethality and chromosomal aberrations in a thelytokous wasp 27
X-ray-induced gene mutations in *Nemeritis canescens* 28
Recessive lethal mutations induced by X rays in the first meiotic prophase of *Habrobracon* oocytes 29
Radiosensitivity of the unfertilized *Habrobracon* egg 30
Effects of polonium alpha particles on hatchability of *Habrobracon* eggs 31
Preliminary data on the irradiation of the cytoplasm and the nuclei of *Habrobracon* eggs with various wave lengths of monochromatic ultraviolet radiation 33
Drosophila Genetics 35
On the structure of the sc^8-Y:bw^+ chromosome of *Drosophila melanogaster* 35
Constitution of recessive lethals induced by radiations of different ion densities 36
Analysis of rod-shaped derivatives of an unstable ring-X chromosome in *Drosophila melanogaster* 38
Importance of the distribution of the heterochromatin within the genotype to the viability of the organism 40
Kinetic activity of centromeres associated with Y^L 43
Regional inhibition of crossing over in the X chromosome in response to the presence of an autosomal inversion 44
Chromosomal interference in attached-X females 47

MICROBIAL PROTECTION AND RECOVERY 49
Radiation Protection and Recovery in Bacteria 49
Protein and nucleic acid synthesis during recovery of bacteria 49

UNCLASSIFIED

On the relation between X-ray protection and induced mutagenesis in *Escherichia coli* auxotrophs 50
Pre- and postirradiation studies with long-ultraviolet and short-visible radiations on *Escherichia coli* B/r 52
Radiation protection by S,β-aminoethylisothiuronium-Br-HBr and related compounds 53
Effect of streptomycin on survival 55
Protective effectiveness of AET and MEA by use of $C_3H \times$ 101 mice 55
Experiments with C_3H mice treated with AET and APT 56

MAMMALIAN RECOVERY 59

Modification of Radiation Injury in Mice 59
Recovery of X-irradiated mice injected with whole leukemoid blood or with leukocyte-platelet suspensions 59
Relation of lymphatic tissue regeneration to survival of bone marrow-treated irradiated mice 61
Bone marrow response of X-irradiated mice receiving varying amounts of isologous bone marrow suspensions 61
Preliminary data from studies of delayed effects in mice surviving total-body exposures to massive doses of X radiation 61
Screening of tissues for new recovery agents 63
Harderian gland test for transplantation of homologous bone marrow cells following total-body X irradiation 63

MAMMALIAN GENETICS AND DEVELOPMENT 65

Genetic and Developmental Effects of Radiation in Mice 65
Radiation-induced mutations in the mouse 65
Gamma-ray sensitivity of spermatogonia of the mouse 66

PATHOLOGY AND PHYSIOLOGY 69

Pathologic and Physiologic Effects of Radiation 69
Radiation-induced myeloid leukemia 69
Mechanism of radiation recovery by marrow transfusion – evidence of repopulation 69
Effects of radiation on megakaryocytes and platelets – uptake of S^{35}-labeled sulfate 72
Transplanted chloromyeloid leukemia in rats 73
Binding of S^{35}-labeled sulfate in rat serum 73
Effects of X radiation on the distribution of $Na_2S^{35}O_4$ in rat plasma 75
Role of the thyroid-pituitary axis in resistance to lymphoid leukemia 75
Effects of radiation on the plasma iron concentration in intact and splenectomized rats 75

MICROBIOLOGY 79

Tracer Studies in Intermediary Metabolism 79
Succinic acid decarboxylase system 79
DPNH-flavoprotein complex of DPNH peroxidase 82

BIOCHEMISTRY 85

Studies on Nucleic Acids, Enzymes, and Radiation-protective Compounds 85
Nucleotide sequences in deoxyribonucleic acid 85
Catalytic reduction of pyrimidine nucleotides 85
Nucleic acid turnover in cell-free extracts of *Escherichia coli* 85
Nucleotide sequences in bacteriophage deoxyribonucleic acid 86
Stability of sugars in the presence of alkaline borate 86

UNCLASSIFIED

Enzyme substrate binding 87
Preliminary studies on the metabolism of S,β-aminoethylisothiuronium-Br-HBr (AET) with S^{35}-labeled compound 88
Structure and activity in radiation-protective sulfhydryl compounds 88
Microbial utilization of heparin 90

ENZYMOLOGY AND PHOTOSYNTHESIS 95

Enzymology 95
Activity of compounds related to β-mercaptoethylamine as inhibitors of enzymic acetylation 95
Fluorescence polarization 95
A requirement for pyridoxal in the interconversion of glycine and serine 97
Reduction of methemoglobin by phenylhydrazine 98

PLANT BIOCHEMISTRY 101
Effect of ionizing and ultraviolet radiation on photosynthesis in plants 101
Radiation sensitivity in formation of the photosynthetic process 103
Photosynthesis in anthocyanin-containing leaf tissue 106
Filter effect of anthocyanins 106
Rate of photosynthesis after ultraviolet irradiation of anthocyanin-containing leaves 107
A form of phosphorus storage and transport in plants 109
Photosynthesis and metabolism in *Sedum spectabile* 111
Glutamic acid metabolism in barley leaves 112
Characterization of an unidentified compound formed in the metabolism of biosynthesized sedoheptulose-C^{14} in plant leaves 114
Growth substance activity of serine 115
Isolation of a photosynthetic oxidation-reduction coenzyme from green plants 115

GENERAL PHYSIOLOGY 117
Horizontal tubeless centrifuge head 117
Organization of the soluble phase of the rat liver cell 117
Production of density gradients in the horizontal tubeless centrifuge head 118
Purity of nuclear fractions of tissue homogenates 119
An analysis of the *in vivo* effect of nucleoprotein from Ehrlich ascites cells 120
Difference in mean survival with time after a single pretreatment with NP 120
Modification by a second injection of NP 120
Modification by an injection of killed ascites cells or ascites fluid 120
Modification by cortisone 121
Difference in mean survival time as a function of the amount of NP injected 121
Specificity of the reaction 122
Effects on tumor cells 123
Tests for NP-induced sensitivity 123
Antigenicity of ascites tumor-cell components 123
Cytotoxicity of different antisera to ascites tumor cells 125
In vivo exposures 125
In vitro exposures 125

BIOPHYSICS 129

Molecular Biophysics and Radiological Physics 129
Molecular biophysics 129
Effect of radiation on *Tradescantia* 129
Amphibian radiobiology 129

Metamorphosis in *Gyrinophilus palleucus* 129
Cytological studies on the pituitary of thyroidectomized newts 129
Radiological physics 130
Cockcroft-Walton fast neutron facility 130
Neutron dosimetry 131
Fast neutron biological exposures with an internal target cyclotron 132

BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

PUBLICATIONS AND LECTURES

Publications. – Publications for the period ranged from abstracts to the 321-page Symposium on Genetic Recombination. In addition to the Symposium, 31 full-length papers and 29 abstracts and book reviews have appeared in open publication. Thirty-two papers and 29 abstracts are in press.

The material comprising the proceedings of the April 1955 Biology Research Conference in Gatlinburg, the Symposium on Enzyme and Protein Structure, is nearly complete. Preparation for publication as another supplement to the Journal of Cellular and Comparative Physiology is progressing.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	Brei fractionation	*Science* 121, 775–776 (1955)
————————	Studies on isolated cell components. VII. A reexamination of the preparation and properties of rat liver homogenates	*Exptl. Cell Research* 8, 91–100 (1955)
Arnold, W. A.	On the light saturation of delayed light production in green plants	*The Luminescence of Biological Systems*, ed. F. H. Johnson, Academic Press, p 47–50 (1955)
Atwood, K. C., and T. H. Pittenger	The efficiency of nuclear mixing during heterokaryon formation in *Neurospora crassa*	*Am. J. Botany* 42, 496–500 (1955)
Baker, W. K., and E. S. Von Halle	Evidence on the mechanism of the oxygen effect by use of a ring chromosome	*J. Cellular Comp. Physiol.* 45, Suppl. 2, 299–307 (1955)
Brown, J. R. C.	Contamination of nuclear fractions of thymus homogenates with whole cells (Comm. to ed.)	*Science* 121, 511–512 (1955)
Carlson, J. G., and N. G. Harrington	X-ray-induced "stickiness" of the chromosomes of the *Chortophaga* neuroblast in relation to dose and mitotic stage at treatment	*Radiation Research* 2, 84–90 (1955)
Doermann, A. H., M. Chase, and F. W. Stahl	Genetic recombination and replication in bacteriophage	*J. Cellular Comp. Physiol.* 45, Suppl. 2, 51–74 (1955)
Doherty, D. G., and W. T. Burnett, Jr.	The protective effect of S,β-aminoethylisothiuronium-Br-HBr and related compounds against X-radiation death in mice	*Proc. Soc. Exptl. Biol. Med.* 89, 312–314 (1955)
Dolin, M. I.	The DPNH-oxidizing enzymes of *Streptococcus faecalis*. II. The enzymes utilizing oxygen, cytochrome c, peroxide, and 2,6-dichlorophenol indophenol or ferricyanide as oxidants	*Arch. Biochem. and Biophys.* 55, 415–435 (1955)
Gude, W. D., A. C. Upton, and T. T. Odell	Giemsa staining of autoradiograms prepared with stripping film	*Stain Technol.* 30, 161–162 (1955)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Hollaender, A.	Biology Division Semiannual Progress Report for period ending February 15, 1955	Project Report ORNL-1863
————	Symposium on Genetic Recombination (1954)	*J. Cellular Comp. Physiol.* 45, Suppl. 2 (1955)
Hollaender, A., and C. O. Doudney	Studies on the mechanism of radiation protection and recovery with cysteamine and β-mercaptoethanol	*Radiobiology Symposium 1954*, ed. Z. M. Bacq and P. Alexander, Butterworths, London, p 112–114 (1955)
Horn, E. C.	An *in vivo* effect of nucleoprotein from Ehrlich ascites tumor cells	*Biochim. et Biophys. Acta* 16, 440–442 (1955)
Kirby-Smith, J. S., C. W. Sheppard, and D. L. Craig	The relative effectiveness of various ionizing radiations on chromosome breakage in *Tradescantia*	*Radiobiology Symposium 1954*, ed. Z. M. Bacq and P. Alexander, Butterworths, London, p 262–264 (1955)
Oakberg, E. F.	Sensitivity and time of degeneration of spermatogenic cells irradiated in various stages of maturation in the mouse	*Radiation Research* 2, 369–391 (1955)
Odell, T. T., Jr., F. G. Tausche, and W. D. Gude	Uptake of radioactive sulfate by elements of the blood and the bone marrow of rats	*Am. J. Physiol.* 180, 491–494 (1955)
Phares, E. F., and M. V. Long	The complete degradation of carbon-14-labeled succinic acid and succinic anhydride by the Schmidt reaction	*J. Am. Chem. Soc.* 77, 2556–2557 (1955)
Russell, L. B., and W. L. Russell	Pathways of radiation effects in the mother and the embryo	*Cold Spring Harbor Symposia Quant. Biol.* 19, 50–59 (1954)
Schwartz, D.	Speculations on gene action and protein specificity	*Proc. Natl. Acad. Sci. U.S.* 41, 300–307 (1955)
————	Studies on crossing over in maize and *Drosophila*	*J. Cellular Comp. Physiol.* 45, Suppl. 2, 177–188 (1955)
Stapleton, G. E.	Factors modifying the sensitivity of bacteria to ionizing radiations	*Bacteriol. Revs.* 19, 26–32 (1955)
Swanson, C. P.	Effect of oxygen tension on the production of chromosome breakage by ionizing radiations: An interpretation	*Radiobiology Symposium 1954*, ed. Z. M. Bacq and P. Alexander, Butterworths, London, p 254–261 (1955)
————	The oxygen effect and chromosome breakage	*J. Cellular Comp. Physiol.* 45, Suppl. 2, 285–298 (1955)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
______	Relative effects of qualitatively different ionizing radiations on the production of chromatid aberrations in air and in nitrogen	*Genetics* 40, 193–203 (1955)
Tolbert, N. E.	Formic acid metabolism in barley leaves	*J. Biol. Chem.* 215, 27–34 (1955)
Totter, J. R.	Antimetabolite studies on bone marrow in vitro	*Antimetabolites and Cancer*, ed. C. P. Rhoades, AAAS Symposium Series, p 153–162 (1955)
Upton, A. C., and J. Furth	A transmissible disease of mice characterized by anemia, leukopenia, splenomegaly, and myelosclerosis	*Acta Haematol.* 13, 65–76 (1955)
______	Spontaneous and radiation-induced pituitary adenomas of mice	*J. Natl. Cancer Inst.* 15, 1005–1021 (1955)
Wilde, W. S., and C. W. Sheppard	Electrolytes and water in muscles of the rat forelimb after intense local X irradiation	*Proc. Soc. Exptl. Biol. Med.* 88, 249–253 (1955)
Wolff, S., and H. E. Luippold	Metabolism and chromosome-break rejoining	*Science* 122, 231–232 (1955)

Scientific Society Lectures and Traveling Seminars. – The listed 95 lectures represent the Biology Division's participation in the Traveling Lecture Program and presentations from the professional society platform.

Anderson, N. G.	Sigma Xi, The University of Tennessee, Knoxville	Concepts of the origin of life
______	Department of Biology, Spring Hill College, Spring Hill, Ala.	Studies on isolated fractions of living cells
______	Departments of Physiology and Zoology, Florida State University, Tallahassee	Cell particulates and cell physiology
______	Sigma Xi, Florida State University, Tallahassee	Origin of life
Anderson, N. G., and M. L. Anderson	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), San Francisco, Calif.	Quantitative beef fractionation [Abstr., *Federation Proc.* 14, 4 (1955)]
Atwood, K. C.	Department of Zoology, Columbia University, New York, N.Y.	Informal seminar
Atwood, K. C., and T. Pittenger	Radiation Research Soc., New York, N.Y.	X-ray-induced mutations in growing mycelium of *Neurospora*
Auerbach, R.	Am. Assoc. Anat., Philadelphia, Pa.	The development of X-ray-induced "spina bifida" in the mouse [Abstr., *Anat. Record* 121, 258 (1955)]

Baker, W. K.	Brookhaven Symp. No. 8 – Mutations, Upton, N.Y.	The oxygen effect and the mutation process
————	Department of Zoology, University of Kentucky, Lexington	Gene action and gene position
Benedict, W. H., K. W., Christenberry, and A. C. Upton	Assoc. Research Ophthalmol. (A.M.A.), Atlantic City, N.J.	Spontaneous and radiation-induced iris atrophy in mice
Billen, D.	Medical and Psychological Section of the Georgia Academy of Science, Dahlonega	Radiation protection
————	Department of Botany, University of Georgia, Athens	The effects of ionizing radiation on the physiology and cytology of bacterial cells
————	Soc. Am. Bacteriol., Tenn.–Ky. Branch, Lexington, Ky.	Some studies on the effects of X irradiation on microorganisms
Billen, D., C. O. Doudney, and A. Hollaender	Soc. Am. Bacteriol., New York, N.Y.	Studies on the relation between induced mutagenesis and survival in X-irradiated *E. coli* [Abstr., *Bacteriol. Proc.*, p 56 (1955)]
von Borstel, R. C., A. R. Whiting, and K. C. Atwood	Radiation Research Soc., New York, N.Y.	Conditionally delayed dominant lethal mutations in *Habrobracon*
von Borstel, [illegible] and H. Moser	Internatl. Conf. on Radiobiol. Cambridge, England	Nuclear and cytoplasmic effects of ultraviolet radiation on *Habrobracon* eggs
Brumfield, R. T. and D. E. Foard	Assoc. S. E. Biol., Charleston, S.C.	The effect of certain chemical treatments on cell growth in timothy roots [Abstr., *ASB Bulletin* 2, 4 (1955)]
Burnett, W. T., Jr.	Biochemistry seminar, School of Medicine, Vanderbilt University, Nashville, Tenn.	On the examination of chemical protectors for X-irradiated animals
Burnett, W. T., and D. G. Doherty	Radiation Research Soc., New York, N.Y.	Additive effect of S,β-aminoethylisothiuronium-Br-HBr, bone marrow, and streptomycin on gamma-irradiated mice
Carson, S. F.	Division of Biological Sciences, North Carolina State College, Raleigh	Problems in bacterial physiology and biochemistry
————	Department of Botany, University of North Carolina, Chapel Hill	As above
Cohn, W. E., and D. G. Doherty	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), San Francisco, Calif.	Catalytic hydrogenation of pyrimidine nucleotides [Abstr., *Federation Proc.* 14, 194–195 (1955)]

Cohn, W. E., and J. X. Khym	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), San Francisco, Calif.	Ion-exchange behavior of undegraded nucleic acid preparations [Abstr., *Federation Proc.* 14, 235 (1955)]
Cohn, W. E., and E. Volkin	Third Internatl. Congr. Biochem., Brussels, Belgium	Pyrimidine sequences in deoxyribonucleic acids from the analysis of acid digests
Conger, A. D.	Sigma Xi, The University of Alabama Medical Center, Birmingham	Radiobiological studies at atomic bomb detonations
————	Southern Research Institute, Birmingham, Ala.	As above
————	Biological Science Department, University of Georgia, Athens	As above
————	Beta Beta Beta Regional Meeting, Stetson University, Deland, Florida	As above
————	Division of Biological Sciences, North Carolina State College, Raleigh	As above
————	Conf. on Ascites Tumor, N.Y. Acad. Sci., New York	Radiation effects on ascites tumor chromosomes
Conte, F. P., G. S. Melville, M. Slater, and A. C. Upton	Radiation Research Soc., New York, N.Y.	Paired dose studies of residual injury after exposure to fast neutrons and X rays
Cormier, M. J., and J. R. Totter	Am. Chem. Soc., 127th Natl. Meet., Cincinnati, Ohio	Studies on the luminescence of the fungus *Omphalia flavida* [Abstr. of Papers of 127th Natl. Meet. ACS, p 11C (1955)]
Dent, J. N., J. S. Kirby-Smith, and D. L. Craig	Am. Assoc. Anat., Philadelphia, Pa.	Induction of metamorphosis in *Gyrinophilus palleucus* [Abstr., *Anat. Record* 121, 429 (1955)]
Doherty, D. G.	Chemistry Department Journal Club, University of North Carolina, Chapel Hill	The hydrolysis of carbon-carbon bonds by α-chymotrypsin
Doherty, D. G., and J. F. Christman	Soc. Am. Bacteriol., New York, N.Y.	Microbial utilization of heparin [Abstr., *Bacteriol. Proc.*, p 141 (1955)]
Doherty, D. G., and R. Shapira	Third Internatl. Congr. Biochem., Brussels, Belgium	A kinetic study of the hydrolysis of carbon-carbon bond by α-chymotrypsin
Dolin, M. I.	Soc. Am. Bacteriol., New York, N.Y.	DPNH peroxidase, a new flavoprotein [Abstr., *Bacteriol. Proc.*, p 116–117 (1955)]

———————	Enzyme Club of the Departments of Biochemistry and Bacteriology, University of Illinois, Champaign	DPNH peroxidase, a new flavoprotein
Doudney, C. O., and A. Hollaender	Soc. Am. Bacteriol., New York, N.Y.	The effect of cysteamine and mercaptoethanol on mutation induction by X rays in *Escherichia coli* [Abstr., *Bacteriol. Proc.*, p 56–57 (1955)]
Gude, W. D., T. T. Odell, Jr., and A. C. Upton	Am. Assoc. Anat., Philadelphia, Pa.	Cytochemical studies of the human and rat platelet [Abstr., *Anat. Record* 121, 303 (1955)]
Hinton, C. W.	Assoc. S. E. Biol., Charleston, S.C.	Evidence for anaphase bridge formation in the cleavages of *Drosophila melanogaster* [Abstr., *ASB Bulletin* 2, 7 (1955)]
Hollaender, A.	Natl. Acad. Sci. – 92nd Annual Meet., Washington, D. C.	Studies on protection by treatment before and after exposure to X and gamma radiation [Abstr., *Science* 121, 624 (1955)]
———————	Internatl. Conf. on the Peaceful Uses of Atomic Energy, Geneva, Switzerland	Studies on protection by treatment before and after exposure to X and gamma radiation
———————	Am. Nuclear Soc., State College, Pa.	Chemical protection of biological materials from radiation effects
Hollaender, A., D. Billen, and C. O. Doudney	Radiation Research Soc., New York, N.Y.	The modification of X-ray-produced mutations in *Escherichia coli* by pre- and posttreatment
Horn, E. C.	Assoc. S. E. Biol., Charleston, S.C.	An *in vivo* effect of nucleoprotein on ascites tumor-induced mortality in the mouse [Abstr., *ASB Bulletin* 2, 7 (1955)]
Horn, E. C., and M. E. House	North Carolina Acad. Sci., Davidson, N.C.	(1) A test of specific uptake from organ homogenates by homologous organs in the young mouse (2) Some serological observations on fractions of Ehrlich ascites tumor cells
Hurst, G. S., W. A. Mills, F. P. Conte, and A. C. Upton	Radiation Research Soc., New York, N.Y.	Neutron and gamma-ray dosimetry in lethality studies with a cyclotron

Khym, J. X., and W. E. Cohn	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), San Francisco, Calif.	Ion-exchange behavior of undegraded nucleic acid [Abstr., *Federation Proc.* 14, 235 (1955)]
Kimball, R. F.	Radiation Research Soc., New York, N.Y.	X-ray mutagenesis during various stages of the interdivision cycle in *Paramecium aurelia*
Kimball, A. W., and W. T. Burnett, Jr.	Radiation Research Soc., New York, N.Y.	Sampling methods for screening compounds in radiation protection studies in mice
Kirby-Smith, J. S.	Brookhaven Symp. No. 8 – Mutations, Upton, N.Y.	Effects on the genetic material by radiations of different linear energy transfer
________	Physics Department, University of Alabama, Tuscaloosa	Application of magnetic resonance techniques to biological problems
________	School of Veterinary Medicine, Tuskegee Institute, Ala.	As above
Kroll, A. R.	127th Natl. Meet. Am. Chem. Soc., Cincinnati, Ohio	Cytochrome oxidase dependence of photosynthetic phosphate transfer [Abstr. Papers at 127th Natl. Meet. ACS, p 43C (1955)]
Kroll, A. R., and N. E. Tolbert	127th Natl. Meet. Am. Chem. Soc., Cincinnati, Ohio	Light-catalyzed uptake of carbon monoxide by barley [Abstr. Papers at 127th Natl. Meet. ACS, p 10–11C (1955)]
Lindsley, D. L.	The University of Missouri, Columbia	Mapping the centromere in the axolotl
Morris, D. M., A. C. Upton, and F. F. Wolff	Am. Assoc. Cancer Research, San Francisco, Calif.	The influence of the thyroid gland on the susceptibility of AK/R mice to transplanted lymphoid leukemia [Abstr., *Proc. Am. Assoc. Cancer Research* 2, 36 (1955)]
Odell, T. T., Jr., W. D. Gude, and F. G. Tausche	Radiation Research Soc., New York, N.Y.	Uptake of $Na_2S^*O_4$ by megakaryocytes and platelets of irradiated rats
Phares, E. F., and S. F. Carson	Soc. Am. Bacteriol., New York, N.Y.	Succinic acid decarboxylase system [Abstr., *Bacteriol. Proc.*, p 112 (1955)]
Randolph, M. L., E. B. Darden, Jr., and C. W. Sheppard	Radiation Research Soc., New York, N.Y.	Fast neutron facility for quantitative radiobiology

Russell, L. B. and W. L. Russell	Internatl. Conf. on Peaceful Uses of Atomic Energy, Geneva, Switzerland	Hazards to the embryo and fetus from ionizing radiation
————	Internatl. Conf. on Radiobiol., Cambridge, England	The sensitivity of different stages in oogenesis to the radiation induction of dominant lethals and other changes in the mouse
Russell, L. B., and R. J. Spear	Radiation Research Soc., New York, N.Y.	Relation between dominant lethal incidence and stage in oogenesis irradiated
Russell, W. L.	Brookhaven Symp. No. 8 – Mutations, Upton, N.Y.	The mutational characteristics of specific loci
————	Internatl. Conf. on the Peaceful Uses of Atomic Energy, Geneva, Switzerland	Genetic effects of radiation in mice and their bearing on the estimation of human hazards
————	4th Regional Symp. on Atomic Energy and Science (sponsored by Duke Univ., Durham; N. C. State College, Raleigh; and Univ. of N.C., Chapel Hill, in cooperation with ORNL and ORINS)	Genetics
St. Amand, G. S., N. G. Anderson, and M. E. Gaulden	Assoc. S. E. Biol., Charleston, S.C.	The effect of ergotamine on mitosis in the neuroblasts of the grasshopper, *Chortophaga viridifasciata* [Abstr., *ASB Bulletin* 2, 10 (1955)]
St. Amand, W.	Assoc. S. E. Biol., Charleston, S.C.	Anaphase abnormality in the Ehrlich mouse ascites tumor [Abstr., *ASB Bulletin* 2, 10 (1955)]
————	Radiation Research Soc., New York, N.Y.	X-ray-induced mitotic and chromosomal effects in the grasshopper neuroblast and in the Ehrlich ascites carcinoma
Sborra, A. J., G. E. Stapleton, and A. Hollaender	Soc. Am. Bacteriol., New York, N.Y.	Partial recovery of *Escherichia coli* B/r from the lethal effects of ionizing radiations on a chemically defined medium [Abstr., *Bacteriol. Proc.*, p 56 (1955)]
Stapleton, G. E.	Oklahoma A & M College, Stillwater	The modification of the effects of ionizing radiation by postirradiation treatment

————	School of Medicine and the University Hospital, University of Oklahoma, Oklahoma City	As above
————	Department of Plant Science, University of Oklahoma, Norman	As above
————	University of Colorado Medical School, Denver	As above
————	Science Club, Regis College, Denver, Colorado	Radiobiological studies at atomic bomb detonations
Stapleton, G. E., A. J. Sbarra, and A. Hollaender	Soc. Am. Bacteriol., New York, N.Y.	Effects of the culture medium on the sensitivity of *E. coli* B/r to ionizing radiations [Abstr., *Bacteriol. Proc.*, p 56 (1955)]
————	Radiation Research Soc., New York, N.Y.	Recovery of bacteria from X- or gamma ray inactivation
Tolbert, N. E.	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), San Francisco, Calif.	Excretion of glycolic acid by algae during photosynthesis [Abstr., *Federation Proc.* 14, 292–293 (1955)]
Totter, J. R.	School of Medicine, University of Arkansas, Little Rock	Luminescence in the study of enzyme reactions
————	Department of Biochemistry, College of Medicine, Baylor University, Houston, Texas	(1) The metabolism of formate (2) Bioluminescence as a tool
————	Biochemistry and Physiology Departments, School of Medicine, Tulane University, New Orleans, La.	(1) The metabolism of formate (2) Bioluminescence as a tool in enzyme studies
Totter, J. R., and C. L. Comar	Johns Hopkins Symp. on Inorganic Nitrogen Metabolism: Function of flavoproteins	Function of molybdenum in xanthine oxidase
Totter, J. R., and M. J. Cormier	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), San Francisco, Calif.	Purification of luciferase from luminous bacteria [Abstr., *Federation Proc.* 14, 293 (1955)]
Upton, A. C.	Medical School, The University of Tennessee, Memphis	Radiation injury
Upton, A. C., and F. P. Conte	Radiation Research Soc., New York, N.Y.	The relative biological effectiveness of fast neutrons, X rays, and gamma rays for acute lethality in mice

Upton, A. C., T. T. Odell, Jr., and W. D. Gude	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), San Francisco, Calif.	The incorporation of sulfur-35-labeled sulfate in healing wounds and in platelets of normal and scorbutic guinea pigs [Abstr., *Federation Proc.* 14, 421 (1955)]
Whiting, A. R., and W. E. Murphy	Radiation Research Soc., New York, N.Y.	Responses of irradiated *Habrobracon* eggs and sperm to anoxia and their theoretical significance
Wolff, S., and H. E. Luippold	Radiation Research Soc., New York, N.Y.	The role of oxidative metabolism and ATP production on the rejoining of radiation-induced chromosome breaks
____________	Internatl. Conf. on Radiobiol., Cambridge, England	The biochemical aspects of chromosome rejoining
Zill, L. P.	Biochemistry Seminar, Vanderbilt University School of Medicine, Nashville, Tenn.	Chemical and biochemical studies of sedoheptulose

Visiting Lecturers. – It has been the privilege of Division members to hear lectures by a number of eminent scientists on the Biology Seminar Series. Listed below are the speakers and the subjects of their discussions.

S. G. Stephens	Genetics Department, North Carolina State College, Raleigh	(1) Natural crossing in cotton, its measurement and significance (2) Some possible mechanisms of speciation in the genus *Gossypium*
C. B. Metz	Department of Zoology, Florida State University, Tallahassee	The nature and role of specific egg and sperm substance in fertilization
S. Rogers	Department of Pathology, Duke University School of Medicine, Durham, N.C.	Studies of the mechanisms of oncogenesis, particularly as concerns pulmonary tumors in mice
E. L. Green	Division of Biology and Medicine, Atomic Energy Commission, Washington, D.C.	Hazards from atomic weapons tests
J. F. MacKey	Swedish Seed Association, Svalöf, Sweden	The evolutionary significance of induced mutations in agricultural plants
T. M. Sonneborn	Indiana University, Bloomington	Aging and its genetic consequences in *Paramecium*
S. Karkas	Department of Biochemistry, Duke University, Durham, N.C.	Enzyme reactions

L. G. Lajtha	Churchill Hospital, Oxford University, England	Studies on DNA synthesis in human bone marrow cells *in vitro*
L. Weil	U.S. Department of Agriculture, Animal Proteins Section, Philadelphia, Pa.	The action of visible light on biologically active proteins
W. S. Vincent	Anatomy Department, State University of New York, Upstate Medical Center, Syracuse	Chemical structure of nucleoli
R. L. Platzman	Department of Physics, Purdue University, Lafayette, Ind.	Mechanism of inactivation of protein by ionizing radiation
J. A. V. Butler	Chester Beatty Research Institute, The Royal Cancer Hospital, London, England	Recent work of nucleic acid and nucleoproteins
J. A. J. Stolwijk	Department of Plant Physiology and Laboratory for Physics Research, Agricultural University, Wageningen, The Netherlands	Plant growth and light
T. Komai	National Institute of Genetics Misima, Sizuoka-ken, Japan	An actual instance of microevolution observed in an insect population
G. D. Novelli	Department of Biochemistry, Western Reserve University, Cleveland, Ohio	Amino acid activation for protein synthesis
P. Koller	Chester Beatty Research Institute, The Royal Cancer Hospital, London, England	Chemical mutagenesis
S. Hornsey	Experimental Radiopathology Research Unit, Hammersmith Hospital, London, England	The length of the mitotic cycle in *Vicia faba* roots after X radiation
H. Eyring	University of Utah, Salt Lake City	Inflammation and stress
E. Novitski	Department of Zoology, University of Missouri, Columbia, Mo.	The nature of the synaptic mechanism in *Drosophila*
H. Borsook	Division of Biology, California Institute of Technology, Pasadena	Factors accelerating red blood cells and hemoglobin synthesis
J. M. Thoday	Department of Genetics, The University, Sheffield, England	Biological effects of radiations, especially on chromosomes
F. H. Sobels	Genetisch Instituut van de Rijksuniversiteit te Utrecht, The Netherlands	Radiogenetical studies
R. B. Withrow	Smithsonian Institution, Division of Radiation and Organisms, Washington, D.C.	A review of the photochemical reactions in plants which regulate growth and development
R. C. MacCardle	Laboratory of Pathology, National Institutes of Health, Bethesda, Md.	Mitochondrial changes in X-irradiated tissues
C. H. Wang	Department of Chemistry, Oregon State College, Corvallis	The utilization of glucose in microorganisms

P. R. Gross	Department of Biology, New York University, New York, N.Y.	The molecular events of cytoplasmic gelation – an *in vitro* approach
A. Fabergé	Department of Botany, University of Missouri, Columbia	Analysis of induced chromosome aberrations by maize endosperm phenotypes

Education. – Many lectures (listed by title in another part of this section) have been given in the Traveling Lecture program of ORINS-ORNL. In addition to these, a series of lectures and demonstrations at the secondary school level was presented at Oak Ridge High School by the following: W. A. Arnold, S. F. Carson, A. D. Conger, M. J. Cormier, A. L. Kroll, J. R. Totter, and L. P. Zill.

Short courses in radiation biology and biochemistry have been taught by Biology Division members at the Duke University Marine Laboratory at Beaufort, N.C. Participating in this summer work were: N. G. Anderson, A. D. Conger, C. W. Sheppard, J. R. Totter, and A. C. Upton.

Several members of the Division have taken advantage of educational and research opportunities during the period. W. A. Arnold is on leave of absence from the Laboratory to carry out studies, on energy transfer between large molecules in biological systems, at the Institute for Muscle Research, Woods Hole, Massachusetts. Daniel Billen is taking a month's course in tissue culture, sponsored by the Tissue Culture Association. W. E. Cohn is spending a year in England on a Guggenheim Fellowship. He is working with Professor Sir Alexander Todd at the Cambridge University Chemical Laboratory.

Speakers at Professional Meetings, Fall 1955. – During the month of September, 21 papers will be given by Biology Division members at meetings of the American Institute of Biological Sciences in East Lansing, Michigan, and at the American Chemical Society in Minneapolis, Minnesota.

At the American Society of Plant Physiologists (of the AIBS), papers by the following will be given: N. E. Tolbert; L. P. Zill and N. E. Tolbert; A. R. Kroll; C. W. Nystrom, N. E. Tolbert, and S. H. Wender; and A. W. Naylor and N. E. Tolbert.

The program of the Genetics Society of America (AIBS) lists papers by the following Biology members: K. C. Atwood and T. H. Pittenger; R. C. von Borstel, K. C. Atwood, and A. R. Whiting; A. D. Conger; D. L. Craig, J. S. Kirby-Smith, and J. N. Dent; C. W. Edington; C. W. Hinton, Jr.; R. F. Kimball and N. Gaither; J. S. Kirby-Smith and D. L. Craig; E. F. Oakberg; T. H. Pittenger; D. Schwartz; J. V. Slater; W. J. Welshons; and A. R. Whiting and S. Caspari.

At the American Chemical Society, papers will be presented by J. X. Khym, L. P. Zill, and W. E. Cohn; and E. F. Phares and S. F. Carson.

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball

K. C. Atwood	S. Wolff
R. T. Brumfield[1]	C. E. Bay
A. D. Conger	D. E. Foard[1]
T. H. Pittenger	N. Gaither
D. Schwartz	A. H. Johnston
J. V. Slater[2]	H. E. Luippold

Effect of Postirradiation Temperature on Mutation Induction in *Paramecium aurelia*

R. F. Kimball N. Gaither

Kimball[3] reported briefly that exposures of *Paramecium aurelia* to H_2O_2 following X irradiation resulted in less mutation, as measured by the percentage of exautogamous descendants that grew normally. There are now ten experiments in which this effect has been checked and, though the effect is small, it has been found in all but two of them. The average percentage of normal exautogamous clones (inversely related to the number of mutations) was 57.4 in the untreated group and 65.1 in the peroxide-treated group.

[1]Consultant.

[2]Research participant.

[3]R. F. Kimball, *Ann. N. Y. Acad. Sci.* **59**, 638–647 (1955).

Among other actions, H_2O_2 slows the division rate, on the average, to about three-fourths of its normal value in the first 24 hr. It seemed possible that this delay was causally related to the decrease in detectable mutation. Two other methods of causing delay in division have been tried, low temperature for 24 hr and starvation for a few hours after irradiation. The results of the starvation experiments have been equivocal so far although the amount of delay was nearly the same as that induced by H_2O_2. The temperature experiments have yielded clearly positive results on the small effect produced (Table 1). Although the data are variable, there seems to be little question that there are less normal clones (more mutation) at the higher temperatures than at the lower ones. Several experiments were carried out during a period of poor growth for considerable numbers of the exautogamous clones from unirradiated controls. There is some indication that the effect of temperature in this period was greater than at other times.

A temperature of 16°C reduces the division rate to about one-fourth its value at 27°C. Thus it is more effective in this regard than H_2O_2. However, it is no more effective in reducing the mutation rate. Whatever is involved in the reduction of the mutation rate, it is fairly clear that it cannot be simply a matter of increasing the time to the first division without regard to other changes in the cell, or else the effect is already at a maximum

TABLE 1. EFFECT OF POSTIRRADIATION TEMPERATURE ON THE PERCENTAGE OF NORMAL EXAUTOGAMOUS CLONES (AN INVERSE MEASURE OF THE AMOUNT OF RECESSIVE MUTATION) IN *PARAMECIUM AURELIA*

Expt. No.	Number of Treated Animals per Group	Number of Normal Exautogamous Clones (%) in Irradiated Animals Posttreated at Indicated Temperatures (°C)							Combined Unirradiated Controls
		6	9	16	20	27	31	37	
1	27			58.5	55.5	52.3	49.3		95.2
2	20		45.6			46.2	39.8		96.6
3	20	53.4				47.8		46.4	93.3
4	80			10.3			5.6		42.4
5	80			22.4		17.7	15.0		55.4
6	40			42.8		31.4			67.6
7	20			32.8		39.8	30.4		96.8

when the division time is only slightly increased. However, the positive effects with H_2O_2 and low temperature and the slightly positive, though still equivocal, effects of starvation do suggest that division time may be involved.

The possibility cannot be excluded as yet that the effect is not really on the production of mutations but on the likelihood that they will come to expression. At the moment, all that can be done is to point out that quite a few cell divisions elapse between the time of treatment and autogamy. During this intervening time, all groups were treated alike. Thus any effect on expression of mutations would have to be transmitted for many cell divisions, and this seems somewhat improbable. However, the considerable variability of the results makes it very desirable to check this possibility further before accepting the view that posttreatments have caused a decrease in mutations.

Effect of X Rays on Nuclear Exchange at Conjugation

R. F. Kimball N. Gaither

It has been reported[4] previously that fewer recessive mutations, as detected by postautogamous death and reduced growth rate, are found in *Paramecium aurelia* irradiated within the first 2 or 3 hr than in those irradiated in the last 2 hr of the interdivision interval. This has now been shown to be true for inhibition of nuclear exchange at conjugation[5] as shown in Fig. 1. Animals having the dominant marker gene cl^+ were irradiated and mated to animals homozygous for the recessive allele of this gene. An exconjugant clone from the unirradiated conjugant was considered not to have obtained a gamete nucleus from its irradiated mate if it grew well but failed to develop the dominant character. The cause of this abnormality in nuclear behavior has not been fully established, but it appears to involve an inherited effect on the nucleus associated with the production of dominant lethality. Thus the latter part of the division cycle is an insensitive period for the induction of both recessive mutations and nuclear effects.

[4]R. F. Kimball, N. Gaither, and M. K. King, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 12–13.

[5]R. F. Kimball, N. Gaither, and M. K. King, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 13–14.

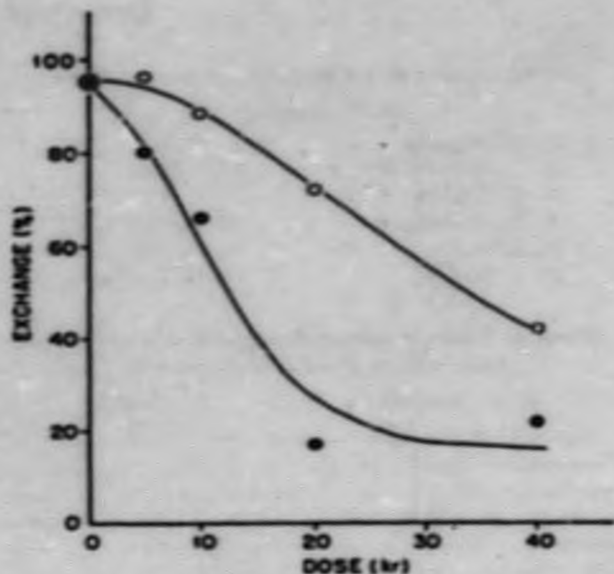

Fig. 1. Percentage of Gamete Nucleus Exchange at Conjugation Between Irradiated and Unirradiated Paramecia. ●, Animals irradiated in the first part of the interdivision interval; O, animals irradiated late in the interdivision interval.

Stationary-phase animals have been used in previous experiments on nuclear exchange and log-phase animals were used in the present experiments. The dose-response curve for stationary-phase animals[5] is nearly the same as the curve for log-phase animals late in the interdivision interval. However, previous experiments[6] have shown that recessive mutations are induced in stationary-phase animals at nearly the same rate as in log-phase animals in the early part of the interdivision interval. This shows that the two phenomena do not have the same physical basis despite certain similarities in their response. This situation is further shown by the failure[3] to obtain an oxygen effect on inhibition of nuclear exchange in contrast with the marked oxygen effect on recessive mutation.

[6]R. F. Kimball, N. Gaither, and M. K. King, *Biol. Quar. Prog. Rep. Feb. 15, 1951*, ORNL-969, p 11–12.

Studies on Cobalt Transport in *Tetrahymena*

J. V. Slater

It has been demonstrated that cobalt is essential for growth in *Tetrahymena*[7] but there is no information of any sort regarding ion transport in this ciliate. The prime function of cobalt in metabolism has been suggested to be that of serving as part of the vitamin B_{12} molecule,[8] but a number of functions have been attributed to this element per se. It is an exceedingly specific activator of glycylglycine hydrolysis[9] and it also strongly activates the hydrolysis of sarcosylglycine.[10] In general, in hydrolytic reactions, this element probably acts as a bridge in the formation of the enzyme-substrate complex.

The present experiments were undertaken with the view of closely delineating the movement of cobalt in this protozoan and of attempting to elucidate some of the factors regulating this transport. All experiments were repeated at least once.

Strain E of *Tetrahymena pyriformis* was grown in synthetic medium as previously described, with the exception that calcium, uracil, and adenylic acid were omitted. This medium resulted in full growth. In the first series of experiments, Co^{60} at a final concentration of 0.01 μc/ml was introduced at the beginning of the experiment in order to trace the movement of this element during growth. Spectrographic analysis revealed that the added Co^{60} amounted to 3.7 μg/ml (final concentration). No growth effects were observed from Co^{60} at this concentration. Cultures were harvested at appropriate time intervals by means of centrifugation in constriction tubes, which permitted a quantitative removal of all the cells. All concentrates were washed once with synthetic medium prior to volume adjustment. Experiments on the effect of washing showed that negligible amounts of the Co^{60} were left behind from the supernatants and that cobalt did not wash out of the animals. The presence of histidine in the synthetic medium aided materially in removal of Co^{60} from the glass tubes. The suspensions were adjusted to 2.0 ml and checked for activity in a deep-well scintillation counter. Adjustment to exactly this volume was found to be critical for reproducibility of results. All counts were made for 10-min intervals. Concentrations of 2×10^6 animals had no effect on activity counts.

Uptake of Co^{60} was steady during growth of the animals (Fig. 2) and release was abrupt shortly after growth reached the stationary phase. The uptake during growth amounted to 4.6×10^{-8} % of the total uptake of Co^{60} per hour per organism. Turnover during the stationary phase was studied for only two days so as to avoid the possibility of measuring cobalt release from disintegrated animals. Fifty per cent turnover was observed in 36 hr and the rate amounted to $(1.4 \times 10^{-6}$ %/hr)/organism. Since it was shown that stationary-phase organisms occupy approximately 0.015 ml when packed by centrifugation at 100 *g*, it was possible to calculate the concentrating ability of these organisms for cobalt. In medium containing 3.7 μg of added cobalt per milliliter, the ciliates concentrated this ion to the extent of 19 times that contained in a comparable volume of supernatant. When the added

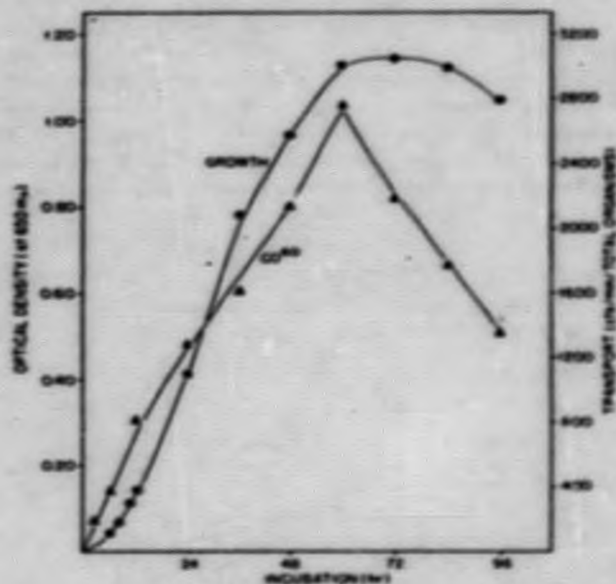

Fig. 2. Transport of Co^{60} During Growth of *Tetrahymena*. Temperature, 27.5 ± 0.5°C; pH, 7.5; synthetic medium.

[7] J. V. Slater, *Physiol. Zool.* 25, 323–332 (1952).

[8] H. R. Marston, *Physiol. Revs.* 32, 66–121 (1952).

[9] M. J. Johnson and J. Berger, *Advances Enzymol.* 2, 69–92 (1942).

[10] E. L. Smith, *J. Biol. Chem.* 176, 21–32 (1948).

cobalt level amounted to only 0.074 μg/ml, however, the concentration ability increased to 45 times.

It can be seen in Table 2 that the number of animals remained constant for 6–12 hr although the optical density measurements steadily increased. The uptake of Co^{60} during this period (Fig. 2) is most likely associated with an increase in volume of the individual organism, which is known to occur under these conditions. The volume reaches a maximum at about the middle of the log phase and falls off to about one-half this value upon reaching the stationary phase.

The turnover of Co^{60} was also studied in medium deficient in essential growth factors, glucose and the salts. Log-phase populations of the order of $8–9 \times 10^5$ animals were incubated for 24 hr in synthetic medium containing 0.01 μc of Co^{60} per milliliter and then removed, washed, and placed in deficient medium. This latter medium was employed in order to prevent growth effects. Two mechanisms (Fig. 3) are evident during cobalt release under these conditions. The first is very rapid and takes place within 2 hr at the rate of (13%/hr)/organism. The second is much slower and amounts to (1.7%/hr)/organism. Under these conditions, 50% turnover was attained in 20 hr.

The influence of number of animals on uptake per animal was studied (Fig. 4). Eight-hour periods of time were employed in order to minimize effects from both growth and the piling-up of metabolic wastes. The number of animals present had a definite effect on the uptake per animal. Populations of the order of 10^4 became nearly ten times

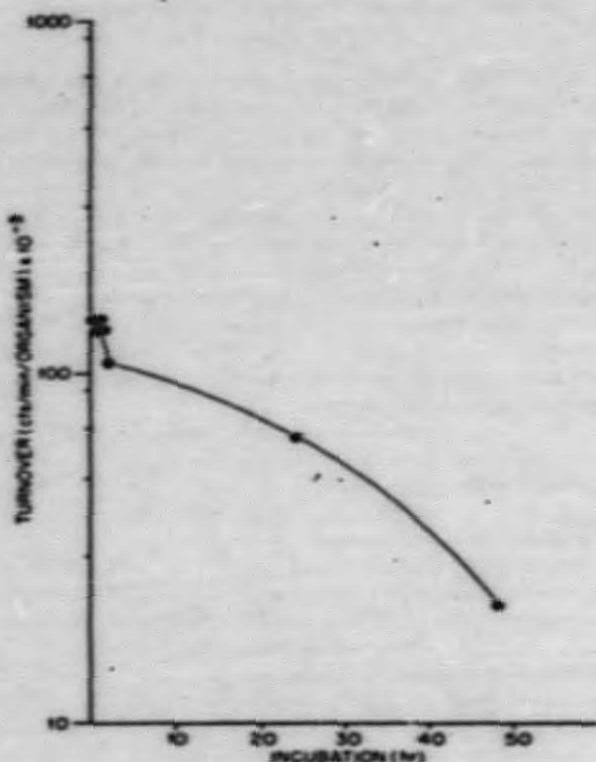

Fig. 3. Turnover of Co^{60} in Synthetic Medium Deficient in Salts and Growth Factors. Temperature, 26 ± 0.5°C; pH, 7.5.

TABLE 2. Co^{60} UPTAKE DURING GROWTH IN FIRST 12 hr

Time (hr)	Optical Density at 650 mμ	Number of Animals	Rate of Uptake in Total Mass of Animals (counts/min)
0		185,000	
15 min			77
2			208
4			252
6	0.04	479,000	381
8	0.07	459,000	489
10	0.11	457,000	743
12	0.14	466,000	806

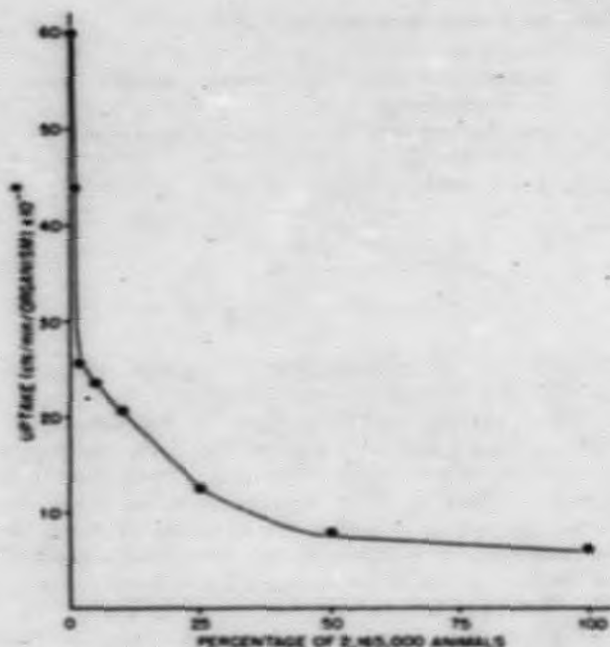

Fig. 4. Influence of Number of Animals on Uptake of Co^{60} in Synthetic Medium. Temperature, 26.5 ± 0.5°C; pH, 7.5; 8 hr.

as radioactive as populations of 2×10^4 organisms. At these high densities it is not unlikely that there was a great deal of competition for oxygen and also that harmful metabolic wastes resulted in inhibitory effects. Population densities of 2×10^5, however, were far from being crowded under these conditions and yet contained only one-third the activity of the lowest concentration.

Uptake was proportional to Co^{60} concentrations of 0.0005 and 0.01 μc/ml for a period of 12 hr. As the concentration of cobalt increased tenfold, the uptake increased twelvefold (Fig. 5). In some studies on the total uptake at peak growth with Co^{60} concentrations of 0.0002–0.01 μc/ml (Table 3), the uptake per organism increased only seventeenfold, whereas the cobalt concentration increased fiftyfold.

Autoradiograms are being prepared in attempts to locate the cellular site of Co^{60} in *Tetrahymena*. In a study on cobalt localization in the cells of the white mouse, Rosenfeld and Tobias[11] reported

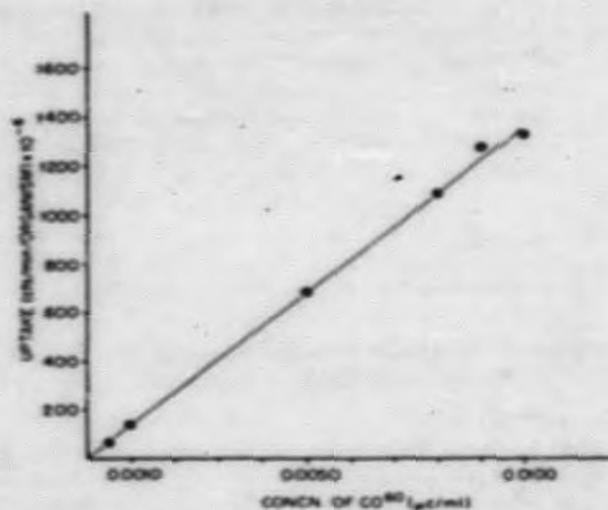

Fig. 5. Effect of Concentration of Co^{60} on Uptake in 12 hr. Temperature, 27.7 ± 0.2°C; pH, 7.5; synthetic medium; initial inoculation, 558,000 ± <5%; final concentrations of organisms, 832,000 ± <5%.

that most of the element was present in the cytoplasm and about 1% of it was firmly bound to cellular protein. Most of the association was with globulin in the bound fraction.

The slowly turning over fraction of Co^{60} in *Tetrahymena* (Fig. 3) may be associated with a firmly bound fraction of this type. This fraction appears to be about 75% of the total, however.

Tanaka *et al.*,[12] using *Bacillus subtilis*, demonstrated a steady uptake of radiocobalt and presented data which may possibly be interpreted as showing a turnover during the stationary phase of growth.

Appreciation is expressed to W. T. Burnett, Jr., and C. W. Sheppard for considerable aid and the use of facilities. N. G. Anderson was also very helpful in executing a successful design for a constriction-chamber centrifuge tube. Gratitude is expressed to W. D. Gude for an introduction to the preparation of autograms.

[11] I. Rosenfeld and C. A. Tobias, *J. Biol. Chem.* **191**, 339–349 (1951).

[12] S. Tanaka, Y. Sawada, and T. Yamamoto, *Kyoto Univ. Inst. Chem. Research Bull.* **30**, 52–53 (1952).

TABLE 3. EFFECT OF CONCENTRATION ON TOTAL UPTAKE OF Co^{60}

Co^{60} Added Initially (μc/ml)	Ratio – Uptake Rates at High and Low Concentrations	Co^{++} Added Initially (μg/ml)	Rate of Uptake per Organism (counts/min $\times 10^{-6}$)	Ratio – Uptake Rates at High and Low Concentrations
0.01		3.7	2292	
	50.1			17.1
0.0002		0.074	136	

Effect of Oxygen on Primary Breakage and Fusion in *Tradescantia* Chromosome Aberrations

A. D. Conger A. H. Johnston

An analysis has been made[13] of the effect of oxygen on primary breakage rate and fusion rate in *Tradescantia* X-ray-induced chromatid aberrations. The analysis showed that although oxygen increased the primary breakage rate, it also increased the fusion rate; therefore, for an equal number of primary breaks, a smaller proportion of breaks turned into visible aberrations. The observed air/nitrogen ratios of aberration production were explicable by the analysis.

It would be valuable if the same type of fusional analysis could be extended to the chromosome aberrations – which arise from irradiation in interphase when the chromosomes are single – since the bulk of the work reported in the literature is based on chromosome aberration results. It develops that the exact fusional analysis applied to the chromatid aberrations is not possible with the chromosome type, but an approximate analysis can be applied, and it is sufficient to indicate that oxygen has the same effect on fusion for chromosome aberrations as for the chromatid type. The problem is to find, among chromosome aberrations, criteria which will indicate a change in the fusion rate of radiation-induced chromosome breaks. The following are suitable criteria.

Incompleteness of Exchanges. – Chromosome exchanges (dicentric and ring chromosomes) can be completely fused (at both ends) or incompletely fused (at one end only). If oxygen has an effect on the fusion of breaks, it should result in a change in the fraction of exchanges which are incomplete. This fraction is independent of any effect that the oxygen may have on primary breakage rate.

Ratio of Terminal Deletions to Exchanges. – Chromosome terminal deletions arise from nonfusion of breaks; chromosome exchanges arise from the fusion of breaks. The ratio of terminal deletions to exchanges is therefore an estimate of the ratio of nonfusion to fusion events. A valid comparison of fusion in different oxygen concentrations can be made by comparing, for equal exchange frequencies, the ratio of terminal deletions in the different oxygen concentrations.

In two experiments, X radiation was delivered at 250 kvp (hvl, 1.45 mm of Cu) at rates of over 600 r/min to *Tradescantia* inflorescences in air and in nitrogen, and slides were made four days later for chromosome aberrations. Two doses each were given in air and nitrogen in the first experiment, and five doses in each gas in the second. Cells were analyzed for frequency of completely and incompletely fused exchanges, of interstitial deletions, and of terminal deletions.

Data on the incompleteness of exchanges are given in Table 4. The microscopic detection of distally incomplete exchanges is more accurate than that of proximally incomplete exchanges. An exchange which is distally incomplete can be detected because it produces two fragments instead of the single fragment of a completely fused exchange. Since the two classes should be equal, the total number of incomplete exchanges is taken as two times the number of the accurately observable distally incomplete exchanges (Table 4). Exchanges are about twice as incomplete in nitrogen as in air. This would mean that for equal numbers of breaks about twice as many turn into terminal deletions in nitrogen as in air.

By comparison of the ratios of terminal deletions to exchanges in nitrogen and in air, it can be

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[13] A. D. Conger and A. H. Johnston, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 16–21.

TABLE 4. FRACTION OF EXCHANGES WHICH ARE INCOMPLETELY FUSED, AFTER X IRRADIATION IN NITROGEN AND IN AIR

Expt. No.	Air		Nitrogen		Ratio of Incompleteness N_2:Air
	Total Exchanges	Fraction* Incomplete	Total Exchanges	Fraction* Incomplete	
1	420	0.13	273	0.19	1.5:1
2	664	0.06	531	0.14	2.1:1

$$\text{*Fraction incomplete} = \frac{\text{Inc}_p + \text{Inc}_d}{\text{total}} = \frac{2(\text{Inc}_d)}{\text{total}} .$$

shown by a different method that fusion of breaks is less in nitrogen than in air. Terminal deletions arise from nonfusion of breaks, exchanges from fusion. If nitrogen decreases fusion, then for an equal number of breaks in each gas, more terminal deletions and fewer exchanges would be found in nitrogen than in air. The estimate of fusion is not direct, as for the former case, because exchanges increase as a power of the dose, whereas terminal deletions increase linearly. However, a comparison of the frequencies of terminal deletions in air and in nitrogen, for equal exchange frequencies in the two gases, gives an approximate estimate of fusion, or rather, the ratio of fusion in the two gases. The values for this comparison, given in Table 5, were made from fitted dose curves for the aberrations concerned.

Experiment 1 is based on only two dose points in each gas, and is therefore less accurate than the results from experiment 2, based on five dose points in each gas. Here again the evidence indicates that fusion is less in nitrogen than in air; the consequences of less fusion in nitrogen would be a higher yield of terminal deletions in nitrogen than in air for an equal number of primary breaks.

TABLE 5. RATIO OF TERMINAL DELETIONS IN NITROGEN AND IN AIR, FOR EQUAL EXCHANGE FREQUENCY IN THE TWO GASES

Expt. No.	Ratio of $(TD)_{N_2}$:$(TD)_{air}$* When Frequency of Exchanges Is:			
	0.25	0.50	0.75	Average
1	1.2:1	1.2:1	1.2:1	~1.2:1
2	1.4:1	1.5:1	1.5:1	~1.5:1

*TD = terminal deletions.

By applying the analysis developed for chromatid aberrations[13] to the chromosome aberration data of these experiments, estimates of primary breakage rates and fusion rates and their ratios in nitrogen and in air can be made. Absolute values for these rates are only approximate, but the ratios in nitrogen and air are more accurate. Only the data of experiment 2 (five doses in each gas) are considered in the analysis in Table 6. Definition of terms and method of calculation are given in the previous report.[13]

The f values mean that about 7% of the primary breaks induced in nitrogen become terminal deletions detected at metaphase, only 3% – about half as many – in air. From the f values for terminal deletions and the ϵ values for exchanges, it is estimated that primary breaks in air and nitrogen are in the ratio of about 3:1 to 3.7:1 in this experiment. The approximate agreement indicates that the air/nitrogen fusion ratios have some validity, and explains how exchanges and terminal deletions can have different air/nitrogen ratios in the same experiment. The conclusions are substantially the same as those for chromatid aberrations.

Ascites Tumor Production by Single-Cell Inocula

A. H. Johnston

Ascites tumors have been used extensively in the last few years for many experimental purposes.

TABLE 6. ABERRATION RATIOS AND ESTIMATED FUSION RATES AND BREAKAGE RATES IN AIR AND IN NITROGEN; DATA FROM EXPERIMENT 2

Gas	Fraction of Incomplete Exchanges	Estimated f^*	Estimated c^{**}	Aberration Ratio Observed for:		Primary Breakage Ratio from:	
				TD	Exchanges	TD	Exchanges
N_2	0.14	0.073	0.97				
Air	0.06	0.033	0.99				
Air:N_2	0.47:1	0.45:1	1.02:1	1.5:1	3.9:1	3.0:1	3.7:1

*f = fraction of primary breaks which become terminal deletions seen at metaphase.

**c = relation between yield of exchanges and the value of f (number of breaks constant).

The ancient Ehrlich mouse ascites tumor, a favorable strain in many respects, has the disadvantage of considerable heterogeneity in the cell population, which is perpetuated by the approximately 10^6 cells used for routine transfer; the same comment applies to some other commonly used ascites tumor strains. Starting a new subline from a single cell would achieve the desirable result of genetic homogeneity. This has been done in the past by Hauschka, Goldman, and others, but at the cost of considerable effort and of mice because of the low percentage of takes. Results of single-cell inoculations made in this laboratory are given in Table 7. Twenty per cent takes in the mice which have been inoculated long enough (about 18–20 days) to develop a tumor is a high degree of success. Seventeen more mice have been inoculated at later dates, but it is still too early to tell whether a tumor will develop. Achieving the desirable result of genetic homogeneity by the simple and relatively easy method used, with its high percentage of successful takes, may be of some practical interest.

TABLE 7. PRODUCTION OF EHRLICH ASCITES TUMOR FROM SINGLE-CELL INOCULA (AS OF JULY 26, 1955)

Time Since Inoculation (days)	Number of Mice Inoculated	Number of "Takes" (Ascites Formed)
32	5	0
20	9	3
18	6	1
Total	20	4
	Percentage: 20	

Inoculations were made by a simple method similar to that of S. Hornsey (M.R.C., Hammersmith Hospital, London; personal communication). Freshly drawn, six-day-old ascitic fluid containing tumor cells was diluted out in cell-free (centrifuged) ascitic fluid to give about 0–10 tumor cells per small droplet delivered onto a glass microscope slide. Under the microscope at about 150 power, a single cell could be sucked up in a hand-held, fine glass, capillary pipette, connected by rubber tubing to the mouth. At the magnification used, obviously, abnormal or degenerate cells could be rejected, yet delicate manual manipulation of the pipette was still possible. To be certain that only a single cell was inoculated, a cell was drawn up into the pipette the first time, expelled on the slide in its droplet of fluid, examined, then sucked up a second time to inject from the same pipette into a day-old mouse. Ease and success of manipulation depended mostly on the size and bore of the capillary pipette. All inoculations had been made within 4 hr at the most, and usually only 1 hr after withdrawal of fluid.

Production of Two Types of X-Ray-Induced Chromosome Breaks

S. Wolff H. E. Luippold

Dose-intensity studies on the seed of *Vicia faba* have indicated that X-ray-induced chromosome breaks stay open for at least 2 hr when the seed

has been soaked in water. If, however, the seed has been soaked in BAL (British Anti-Lewisite) or placed *in vacuo*, then the breaks remain open for only ½ hr before rejoining. These experiments were designed to determine whether or not there was another type of break present which rejoined at a faster rate than those previously described. It should be noted that intensity studies in *Tradescantia* led Catcheside, Lea, and Thoday[14] to speculate on the existence of two different types of chromosome breaks – one type that rejoins relatively rapidly, and another that rejoins at a slower rate.

In the present experiments, the seeds were soaked in water for 24 hr or in water for 23½ hr and then in BAL for ½ hr. They were then irradiated with 600 r of 250-kvp X rays. After germination, the first mitotic root-tip division was examined for 2-hit chromosome aberrations. The results of the studies wherein the radiation was administered at various intensities are given in Fig. 6.

As may be seen, there is a group of breaks that stay open for a relatively long period of time. This is indicated by the plateau exhibited by each curve. If the breaks underwent restitution during this period, then, for a given dose, at any given time, there would be fewer open breaks in the system at low intensities than at high. Consequently, the number of 2-hit aberrations would diminish as intensity decreased. The length of the plateau wherein there is no diminution of 2-hit aberrations is, therefore, a measure of the time the breaks stay open. This time, which is dose dependent, is at least 2 hr in those seeds irradiated in aerated water and ½ hr in those irradiated in BAL. At the end of these periods, rejoining occurs and a typical intensity effect results. However, if the radiation is given at an intensity higher than 200 r/min, an increased number of aberrations above the plateau level is observed. This is true for aberrations produced in both water and BAL. This is interpreted as indicating that there is one type of break produced which rejoins, i.e., restitutes or forms a 2-hit aberration very rapidly. If the radiation is administered at a very high intensity, these breaks are present in the cell simultaneously and are capable of yielding a large number of 2-hit aberrations. In those seeds irradiated at lower intensities, most of these breaks restitute and there remain only those breaks which do not rejoin for a relatively long period of time. The latter are the breaks, described in previous reports from this Laboratory, that are responsible for the plateau on the intensity curves.

It has been postulated that, since respiration and ATP (adenosine triphosphate) are necessary for the rejoining of this latter type of break, the bonds formed are strong bonds, possibly of a covalent nature. Any discussion of the type of bonds formed in the rejoining of the breaks that rejoin very rapidly is, at this time, purely speculative. However, Mazia[15] has found that chelating agents are capable of breaking chromosomes, and Steffensen[16] has found that plants grown without calcium exhibit an increase in the number of spontaneous chromosome breaks. It may be that the breaks described by both Mazia and Steffensen are the same as the type that rejoins rapidly in *Vicia*. These may be breaks not of covalent bonds, but of salt linkages that will rejoin immediately and that will not remain open for 2 hr. In Table 8 are presented the results of experiments showing that chelating agents are capable of breaking the chromosomes of *Vicia*. In these experiments, the seeds were soaked first in water and then for varying periods of time in 0.001 *M* Versene. The total time of soaking, in all cases, was 24 hr.

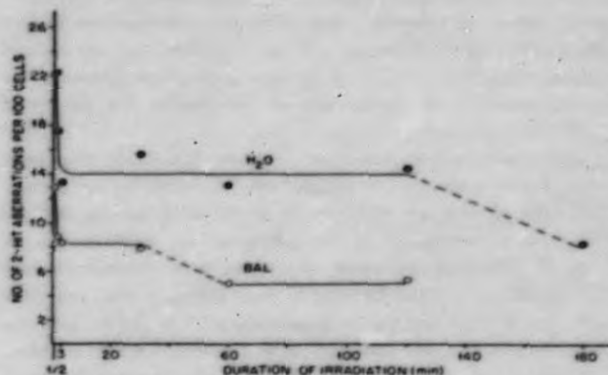

Fig. 6. Effect of High Intensity on Production of 2-Hit Aberrations.

[14]D. G. Catcheside, D. E. Lea, and J. M. Thoday, *J. Genet.* 47, 137–149 (1946).

[15]D. Mazia, *Proc. Natl. Acad. Sci. U.S.* 40, 521–527 (1954).

[16]D. Steffensen, *ibid.* 41, 155–160 (1955).

TABLE 8. PERCENTAGE ABERRATIONS PRODUCED BY SOAKING SEED IN VERSENE (0.001 *M*)

Total Cells	Time in Versene (hr)	Number of Aberrant Cells	Aberrations (%)
650	0	6	0.94 ± 0.38
750	3	27	3.60 ± 0.69
250	6	36	14.40 ± 2.4

These seeds were not irradiated. The ability of chelating agents to cause chromosome breakage in *Vicia* is interpreted as indicating that there are some breaks in the system that are breaks of salt linkages. As yet, these breaks have not been definitely identified as being the same as those that give the high numbers of aberrations when the material is irradiated at very high intensities. However, the existence of two chemically defined types of breaks – one of salt linkages and one of covalent bonds – is consistent with the results of the radiation-intensity experiments and the stated hypothesis.

Further Studies on the Biochemistry of Chromosome Rejoining

S. Wolff H. E. Luippold

Recent experiments in this Laboratory have indicated that postirradiation inhibition of cellular respiration is capable of preventing the rejoining of chromosome breaks induced in the seed of *Vicia faba*. It has also been determined that the application of exogenous ATP causes breaks to rejoin in a shorter period of time than they would in the absence of the ATP.

In Fig. 7 may be seen the results of radiation-intensity experiments performed on seeds soaked for 23½ hr in water and then for ½ hr in either ADP (adenosine diphosphate) or AMP (adenosine monophosphate). In order to allow comparison, Fig. 7 also includes the previous intensity curves obtained when the last ½ hr of soaking took place in either ATP or water. The total dose administered at any point of the curves was 600 r delivered by a G-E Maxitron tube operated at 250 kvp with 3 mm of Al filtration added. It may be noted that, in those seeds soaked in water alone

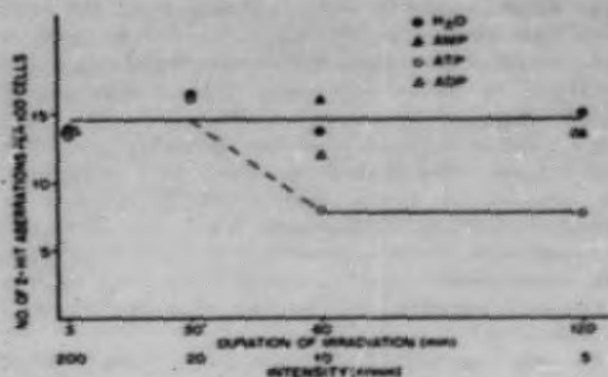

Fig. 7. Effect of Varying the Intensity of Radiation on Yield of 2-Hit Chromosomal Aberrations.

or in water plus either ADP or AMP, there was no decrease in the numbers of 2-hit aberrations when the radiation was administered at low intensity. This indicates that, under these conditions of soaking, there was no chromosome rejoining for at least 2 hr after the onset of irradiation. In contrast, seeds soaked for the last half hour in ATP did show an intensity effect (decrease in 2-hit aberrations), indicating that the chromosome breaks rejoined ½ to 1 hr after the commencement of the irradiation. The only discernible physiological difference between ATP and AMP or ADP is caused by the presence of a readily available high-energy phosphate bond in ATP. Thus this evidence that ATP itself can cause chromosome rejoining whereas its dephosphorylation products cannot is interpreted as being corroborative evidence of our previously advanced hypothesis that ATP with its pyrophosphate bond is necessary as a source of energy to be utilized in chromosome rejoining.

The cumulative studies in this field for the last year have led us to postulate a scheme to represent the train of events that chromosomes follow after irradiation. This scheme is presented in Fig. 8. It may be seen that the radiation has two independent consequences that affect the production of chromosomal aberrations. It both breaks the chromosomes and damages what, at this time, can only be designated as a "rejoining system." In the presence of ATP, the "rejoining system" is able to recover and cause the breaks to rejoin. If the X irradiation is performed *in vacuo*, a typical

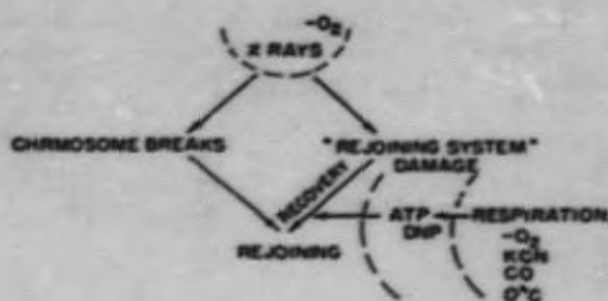

Fig. 8. Schematic Representation of the Effects of X Rays in Producing 2-Hit Chromosomal Aberrations.

"oxygen effect" is achieved; i.e., there is less damage caused by the radiation. This is reflected in both fewer breaks and less damage to the rejoining system, enabling the breaks to rejoin in a shorter time than after irradiation in air. If, however, the oxygen is removed after the irradiation and oxidative metabolism is inhibited, then no ATP is produced and the breaks formed are unable to rejoin.

At present, experiments designed to elucidate the nature of the "rejoining system" are under way. One possibility being explored is that it is connected with nucleic acid synthesis. In Fig. 9 may be seen the results of dose-intensity studies performed on seeds soaked in water for $23\frac{1}{2}$ hr and then $\frac{1}{2}$ hr in the various purine and pyrimidine bases utilized in the formation of nucleic acids. Adenine, guanine, uracil, and cytosine all act as protective agents, and the seeds soaked prior to irradiation in these chemicals exhibit fewer 2-hit aberrations than those soaked only in water. This was not so with thymine; seeds soaked in this substance did not show a significantly different aberration yield from the water controls. Thymine per se, however, is not the precursor of the thymine that becomes incorporated into the nucleic acids of the cell. In these experiments, uracil may be expected to act as the precursor for both incorporated uracil and thymine. It is concluded, therefore, that the time during which the breaks stay open can be influenced by preirradiation treatment with those purines and pyrimidines that are precursors for the purine and pyrimidine bases incorporated into nucleic acids.

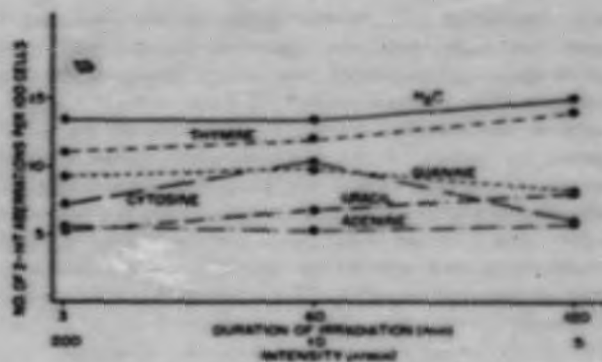

Fig. 9. Intensity Curves After Pretreatment Treatment with Selected Purines and Pyrimidines.

These results do not prove that the "rejoining system" is intimately connected with nucleic acid synthesis. However, they are consistent with such a hypothesis and indicate that further research along these lines is warranted.

Dominance Relations in *Neurospora* Heterokaryons

T. H. Pittenger K. C. Atwood

Heterokaryons between biochemical mutants in *Neurospora crassa* grow at an optimal rate of 5 mm/hr at 30°C on minimal medium provided that the components are of the same mating type and are genetically compatible. When the proportions of the two nuclear types are sufficiently unequal, however, growth will be limited by the ability of the minority component to offset the biochemical defect associated with the majority type, and growth rates will be suboptimal. Under conditions of prolonged stability of the nuclear proportions, a subnormal growth rate may be looked upon as a measure of the degree of dominance of the wild-type allele present only in the nuclei of the minor nuclear component. Thus, in cultures with sufficiently disproportionate ratios, and in the absence of nuclear selection, growth rates will be suboptimal and gene dosage and growth rate can be correlated over a wide range of values.

The opportunity to correlate gene dosage and growth rate is now afforded since recent studies have shown that (1) heterokaryons with a wide range of nuclear ratios can be prepared by varying

the proportion of conidial types in the inoculum[17] and (2) heterokaryons can be constructed with prolonged submaximal rates in which adaptive nuclear changes are absent.[18]

In these studies of the dominance of the *pan*$^+$ gene, a series of 21 heterokaryons with a wide range of nuclear ratios were synthesized in which an albino strain with a requirement for pantothenic acid (*pan,al*-1 A) was used as the major nuclear component and the minor nuclear component was one of three strains (*lys*-3 A, *arg*-6 A, or *nic*-2,*al*-2 A), each having the normal allele of the *pan* mutant but with requirements for lysine, arginine, and nicotinic acid, respectively. The heterokaryons were grown at 30°C in 300-mm growth tubes[19] on Fries minimal medium with washed agar and the growth rates determined. The frequencies of the minor nuclear components were calculated by the approximation of Atwood and Mukai.[20] The nuclear ratios in the proximal and distal ends of the growth tubes were then averaged, a procedure which seemed justified because the differences were usually small. For the purposes of correlating average growth rate with nuclear ratio and thus arriving at an estimate of dominance of *pan*$^+$, the data from the 21 heterokaryons were plotted in Fig. 10. In addition, the growth rates of *pan,al*-1 A homokaryons on various concentrations of calcium pantothenate were similarly determined. These data are also plotted in Fig. 10 with the concentration of calcium pantothenate on the same axis as the frequency of *pan*$^+$ nuclei.

The maximum growth rate of *pan,al*-1 A is reached with 0.5 μg of calcium pantothenate per milliliter and is the same as the growth rate on minimal Fries medium of heterokaryons in which the nuclear proportions are in the optimal range. Below the optimal range, the growth rate falls precipitously with decreasing proportions of *pan*$^+$ nuclei, or with decreasing concentrations of supplement. It is noteworthy that the curves are, at least roughly, of the same form; that is to say, changing the

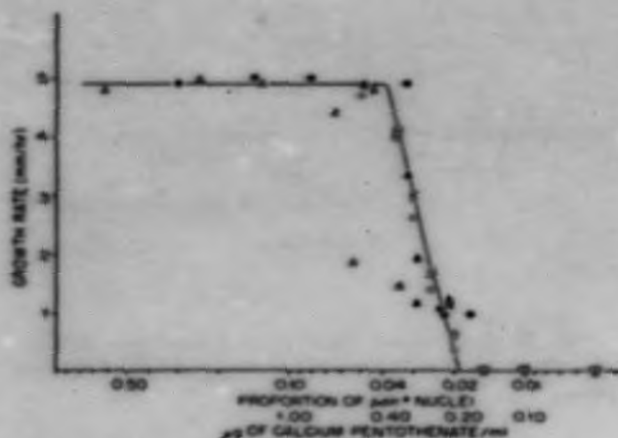

Fig. 10. Stability of Nuclear Proportions During Growth of *Neurospora* Heterokaryons. O, *pan,al*-1; ●, *pan,al*-1 + *nic*-2,*al*-2; △, *pan,al*-1 + *lys*-3; ▲, *pan,al*-1 + *arg*-6.

concentration of pantothenate by a certain factor has the same effect on the growth rate as changing the proportion of *pan*$^+$ nuclei by the same factor. Thus the proportion of *pan*$^+$ nuclei can be equated to the concentration of pantothenate in the medium. From the data in Fig. 10 it appears that 0.1 μg of calcium pantothenate per milliliter is approximately equivalent in growth promoting effects to 1% of *pan*$^+$ nuclei.

The heterokaryon having *lys*-3 as the minor nuclear component appears to require more *pan*$^+$ nuclei for an equivalent growth response than those having either *nic*-2, *al*-2, or *arg*-6. Although at present it is uncertain whether this effect is real, it is possible that this *pan*$^+$ is less dominant than the others, or that modifying factors in the genetic background of *lys*-3 are responsible.

If dominance is defined as the reciprocal of the frequency of the minor component just sufficient to promote normal growth, then the dominance of *pan*$^+$ is approximately 25–30; that is, the maximum activity of *pan*$^+$ is 25–30 times as great as that necessary for a normal growth rate. It would be of interest to learn whether the system always functions with this extra capacity, or whether the peak activity is evoked by the growth-limiting conditions. This cannot be determined with the type of information at hand, but since the growth

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[17]K. C. Atwood, T. H. Pittenger, and A. W. Kimball, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 26–28.

[18]T. H. Pittenger, K. C. Atwood, and A. W. Kimball, *ibid.* p 30–32.

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

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

responses to pantothenate and *pan*$^+$ nuclei were similar, it can be inferred that the activity per nucleus does not change appreciably within the suboptimal range.

Relation Between the Lethal Effects of X Rays on Microconidia and Ascospores

K. C. Atwood T. H. Pittenger

The survival curves of uninucleate microconidia and of macroconidia, which have a distribution of nuclear number, have been discussed previously.[21] It was apparent that the X-ray survival curve of the multinucleate cells could *not* have been predicted from that of the uninucleate, and that a large portion of the damage is prevented by interactions of unknown nature between the nuclei in multinucleate conidia. Another morphological element in *Neurospora* is the ascospore, of interest in these studies because it contains exactly two nuclei. The nuclei in ascospores are some 40 μ apart in a dense, coarsely vacuolated storage material, whereas the nuclei in macroconidia are closely adjacent in a finely granular cytoplasm. X-ray survival curves of ascospores were first obtained by Uber and Goddard in 1934, but these data cannot be used for comparison because the number scored by the individual isolation technique was insufficient to establish the form of the curves. Therefore it seemed important to obtain a survival curve for ascospores by the sorbose plating technique.

Ascospores taken from the cross between *tryp* (10575A) and *ad* (27663a) were irradiated with 250-kvp X rays, 4 mm of Al filtration, and then activated at 60°C for 20 min and plated in a thin top layer on supplemented sorbose agar. Accurate dilutions were made possible by suspending the heavy spores in a viscous 0.1% agar solution. Survival data based on counts of several hundred colonies per point are shown in Table 9.

[21] K. C. Atwood, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 24–26.

The significance of this survival curve is its relation to the curves previously obtained with uninucleate microconidia. The survival of ascospores agrees with that of microconidia on the assumption that the nuclei are independent units of inactivation; that is, that no interactions occur between nuclei in the ascospores and that an ascospore is viable if it contains at least one viable nucleus. On this basis the ascospore survival would be given by

$$s_{asc.} = 1 - (1 - s)^2 \; , \qquad (1)$$

where s is the survival of microconidia. In Table 9 the ascospore survival is compared with that of microconidia and macroconidia. The computed survival according to Eq. 1 is in good agreement with experiment. The macroconidia are much less sensitive; at 100,000 r their survival is 86 times as high as that of ascospores. This is consistent with the notion that the nuclei in ascospores are functionally isolated from one another to an extent which precludes an interaction of the type encountered in macroconidia.

Radiation-Induced Mutations in Maize

D. Schwartz C. E. Bay

Preliminary studies[22] have been reported on the mutagenic action of ionizing radiations at the

[22] D. Schwartz and G. M. Chenian, *Biol. Quar. Prog. Rep. Feb. 10, 1952*, ORNL-1244, p 24–25.

TABLE 9. SURVIVING FRACTIONS OF MICROCONIDIA, ASCOSPORES, AND MACROCONIDIA AS A FUNCTION OF X-RAY DOSE

Dose (10^3 r)	Survival, s, of Microconidia	$1 - (1 - s)^2$	Survival of	
			Ascospores	Macroconidia
20	0.62	0.86	0.90	0.82
40	0.20	0.36	0.58	0.50
60	0.026	0.051	0.066	0.35
80	0.0030	0.0060	0.0074	0.17
100	0.00034	0.00068	0.0011	0.095

Yg_2 locus in maize. Further testing has established that only two of the five yellow-green seedlings and only one of the mosaics found were true mutants. The others became fully green as they matured, and failed to transmit the mutant character to their offspring. The final tabulation gave two yellow-greens of the 33,934 seedlings grown. Since the female parent was heterozygous for the *wd* deficiency, only half the seedlings were of the proper genotype for the expression of the mutant phenotype. When the correction is made, the mutation rate of the Yg_2 gene at 1000 r of γ rays is calculated to be 1.18×10^{-4} (2/16,967). The mutation rate in the unirradiated control is 0.37×10^{-4} (2/54,677). The difference between these is not statistically significant.

From their X-ray studies on the *a* locus, Stadler and Roman[23] concluded that ionizing radiations produce few if any true gene mutations in maize. All the apparent gene mutations which they found were proved conclusively to be minute deletions. The present study was undertaken to determine whether this was peculiar to the *a* locus or was true for other loci as well. The data presented here support the conclusion of Stadler and Roman.

As the experiment was set up, yellow-green seedlings could result from either gene mutation or a small internal deficiency which did not affect the loci on either side, *w* and *py*. However, the absence of pale yellow-seedlings in the irradiated population points to mutation as the agent responsible for the yellow-green seedlings. The data suggest that all deficiencies in the chromomere must have been gross enough to delete the *w* locus as well, resulting in a white seedling. One pale yellow seedling was found in the control.

The mutable yellow-green (mosaic) plant is of considerable interest and is being studied in great detail. It was not included with the yellow-greens in calculating mutation rate since it appears to involve a chromosomal aberration similar to the *Ac-Ds* material described by McClintock. Different states of mutability which affect the time of mutation have been isolated. The mutability is associated with a change in the morphology of the terminal knob on chromosome 9. The knob is normally round and, in the line used here, is also very large. The mutable yellow-green plants contain both the round and an extremely elongated knob. Both knob types are found in the same anther. The factor responsible for this mutability does not activate McClintock's *Ds*.

Effects of Chemical Treatments and Radiations on the Growth of Timothy Roots

R. T. Brumfield D. E. Foard

In an earlier report[24] it was shown that 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) inhibits curvatures induced in timothy roots by unilateral exposure to ultraviolet radiation. The same compound was also found to inhibit completely the geotropic response of the root.[25] Indoleacetic acid (IAA) does not modify the curvatures induced by ultraviolet or the geotropic curvature.[24,25] In continuing this research, the effects of other compounds on curvatures in the timothy root have been tested. The methods used have been the same as those described in the earlier reports except that a constant-temperature box to house the microscope and growing root has been constructed and put into use. The additional compounds tested up to the present time are 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-butyric acid (IBA), and maleic acid hydrazide (MH), all in a wide range of concentrations.

The 2,4,5-T and 2,4-D in concentrations of 5 mg/liter inhibit the geotropic curvature but reduce growth to about one-third that of controls. IBA has essentially the same effect as IAA, i.e., a temporary inhibition of root growth, but the curvature per unit of growth is about the same as that of untreated controls. MH (800 mg/liter) had no apparent effect on growth or the geotropic curvature for the interval of observation (100 min). Experiments concerning the effects of these compounds on the curvatures induced by ultraviolet are still in progress.

In the experiments with 2,4,6-T on curvatures in the root, there was some indication that treatment with 2,4,6-T (10 and 20 mg/liter) immediately stimulated root growth in addition to its effect on the geotropic response. This possibility was more closely investigated by keeping individual

[23] L. J. Stadler and H. Roman, *Genetics* 33, 273–303 (1948).

[24] R. T. Brumfield, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 24–27.

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

roots under observation for 4 hr and then treating them with varied concentrations of 2,4,6-T and comparing rates of growth before and after treatment. The rate of root growth is essentially constant under uniform environmental conditions for about 24 hr. Therefore, the measurement of the growth of each root prior to treatment serves as a control to detect a stimulation or inhibition of any applied treatment. The 2,4,6-T in 10- and 20-mg/liter concentrations caused a slight temporary inhibition of growth and 5 mg/liter had no evident effect. It is concluded for the present that the stimulation indicated in the earlier experiments was caused by normal differences in growth rates of different groups of roots. This phase of the research is still being investigated.

Effects of Alpha-Particle Irradiation. – Since the timothy root is of small size and has proved to be a suitable test object for ultraviolet radiation, which is of limited penetration, it appeared likely that it might also serve as material for the study of the effects of α irradiation on cell division and growth. In preliminary experiments, roots were exposed unilaterally for 2, 4, 6, and 8 min to a polonium α-particle source delivering 1.5×10^5 α particles/sec/mm^2 at the surface of the root. All these dosages resulted in curvatures in the root. These curvatures are however quite different from those induced by ultraviolet radiation. Ultraviolet induces two curvatures in the root – one is toward the source, reaching a maximum about 40 min after irradiation, and is apparently caused by the inhibition of cell growth in that part of the root generally referred to as the region of elongation. The second curvature is away from the source, reaches a maximum about 80 min after irradiation, and is nearer the root tip than the first curvature. The second curvature evidently results from a stimulation of growth of cells on the irradiated side and nearer the tip than in the case of the first curvature. The curvatures induced by α radiation develop about 4 hr after irradiation and are toward the source. It is not known at present whether the curvatures induced by α radiation result from an inhibition of growth of cells in the region of cell division or whether dividing cells are so affected that their growth is inhibited when they reach the region of elongation. The curvature of the root straightens out during subsequent growth, possibly an effect of gravity since the roots were kept in a vertical position.

Another effect of α radiation is the production of greatly elongated cells on the irradiated side of the root, which becomes evident about 12–18 hr after exposure. The elongated cells are quite similar in appearance to the elongated cells appearing after ultraviolet irradiation which have been described elsewhere.[26] The elongated cells presumably result from an inhibition of cell division while the elongation of the cell continues. These experiments are currently in progress.

INSECT CYTOLOGY AND GENETICS

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R. W. Rogers[2] | G. S. St. Amand

X-Ray-Induced Dominant Lethality and Chromosomal Aberrations in a Thelytokous Wasp

B. R. Speicher K. G. Speicher

The ichneumon wasp, *Nemeritis canescens*, reproduces by female parthenogenesis, males being almost unknown. Diploidy of the matured, unfertilized egg is assured by an ineffective first maturation division which causes the abortive first polar nucleus to reunite with the second oocyte nucleus on a common metaphase II plate. Completion of the second maturation division produces a female pronucleus and a single polar nucleus, each with 2n (22) chromosomes.

If a mature female is witheld for a day or two from its host larva, the Mediterranean flour moth, *Ephestia kuehniella*, as many as 75 unlaid eggs will collect in the uteri. Such eggs have all passed through prophase and metaphase of the first meiosis and their chromosomes are uniformly fused into a compact mass, a condition which is common to the eggs of many Hymenoptera but distinctly different from *Habrobracon*. Chronologically at least, they are in a stage of arrested and modified anaphase I. They do not resume their maturation divisions until after oviposition.

In order to determine the sensitivity of these unusual eggs to radiation-induced dominant lethality, stock females were X-irradiated at doses ranging from 100 to 1200 *r* by increments of 100. Immediately after exposure they were allowed to oviposit into their host larvae for 3 or 4 hr, a

[26]R. T. Brumfield, *Proc. Natl. Acad. Sci. U.S.* **39**, 366–371 (1953).

period sufficiently brief to include only those eggs present in the uteri (i.e., in early anaphase I) at the time of irradiation. Near hatching time (51 hr at 30°C) the laid eggs were dissected from the host and the percentage of viable eggs was determined.

Results (Fig. 11), corrected to an inviability normal to the stock of about 1.5%, show a 50% survival at about 400 r and essentially no survival at 1200 r. These data agree substantially with those obtained for *Habrobracon* by A. R. Whiting[27] for eggs treated in late metaphase I. When plotted semilogarithmically, however, the survival slope for *Nemeritis* eggs appears not to be based on a simple one-event phenomenon.

Cytological examination was made of about 160 eggs, varying in stage at the time of fixation from anaphase I to completion of the second maturation division, but all irradiated at 600 r while in early anaphase I. Chromatid bridges and fragments were seen in all stages, although their frequency in anaphase I was impossible to determine. The presence of bridges in anaphase I, together with chromosome clumping, indicates that chromosome stickiness is present. Bridges seen in anaphase II could be caused by chromosome breakage and fusion of either sister strands or nonhomologous chromatids. About 135 eggs covering the same stages, but not irradiated, showed no unequivocal bridges, fragments, or clumping.

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

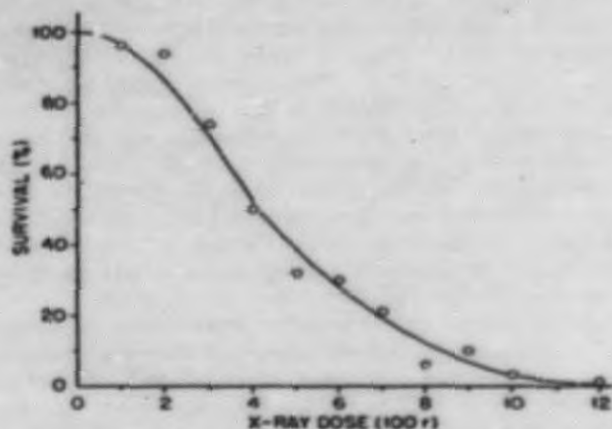

Fig. 11. Survival of *Nemeritis* Eggs Treated in Anaphase I to Increasing Doses of X Rays.

These results indicate that although the egg of *Nemeritis* is about equal in X-ray sensitivity with that of *Habrobracon*, there is some difference in (1) the cause of some dominant lethality and (2) the presence of chromosome stickiness during anaphase I. Both differences could be ascribed to the more compact, almost fused, condition of the uterine egg nucleus.

X-Ray-Induced Gene Mutations in *Nemeritis canescens*

B. R. Speicher K. G. Speicher

Wild-type females were X-irradiated with doses of 200–1000 r to produce mutations that could be used to analyze chromosome segregation in the atypical meiosis of *Nemeritis canescens*. The females were left with host caterpillars for only 4 hr after X-ray exposure so they would lay only those eggs which were irradiated at early anaphase I, and thus ensure a uniformly treated F_1 population. One visible mutation was found in the 1348 F_2 offspring from X-irradiated females which were examined. It has proved of little use because it affects the position of the wings and cannot be recognized in anesthetized specimens, where the wings are extended in normal as well as mutant individuals. Counts are therefore unreliable.

Seven hundred and forty-three eggs laid by F_1 females from irradiated mothers were examined, at a timed interval after laying, to determine whether recessive lethal mutations had been produced. Three such mutations were found and have been followed through three generations by means of egg counts. The ratios of inviable eggs among total eggs examined from heterozygous females are given in Table 10. Data are incomplete and corrections have not been made for inviability found in the stock.

TABLE 10. RATIOS OF INVIABLE EGGS PRODUCED BY THREE RECESSIVE LETHAL MUTATIONS

Mutation	Total Eggs	Number of Inviable Eggs	Percentage Inviability
l_1	97	24	24.7
l_2	111	28	25.2
l_3	155	42	27.1

The authors had previously reared *Nemeritis canescens* in mass culture through more than 100 generations without finding a male, and the species is described in the literature as consisting solely of females, except for one report in 1931 of the occurrence of three males in a laboratory culture.[28] In this experiment one male was found among the progeny of an X-rayed female. Prochromosomes that could be counted in spermatogonial cells were well above the haploid number, indicating that the male was diploid. This was verified by counts of bristles on a measured area of the wing which were lower than comparable bristle counts on female wings, as they are in diploid males of *Habrobracon*. Since males in the order Hymenoptera are normally haploid, this may be considered an exceptional male, and its origin from an irradiated female suggests that it may have been produced by an alteration in a sex-determining locus, or region, during meiosis.

Recessive Lethal Mutations Induced by X Rays in the First Meiotic Prophase of *Habrobracon* Oocytes

R. C. von Borstel K. C. Atwood[29]
A. R. Whiting

It was previously reported[30] that a paradoxical inequality existed in embryo recessive lethal mutation frequency as determined by two different methods. This paradox was resolved by the discovery and demonstration of a new type of dominant lethal mutation which is referred to as a conditionally delayed dominant lethal mutation.[31] The conditionally delayed dominant lethal mutation is expressed by death of the haploid embryo and death of the diploid larvae or pupae and is associated with eggs irradiated in the first meiotic metaphase of oogenesis.

It is the purpose in this report to use the two methods of analysis for recessive lethal mutations on eggs irradiated in the first meiotic prophase. These methods are (1) an F_1 generation test which discloses the frequency of recessive lethal mutations induced by X rays in haploid eggs, and (2) a second-generation (F_2) test which reveals the frequency of recessive lethal mutations in eggs which were fertilized subsequent to irradiation. When eggs in the first meiotic prophase are irradiated, there is no discrepancy between the results of the two methods of analysis, and either test may be regarded as providing an accurate estimate of the frequency of recessive lethal mutations induced by X rays.

Based on the equations reported previously,[30,31]

$$r = 1 - (V_u/V_f),$$

where r is the frequency of chromosome sets having at least one embryo recessive lethal mutation, V_u is the viable proportion of unfertilized eggs from unmated or mated females, and V_f is the viable proportion of fertilized eggs. Since approximately 0.67 of the eggs are normally fertilized, then V_f may be obtained from

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

where m is the viable proportion of eggs from mated females and f is the proportion of fertilized eggs. From these equations, the embryo recessive lethal frequency can be determined based on egg hatchability in the F_1.

Similarly, for total recessive lethals in the F_1, the method can be used to express r', the frequency of genomes bearing at least one recessive lethal mutation expressed at any stage of development,

$$r' = 1 - (V_u'/V_f'),$$

where

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

is the proportion of fertilized eggs reaching the adult stage.

The second method (F_2 analysis) is to isolate unmated females and determine the frequency that these are heterozygous for at least one recessive lethal mutation.

Table 11 contains data from an experiment from which the recessive lethal frequencies are calculated by the F_1 method. In this experiment eggs

[28] E. Deutert-Willmczik, *Zool. Anz.* 93, 274–275 (1931).

[29] Cytogenetic Effects of Radiation Group.

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

[31] R. C. von Borstel, K. C. Atwood, and A. R. Whiting, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 36–39.

TABLE 11. SURVIVAL DATA AND F_1 ANALYSIS OF *HABROBRACON* EGGS IRRADIATED IN THE FIRST MEIOTIC PROPHASE

	Offspring from:	
	Mated Females	Unmated Females
Hatchability (larvae/eggs)	232/531 = 0.437 (m)	211/537 = 0.393 (V_u)
Hatchability (corrected)	0.459 (V_f)	
Adults/eggs	173/531 = 0.326 (m')	150/537 = 0.279 (V'_u)
Adults/eggs (corrected)	0.349 (V'_f)	

were irradiated with 15,000 r in the first meiotic prophase of oogenesis. The values of r and r' as calculated by the F_1 and F_2 method are compared in Table 12. It is apparent that there is no significant difference between the two methods for determining the frequency of recessive lethal mutations when eggs in the first meiotic prophase are irradiated. At present it is unknown why conditionally delayed dominant lethal mutations are restricted to eggs irradiated in the first meiotic metaphase.

TABLE 12. RECESSIVE LETHAL MUTATION FREQUENCY DETERMINED BY F_1 AND F_2 ANALYSIS

	F_1 (Computed from Table 11)	F_2 (Females with Lethals / Females Tested)
Embryo (r)	0.145	17/118 = 0.144
Total (r')	0.201	28/118 = 0.237

Radiosensitivity of the Unfertilized *Habrobracon* Egg

W. St. Amand[29] R. C. von Borstel

In the parasitic wasp, *Habrobracon*, oogenesis is arrested near the end of the first meiotic division and meiosis continues only after passage of the egg through the ovipositor. Having been laid, all eggs develop at approximately the same rate and, within any given egg, cleavage division nuclei are synchronized. Stage of meiosis or mitosis can thus be related to time after laying, and the radiosensitivity of division stages can be determined by the hatchability of eggs treated at known intervals after oviposition.

Eggs from virgin females were collected as soon as laid, placed on numbered plastic cover slips, and the cover slips put in a plastic box which was maintained at 20°C over a water bath. In all cases, the "age" of the egg is known to within 1 min.

Eggs to be used for cytology were fixed in Kahle's fluid and stained with Feulgen. (See data in Table 13.) "Time-range" refers to the interval between oviposition and fixation.

The irradiated eggs received 500 r of X rays (approximately an LD_{50} dose for unlaid arrested eggs) at about 600 r/min (250 kvp, 30 ma; inherent filtration, 1 mm of Al; added filtration, 3 mm of Al, ½ mm of Cu). Data on the hatchability of irradiated eggs have been grouped and are tabulated in Table 14. "Time after laying" refers to the interval between oviposition and irradiation. The hatchability of eggs collected as controls (521 eggs) was 92.1%.

A total of 538 eggs have been irradiated and 163 eggs were examined cytologically. Experiments are being continued. The results obtained to date indicate that (1) eggs just before or just after oviposition are about equally radiosensitive; (2) the radiosensitivity of the meiotic stages from the arrested stage to anaphase II shows no great fluctuations of radiosensitivity; (3) the pronuclear stage is much more radiosensitive than prophase of the first cleavage division; and (4) there is a progressive increase in radiosensitivity from the first to the third cleavage division.

TABLE 13. CYTOLOGY OF UNFERTILIZED *HABROBRACON* EGGS

Stage	Time Range After Laying (min)	Number of Eggs
Anaphase-I	0–4	6
Meion	6–18	21
Prometaphase II	16–28	12
Metaphase II	23–41	27
Anaphase II	43–48	10
Pronucleus	54–61	6
Prophase (1st cleavage div)	73–87	20
Metaphase (1st cleavage div)	88–115	24
Anaphase (1st cleavage div)	101–115	5
Telophase (1st cleavage div)	116–119	4
Interphase	117–119	3
Prophase (2nd cleavage div)	117–127	4
Metaphase (2nd cleavage div)	119–136	8
Anaphase (2nd cleavage div)	133–136	2
Telophase (2nd cleavage div)	127–146	4
Interphase	146	1
Prophase (3rd cleavage div)	157	1
Metaphase	–	–
Anaphase	153–167	5

Effects of Polonium Alpha Particles on Hatchability of *Habrobracon* Eggs

R. W. Rogers   R. C. von Borstel

Newly deposited eggs of virgin female *Habrobracon* were irradiated with polonium α particles in an effort to determine the relative radiosensitivities of nuclear and nonnuclear elements of the egg. At the time of oviposition and for about 10 min following, the egg nucleus is in first meiotic metaphase or anaphase and averages only a few microns in diameter. During this time the nucleus resides within a few microns of the egg surface in the anterior one-sixth or less of the egg length. Thus, in an egg properly oriented, the nucleus is readily accessible to α particles with limited range in tissue.

TABLE 14. HATCHABILITIES OF IRRADIATED EGGS

Time After Laying (min)	Larvae/Eggs	Hatchability (%)
0–10	25/54	46.3
11–20	25/45	55.5
21–30	41/73	56.2
31–40	22/50	44.0
41–50	17/43	39.5
51–60	2/30	6.7
61–70	19/46	41.3
71–80	29/40	72.5
81–90	16/44	36.4
91–100	6/17	35.3
101–110	2/26	7.7
111–120	4/21	19.1
121–130	2/17	11.8
131–175	0/32	0.0

Irradiation of the nucleus was accomplished by orienting small groups of eggs, all of which had been oviposited *not more than 5 min before irradiation*, nuclear side up on a microscope slide, placing the slide on a microscope stage, and irradiating with the source mounted in the microscope nosepiece.[32] The distance between source and eggs was 10.0 mm and exposures were 5–150 sec in length. The dose rate, computed for an average nuclear cross-sectional diameter of 2.5 μ, was (0.015 α particle/sec)/nucleus. Hatchabilities were determined about 40 hr after irradiation. The percentage of hatchability of the irradiated eggs, nucleus exposed, is shown in Fig. 12 and is clearly exponential in nature.

The very low dose rate effective in killing eggs with the nucleus exposed affords an interesting comparison between the experimentally determined percentage hatchabilities relative to dose administered, and the theoretical probability of the nucleus receiving zero α particles during each period of exposure. Thus, in the 5-sec exposure, with a dose rate of (0.015 particle/sec)/nucleus, the average dose is equal to (0.08 particle/5 sec)/nucleus. The probability of receiving zero α particles with this value for average dose a in the

[32]The technique used in the dosimetry for this experiment is similar to that described in a paper by the senior author and which will appear in an early autumn issue of *Radiation Research*.

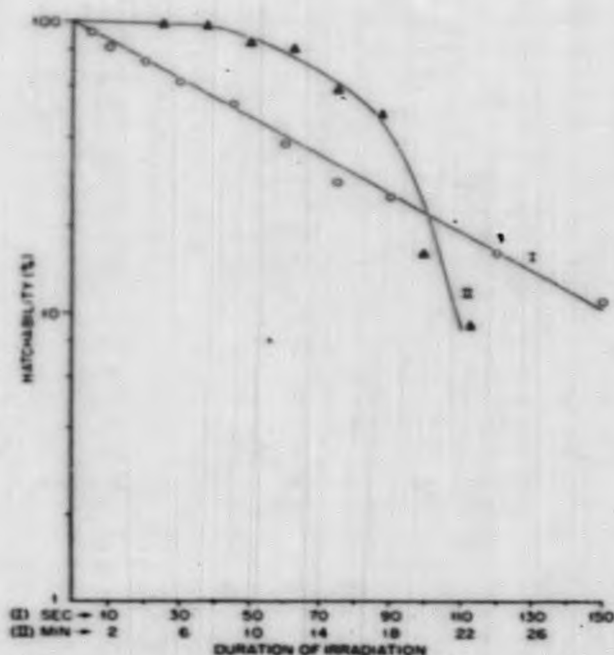

Fig. 12. Dose-Hatchability Curves for *Habrobracon* Eggs Irradiated with Polonium α Particles. O, Nucleus exposed, dose rate ≅ 0.015 α particles/sec/nucleus; ▲, nucleus shielded, dose rate ≅ 1.1×10^6 α particles/min/egg.

Poisson formula $a^x e^{-a}/x!$ is 0.92. The experimental percentage hatchability was also 0.92. Comparison is similarly drawn in Table 15 for all the radiation exposures utilized.

Exposure at the doses, in rad, administered to the nuclei is awkward in that the average doses do not have quite the same significance when applied in other radiations. For example, in the 5-sec dose, only about 8% of the nuclei exposed received any radiation at all, and this in terms of a single α particle. In the 150-sec dose, the maximum number of particles probably received by the nuclei was 6, with a probability of about 0.01. It is, then, perhaps better to express the doses in minimal and maximal probabilities; in this experiment the dose ranged from about 720 to 4320 rad/nucleus, representing 1–6 α particles per nucleus.

Irradiation of the cytoplasmic or nonnuclear end of the egg was done by arranging eggs, concave surface up, around a small hole in a plastic coverslip so that the nucleated half of the egg was shielded and the remainder exposed for irradiation. A more intense polonium source was used at a distance of only 1.0 mm from the eggs. The dose rate was computed to be about (1.1×10^6 particles/min)/egg. Duration of exposure was 5–22½ min, and the experimental results are shown in Fig. 12. The doses ranged from about 2.2×10^5 rad at 5 min to about 9.6×10^5 rad at 22½ min.

TABLE 15. EXPERIMENTAL AND EXPECTED PERCENTAGE OF HATCHABILITIES

Duration of Irradiation (sec)	Average Dose a (No. sec irrad × 0.015)	Probability of Zero α Particles	Exptl Percentage Hatchability
5	0.08	0.92	0.92
10	0.15	0.86	0.82
20	0.30	0.74	0.73
30	0.45	0.64	0.62
45	0.68	0.51	0.52
60	0.90	0.41	0.38
75	1.13	0.32	0.28
90	1.36	0.25	0.25
120	1.80	0.17	0.16
150	2.26	0.11	0.11

A comparison between the 50% lethal doses for nucleus and cytoplasm yolk is complicated somewhat by different shapes of the curves and by the Poisson nature of the nuclear low doses, but the latter difficulty can be generally expressed by the use of an average value for the low dose. The 50% lethal dose in the nuclear irradiations is the 45-sec dose with an average of (0.68 α particle/45 sec)/nucleus. This average actually represents a dose in which about one-half the nuclei would be expected to receive 0 α particles; about one-third receive 1 α particle; about one-tenth receive 2 α particles; and the remaining few per cent receive 3 or 4 α particles. The average dose with this distribution equals about 500 rad/nucleus. The 50% lethal dose in the cytoplasm yolk or nonnuclear exposures is approximately the $17\frac{1}{2}$-min dose, or 7.6×10^5 rad. The ratio of nuclear to nonnuclear doses is of the order of about 1 to 1.5×10^3. That is to say, the nucleus would appear to be over a thousandfold more radiosensitive to α particles than an equivalent volume of nonnuclear elements of the egg when hatchability of the egg is used as the criterion of sensitivity.

Preliminary Data on the Irradiation of the Cytoplasm and the Nuclei of *Habrobracon* Eggs with Various Wave Lengths of Monochromatic Ultraviolet Radiation

R. L. Amy R. C. von Borstel

In a living cell that has been injured by radiation it is of interest to attempt to identify the particular cellular constituent which has been affected adversely. A useful approach to such a study can be made by utilizing ultraviolet light as the damaging agent, since different wave lengths of this type of radiation are selectively absorbed by different chemical groups. Some infomation may be gained as to the identity of the cellular components absorbing the radiation (and thus eliciting the deleterious effect) by comparing action spectra (the relative effectiveness of various wave lengths producing this effect) with absorption spectra of various cellular constituents.

The newly laid haploid *Habrobracon* egg, by virtue of its organization, provides an excellent system for ascertaining separately the action spectra for the nucleus and for the cytoplasm. In the elongate, slightly curved egg, the nucleus is located near the anterior end adjacent to the convex surface and remains in this position until meiosis is completed (30 min).[33] The bulk of the cytoplasm is distributed in a layer just beneath the egg membranes and completely surrounds the large centrally located yolk mass. By exposing only the concave surface of the egg to a source of radiation, cytoplasm is treated, whereas exposure of the convex surface results in irradiation of the nucleus. Since individual irradiation of the two surfaces results in (1) differently shaped hatchability curves, (2) dissimilar sensitivity, and (3) unlike appearance, it seems probable that different cellular constituents are affected in each case.

In a typical experimental run, virgin females were allowed to oviposit on their hosts (*Ephestia* larvae) in Stender dishes. By examining the hosts periodically, fairly large numbers of eggs 0–15 min of age could be collected, positioned on a glass slip, and irradiated. Hatchabilities were recorded two days later. Adequate precautions were taken to prevent photoreactivation.

Mercury lamps of the Daniels-Heidt type[34] or of the G-E H-4 type were employed as sources of radiation. The source in use was focused upon the entrance slit of a Hilger D96 monochromator. A holder, attached to the end of the monochromator, was fashioned so that either a thermopile or an exposure chamber could be fitted into it. With the exposure chamber in place, the glass slip carrying the eggs was inserted in it through a side slit. The incident energy was measured with an Eppley thermopile and galvanometer which had been calibrated previously against an NBS lamp.

Eggs were exposed to various dosages of radiation at five wave lengths between 3022 and 2378 A. In one group, the convex surfaces were irradiated; in another, the concave. The percentages of eggs hatching after exposure to various dose levels are presented in Table 16. Action spectra constructed from these incomplete data show, for both convex and concave surfaces, broad maxima of effectiveness (with respect to killing) in the region 2804–2537 A with points of drastically lowered efficiency at 3022 and 2378 A. The data indicate that at 2804 A, cytoplasmic killing is less efficient than at 2650 or 2537 A, whereas killing is approximately the same at all these wave lengths when the nucleus is irradiated. Dose-hatchability studies

[33] R. C. von Borstel and S. Wolff, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 35–36.

[34] F. Daniels and L. J. Heidt, *J. Am. Chem. Soc.* 54, 2381 (1932).

TABLE 16. PERCENTAGES OF EGGS HATCHING AFTER IRRADIATION

Dose (ergs/mm²)	Hatchability (Larvae/Eggs = %) After Treatment with Monochromatic Ultraviolet at:				
	2378 Å	2537 Å	2652 Å	2804 Å	3022 Å
			Convex (Nuclear) Surface		
29		105/122 = 86	44/54 = 81		
57		68/108 = 63	51/99 = 52	66/112 = 59	49/51 = 96
78			48/114 = 42		
114	85/100 = 85	21/103 = 20	23/109 = 21	22/103 = 21	
209	84/100 = 81				
227		16/101 = 16	17/104 = 16	39/104 = 38	
284		21/72 = 29			
341	27/99 = 27	14/102 = 14	6/99 = 6	14/101 = 14	43/45 = 96
1023					30/33 = 91
2046					32/54 = 59
			Concave (Cytoplasmic) Surface		
557		93/109 = 85	81/90 = 90	111/115 = 97	32/32 = 100
697		90/114 = 79			
836		56/108 = 52	76/99 = 77	69/80 = 86	
929			33/98 = 34		
976		23/82 = 28			
1022		24/103 = 23	24/103 = 23		
1115		11/100 = 11	11/100 = 11	57/100 = 57	33/33 = 100
1257	90/100 = 90	1/103 = 1	1/103 = 1	48/85 = 56	
1393		0/100 = 0	0/100 = 0	7/102 = 7	
1528	73/101 = 72		0/110 = 0	10/106 = 9	48/48 = 100
1671	63/112 = 56		0/99 = 0	0/104 = 0	
2230	9/97 = 9				
2787	4/104 = 4				40/41 = 98
5574					37/41 = 90
	Controls: 1362/1424 = 96				

at additional wave lengths, other dose levels at the wave lengths listed, and absorption studies on the egg membranes are in process or are planned which should make for more precise spectra.

Dose-hatchability relations for both the convex and concave surfaces follow the form of those described by von Borstel and Moser.[35]

The appearance of the nonhatching egg following irradiation has also been studied in the present work in an attempt to correlate it with the developmental condition at the time of death. In general, eggs which do not hatch as a result of convex surface irradiation die very early in development and are identical in appearance with ova which have been exposed to ionizing radiations.[36] Those

[35] R. C. von Borstel and H. Moser, paper read at *Intern. Conf. Radiobiol.*, Cambridge, England (1955).

[36] R. L. Amy, *Radiation Research*, in press.

exposed to radiation on their concave surfaces die later in development and present a much different pattern of structural derangement. Present indications are, therefore, that it is possible to detect under low magnification (X25) whether damage induced by radiation has primarily affected the nucleus or the cytoplasm.

DROSOPHILA GENETICS

W. K. Baker

C. W. Edington	W. J. Welshons[37]
C. W. Hinton, Jr.[37]	P. B. Turpin
D. L. Lindsley[37]	E. S. Von Halle

On the Structure of the sc^8-Y:bw^+ Chromosome of *Drosophila melanogaster*

W. K. Baker

As a result of past studies on the effect of ionizing radiations on the loss of a ring chromosome, X^c, in *Drosophila*[38] it appeared advantageous to study the loss of y^+ and bw^+ markers located on a single Y chromosome. A chromosome fulfilling these qualifications was obtained by Cooper[39] from a crossover between the sc^8-Y and the Y:bw^+. However, the order of the genes on this new chromosome, which is designated sc^8-Y:bw^+, was unknown. With this chromosome it is possible to follow five markers if appropriate crosses are made. These markers are as follows: y^+, bw^+, bb^+, Y^S (the fertility factors located in the short arm of the Y chromosome), and Y^L (the Y^L fertility factors).

Males of the genotype, X^c, y/sc^8-Y:bw^+;bw were X irradiated with doses varying from 450 to 1800 r and mated to attached-X females of the genotype, $\underline{y\ v\ bb}/Y^{Lc}$;$bw$. The resulting F_1 females were examined to determine if either or both of the Y markers, y^+ or bw^+, had been lost or, possibly, mutated. They were not checked for the *bobbed* phenotype unless either y^+ or bw^+ were lost. From these phenotypic examinations it was possible to determine the loss of any of the three visible markers on the Y. The presence or absence of the two fertility markers was determined by a series of crosses which resulted in testing the fertility of two types of males, X·Y^L/Y? and X·Y^S/Y?. If only the first male is fertile, the Y chromosome in question contains Y^S; if only the second type is fertile, the irradiated Y has Y^L; fertility of both males indicates the presence of both fertility factors.

The types and frequencies of aberrant Y chromosomes recovered in these experiments, from a total of 42,370 F_1 flies, are presented in Table 17. Loss of all the markers, genotype 1, could be caused by either complete loss of the irradiated Y chromosome or loss of the X^c chromosome in 3X:2A zygotes. Either of these losses accompanied by primary nondisjunction in the parental female or by nondisjunction in X·Y^L/Y^S male (used to test for fertility factors) would account for genotypes 2 and 3, respectively. Therefore, the first three genotypes provide no information on the structure of the chromosome in question. The relatively high frequency with which bb^+ is recovered without any of the other markers (genotype 4) indicates that this locus is very closely linked to the spindle fiber attachment (sfa) and that the other markers are at some distance from this region. The close linkage of y^+ and Y^S is evident since all the $v\ bw$ females recovered carried the fertility factors of Y^S, and only infrequently was y^+ separated from Y^S (genotypes 9 and 10). Of particular interest are the $y\ v$ females of genotype 9. Cytological examination of two of the four cases showed a ring-Y chromosome. (Stocks of the other two cases died before a cytological check could be made.) This result suggests that y^+ is distal to Y^S and that bw^+ is proximal to Y^L. Finally, the presence of $y\ v\ bb$ females suggests that bb^+ is on the same side of the spindle fiber attachment as Y^S. The types of chromosomes recovered suggest that the order of markers on the sc^8-Y:bw^+ chromosome is the one given at the top of the table.

Other arrangements cannot, however, be excluded solely by these qualitative considerations. Another approach is to determine the order of markers which would require the least total number of breaks in the Y chromosome to produce the rearranged chromosomes observed. In addition, orders which require the fewest number of multibreak events would be favored since the doses administered were below 1800 r. The last column of Table 17 shows the expected number of chromosomes of each type based on the total number of individuals recovered of a particular phenotype and the frequency of the genotypes determined from the

[37] Research Associate.

[38] W. K. Baker and E. S. Von Halle, *J. Cellular Comp. Physiol.* 45, Suppl. 2, 299–307 (1955).

[39] K. W. Cooper, *Drosophila Info. Service* 26, 97 (1952).

sample tested of that phenotype. If the position of bb^+ with respect to the centromere is disregarded, there are 12 possible orders of the y^+ and bw^+ markers with respect to Y^S and Y^L. Calculations of the total number of breaks in the Y chromosome necessary for each of these 12 orders (the assumption is made that 1-break events in the Y can take place – probably chromosome 4 is involved in these cases – and consideration is taken of the fact that the chromosomes of genotype 9 are rings) show that the three orders shown in Table 18 have the fewest number of breaks. The data presented in this table clearly demonstrate that the first order is probably correct since this order not only has the fewest number of breaks but also the lowest frequency of 3- and 4-break rearrangements.

Constitution of Recessive Lethals Induced by Radiations of Different Ion Densities

C. W. Edington

If sex-linked recessive lethals arise as the result of chromosome breakage with few, if any, as the result of point mutations (intragenic changes) or chromosome rearrangement (position effect), some increase in the frequency of induced recessive lethals with increasing ion density would be expected. A decrease would also be expected in

TABLE 17. GENOTYPES OF THE LOST OR FRAGMENTED Y CHROMOSOMES RECOVERED FROM AN X-IRRADIATED sc^8-Y:bw^+ CHROMOSOME

Phenotype of F_1 Female	Genotypic Designation	sfa					Number Tested	Number Expected
		Y^L	bw^+	bb^+	Y^S	y^+		
y v bb bw (429)*	1	–	–	–	–	–	271	412
	2	+	–	–	–	–	8	12
	3	–	–	–	+	–	3	5
y v bw (95)	4	–	–	+	–	–	58	89
	5	+	–	+	–	–	4	6
v bw (14)	6	–	–	+	+	+	13	13
	7	+	–	+	+	+	1	1
y v (22)	8	–	+	+	–	–	8	14
	9	–	+	+	+	–	4	6
	10	+	+	+	+	–	1	2
y v bb (9)	11	–	+	–	–	–	4	7
	12	+	+	–	–	–	1	2

*The numbers in parentheses indicate the observed total number of individuals of that particular phenotype.

TABLE 18. ORDERS OF THE MARKERS ON THE sc^8-Y:bw^+ CHROMOSOME WHICH APPEAR MOST LIKELY

Order	Number of Breaks Necessary				Total Breaks
	1	2	3	4	
Y^L bw^+ (sfa-bb^+) Y^S y^+	17	28	6	0	91
Y^L bw^+ (sfa-bb^+) y^+ Y^S	15	24	6	6	105
Y^L (sfa-bb^+) bw^+ Y^S y^+	10	28	13	0	105

the frequency of recessive lethals induced in a ring-X chromosome, as compared to the frequency induced in a rod-X chromosome, because torsional restitution of broken chromosome ends results in loss of the ring, whereas a rod-X chromosome will not be lost after torsional restitution occurs.[40]

It has been shown previously that fast neutrons are more effective than Co^{60} γ rays[41] and X rays[42,43] in producing recessive lethals, and that the frequency of neutron-induced recessive lethals in a ring-X chromosome is significantly lower than the frequency of recessive lethals induced in a rod-X chromosome.[41] Since earlier reports in the literature state that the frequency of recessive lethals induced by X rays in a ring and rod chromosome are the same,[44,45] it was necessary to determine whether this difference in response to fast neutrons and X rays was real.

Males of *Drosophila melanogaster* bearing a ring-X chromosome, X^{c1}, and males bearing a rod-X chromosome, In(1)EN, were exposed simultaneously to 250 kvp X rays (30 ma; 3 mm of Al; hvl, 0.55 mm of Cu) at an intensity of 250 r/min, and the frequency of recessive lethals determined at several dose levels. When the slopes of the regressions of recessive lethals on dose are compared for the ring, 2.3×10^{-3}, and the rod, 2.5×10^{-3} (see Fig. 13), it is found that there is no significant difference in the frequency of recessive lethals induced by X rays in a ring-X and a rod-X chromosome. It is evident from an examination of Fig. 14 that there is an increase in the frequency of F_1 males observed in the offspring of irradiated ring-bearing males. This shift in the sex ratio occurs as the result of torsional restitution of the broken chromosome ends, with subsequent loss of the ring. It is of interest that, in these experiments as well as in the earlier neutron experiments,[41] no shift in the sex ratio of the offspring from irradiated rod-X-bearing males was observed. This finding indicates that dominant lethals involving the X and

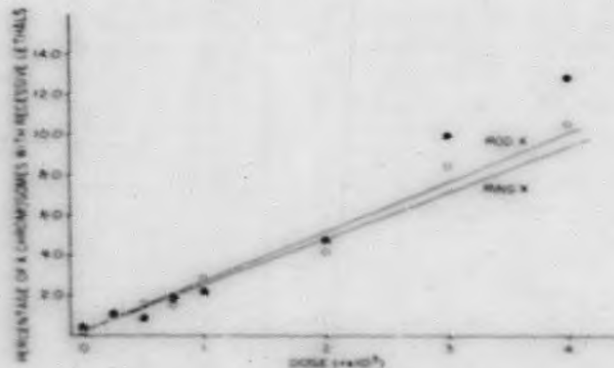

Fig. 13. Frequency of Recessive Lethals Induced by X Rays in Rod-X and Ring-X Chromosomes.

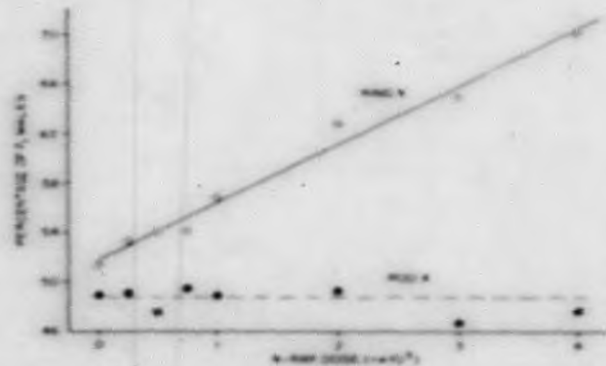

Fig. 14. Frequency of F_1 Males from Irradiated Rod-X- and Ring-X-Bearing Males.

the Y chromosomes occur with equal frequency. At any rate, the sex ratio shift observed in the ring-X offspring at low doses occurs as the result of torsional restitution of the broken chromosome ends; at higher doses it will be produced by a combination of this effect and the frequency of all ring-X autosome interchanges which also contribute to the total frequency of induced X-chromosome dominant lethals.

In order to explain the differential effect of radiations of different ion densities in producing recessive lethals in ring and rod chromosomes, it is necessary to postulate that recessive lethals

[40] D. G. Catcheside and D. E. Lea, *J. Genet.* 47, 25–40 (1945).

[41] C. W. Edington, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 42–44.

[42] G. H. Mickey, *Am. Naturalist* 88, 241–255 (1954).

[43] P. T. Ives, R. P. Levine, and H. T. Yost, Jr., *Proc. Natl. Acad. Sci. U.S.* 40, 165–171 (1954).

[44] H. J. Muller, *J. Genet.* 40, 1–66 (1940).

[45] E. Novitski, *Drosophila Info. Service* 26, 115 (1952).

are induced by fast neutrons and X rays in qualitatively different ways or that differential rejoining (i.e., restitution versus restitution plus torsional restitution) of the broken chromosome ends occurs after neutron and X-ray exposure. On the former hypothesis, the assumption is made that torsional restitution (asymmetrical restitution) and true restitution (symmetrical restitution) occur with equal frequency. If this assumption is valid, it would be expected, on the breakage hypothesis of lethal origin, that at least twice as many recessive lethals would be recovered in the rod-X chromosome as in the ring-X chromosome (Fig. 15) and that few, if any, recessive lethals arise as the result of point mutations. On the other hand, interpretation of the X-ray data for the ring-rod experiments necessitates the conclusion that the majority of X-ray-induced recessive lethals arise as the result of point mutation and that few, if any, arise as the result of chromosome breakage.

On the differential rejoining hypothesis, it must be assumed that restitution of broken chromosomes can occur in the sperm. If this assumption is true, then after X-ray exposure broken chromosomes are capable of restituting in the normal manner in the sperm; whereas, after neutron exposure, the majority of the chromosome breaks remain open until after fertilization, at which time torsional restitution can occur with subsequent loss of the ring chromosomes in which it occurred. Therefore, a decrease would be observed in the frequency of recessive lethals induced by fast neutrons in a ring chromosome as compared to that induced in a rod, but little or no change would be seen in the frequency of lethals induced by X rays in a ring as compared to a rod.

ONE-BREAK EVENTS

Type of Reunion	Probability	Ring X: Structure	Ring X: Fate	Rod X: Structure	Rod X: Fate
SU	$(1-R)$		L		L
TR	$R/2$		L		V
R	$R/2$		V		V
TWO-BREAK EVENTS WITHOUT DELETION					
(SU)(SU)	$(1-R)^2$		L		L
(SU)(TR)	$R(1-R)$		L		L
(SU)(R)	$R(1-R)$		L		L
(TR)(R)	$R^2/2$		L		V
(TR)(TR)	$R^2/4$	INTERLOCKED	L or V		V
(R)(R)	$R^2/4$		V		V

SU – SISTER UNION; TR – TORSIONAL RESTITUTION; R – RESTITUTION; V – VIABLE; L – LOST

Fig. 15. Types of Rejoining in Rod-X and Ring-X Chromosomes.

Analysis of Rod-shaped Derivatives of an Unstable Ring-X Chromosome in *Drosophila melanogaster*

C. W. Hinton

Instability of the w^{vC} ring-X chromosome leads to the production of gynandromorphs, XO males, dominant lethals, and (rarely) small unstable rings consisting chiefly of the w^{vC} centric and heterochromatic regions. Formation of anaphase bridges by the w^{vC} chromosome accounts for these results;[48] thus, gynandromorphs and XO males register bridge loss, whereas dominant lethals and small rings arise by inclusion of bridge breakage products in the cleavage nuclei. Sister-strand crossing over has been proposed as the basis for anaphase bridge formation by the ring chromosomes of maize. To test whether this mechanism explains w^{vC} instability, metacentric rod chromosomes were constructed having the w^{vC} centric and heterochromatic regions intact; sister-strand exchanges in rod chromosomes should not produce anaphase bridges. It was reported that these derived w^{vC} rod chromosomes were stable, but further investigations have not substantiated that preliminary conclusion.

Five different w^{vC}-B^S chromosomes were obtained, and from each of these w^{vC}-dl-49 compound-X chromosomes and generated w^{vC} ring chromosomes were recovered according to the scheme shown in Fig. 16. In three w^{vC}-B^S lines, the generated w^{vC} rings were stable, whereas the other two lines generated unstable w^{vC} rings (Table 19). The behavior of the w^{vC}-B^S chromosomes which generated stable rings and those which generated unstable rings is compared in Table 20. Both lines produce very few gynandromorphs. However, the incidence of XO males and the deficiency of

[48] C. W. Hinton, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 41–42.

w^{vC}-B^5 offspring are significantly greater in the unstable line than in the stable line. Part of the w^{vC}-B^5 offspring deficiency may be explained by hyperploidy of the B^5 duplication; however, this does not account for the viability difference of about 30% between the two lines. Crosses similar to those of Table 2 have shown that primary exceptional females are more frequent in the unstable w^{vC}-B^5 line just as are XO males, indicating primary nondisjunction rather than loss of the w^{vC}-B^5 chromosome as the source of the XO males. A similar comparison of the compound w^{vC}-dl-49 chromosomes in the stable and unstable lines is presented in Table 21. Here, also, the incidence of XO males and deficiency of w^{vC}-dl-49 offspring is higher in the unstable line, but most of the XO males probably result from unstable generated ring loss.

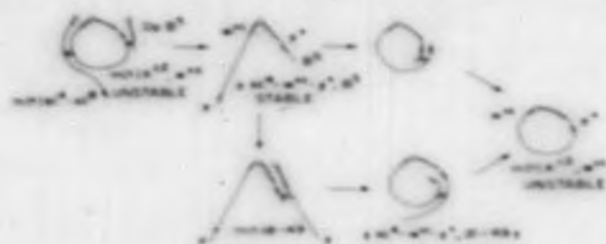

Fig. 16. Diagram of the Chromosomes Employed to Construct Rod-w^{vC} Chromosomes Which in Turn Generate the Original Ring-w^{vC} Structure. The meiotic pairing configurations are shown in the two-strand stage and points of exchange are indicated by X between the strands. Euchromatin is shown as a straight line and heterochromatin is denoted by a zigzag line.

These data show that, although the w^{vC}-B^5 and w^{vC}-dl-49 chromosomes which generate unstable w^{vC} rings do not experience loss, they nevertheless manifest instability as shown by the deficiency of w^{vC} B^5 or w^{vC}-dl-49 offspring; that is, these chromosomes still produce dominant lethality. If this evidence for instability is accepted and the anaphase bridge model of w^{vC} instability is retained, then the hypothesis that anaphase bridges arise by sister-strand exchange becomes invalid. Alternatively, it may be supposed that sister-strand fusion occurs in both the ring and rod w^{vC} chromosomes; the resulting bridges in the ring would consist of two members, and those in the rod, of only one member. This difference in

TABLE 19. BEHAVIOR OF w^{vC} RING CHROMOSOMES GENERATED FROM w^{vC} RODS

Cross: w^{vC}/dl-49, y w lz^s × dl-49, y w v f car/Y

Line	y w ♀	w^{vC} ♀	Gy	XO ♂	w^{vC} ♀ / y w ♀	Gy / y w ♀	XO ♂ / y w ♀	Remainder
Stable	2165	2013	39	18	0.929	0.018	0.008	0.045
Unstable	2443	1064	338	166	0.435	0.138	0.068	0.359

TABLE 20. BEHAVIOR OF THE w^{vC}-B^5 CHROMOSOMES

Cross: w^{vC}-B^5/dl-49, y w lz^s × dl-49, y w v f car/Y

Line	y w ♀	w^{vC}-B^5 ♀	Gy	XO ♂	w^{vC}-B^5 ♀ / y w ♀	XO ♂ / y w ♀	Remainder
Stable	5466	4623	1	26	0.846	0.005	0.149
Unstable	4640	2273	5	214	0.492	0.046	0.462

TABLE 21. BEHAVIOR OF THE w^{vc}-dl-49 COMPOUND-X CHROMOSOMES

Cross: w^{vc}-dl-49/sc^{8}·Y × dl-49, y w B/Y

Line	y^+ ♂	w^{vc}-dl-49 ♀	X0 ♂	Generated Rings		w^{vc}-dl-49 ♀ / y^+ ♂	Rings / y^+ ♂	X0 ♂ / y ♂	Remainder
				w^{vc} ♀	Gy				
Stable	2021	1795	18	164	1	0.887	0.082	0.009	0.022
Unstable	3852	2071	124	161	63	0.537	0.058	0.032	0.373

bridge structure might be correlated with the difference in instability manifestation by the w^{vc} ring and rod on the assumption that the two-membered bridges are susceptible to loss as well as breakage, but that the single-membered bridges always break.

Importance of the Distribution of the Heterochromatin Within the Genotype to the Viability of the Organism

D. L. Lindsley

From females of the constitution Y^S y cv v f·/y^2 w^a(·Y^L)y^+ (center point indicates the centromere and parentheses indicate that order of included elements unknown), an attempt was made to recover the single exchange product, $Y^S X(\cdot Y^L)$. This product was seldom recovered with the expected frequency when y^2 w^a(·Y^L)y^+ chromosomes were set as follows:

$$Y^S\ y\ cv\ v\ f\cdot/y^2\ w^a(\cdot Y^L)y^+ ♀ \times Y^S\ y\ B\cdot Y^L/0\ ♂♂$$

The y^2 w^a(·Y^L)y^+ chromosomes were obtained as spontaneous detachments of XY^L·X with small marked heterochromatic X duplications (see Fig. 17); possible points of exchange in the attached X are represented by asterisks in the following notation: X*Y^L·*X. Table 22 shows the progenies of these crosses. The wide discrepancies in the recovery of reciprocal single crossover classes, as great as 343:0, must be explained. It might be considered that the inequality of reciprocal classes is a function of nonrandom disjunction as described by Novitski;[47] this phenomenon is certainly responsible for some of the inequality, but it fails fully to account for the observations. Nonrandom disjunction is a manifestation of the orientation of the dyads at the second meiotic division, the first division being random in orientation. Consequently, nonrandomness is observed for terminal loci but not for centromere regions, the latter being recovered in the expected 1:1 ratio. In the present data, however, the y^+ centromere region is recovered with a greatly reduced frequency. This cannot be a function of the centromere region alone, however, since among the noncrossovers the y^+ centromere is recovered in excess of the homologous centromere; this excess may be in part a function of nonrandomness, i.e., preferential recovery of y^+ noncrossover strands rather than y^+ crossover strands. It was hypothesized that

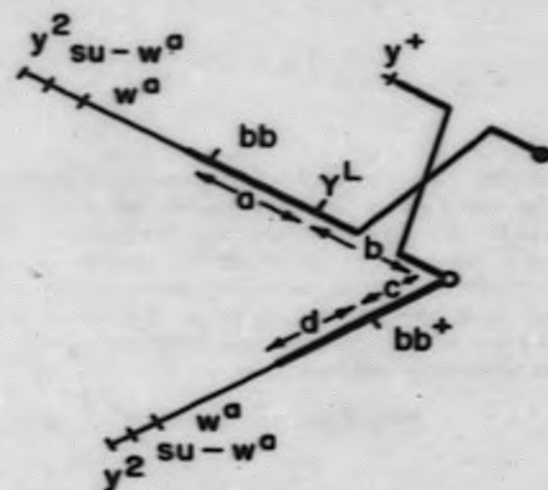

Fig. 17. Attached-X Chromosomes from Which Detachments Discussed Were Derived and the Crossover Event Thought to Give Rise to XY^L. (Heterochromatin is designated by heavy line.)

[47] E. Novitski, *Genetics* 36, 267–280 (1951).

TABLE 22. PROGENY FROM $Y^S y\ cv\ v\ f/y^2\ w^a(\cdot Y^L)$ ♀ × $Y^S y\ B\cdot Y^L/O$ ♂♂

The results are divided into two rows with classes carrying recovered chromosomes with the same centromere region as the chromosome in the corresponding row of the maternal constitution

Maternal Constitution	Females			Males													
	Non-co		Co	Non-co	Single Co in Regions:				Total Singles	Double Crossovers in Regions:						Total Doubles
					1	2	3	4		1 & 2	1 & 3	1 & 4	2 & 3	2 & 4	3 & 4	
$X(\cdot Y^L)3a$		532		444	37	67	70	24	198	1	8	3	17	10	9	48
$Y^S X\cdot$	405		444	306	42	138	199	70	449	1	16	5	24	11	10	67
$X(\cdot Y^L)3b$		554		530	32	69	72	18	191	1	4	1	22	17	6	51
$Y^S X\cdot$	343		457	289	36	152	200	81	469	3	9	6	14	13	3	48
$X(\cdot Y^L)3c$		659		658	4	4	3	0	11	4	4	0	15	9	2	34
$Y^S X\cdot$	526		598	475	47	200	224	62	535	1	10	6	15	8	5	45
$X(\cdot Y^L)118a$		380		445	0	1	1	0	2	2	6	1	15	16	5	45
$Y^S X\cdot$	316		380	296	35	159	178	61	433	0	13	3	9	8	6	39
$X(\cdot Y^L)122a$		233		356	0	0	0	0	0	1	2	1	11	7	5	27
$Y^S X\cdot$	277		372	249	24	109	158	52	343	0	12	5	12	9	7	45
$X(\cdot Y^L)122b$		497		553	5	2	6	1	14	3	7	5	21	17	14	67
$Y^S X\cdot$	469		459	407	40	146	212	95	493	8	14	7	20	13	6	68
$X(\cdot Y^L)122c$		671		713	5	11	11	4	31	8	13	8	39	24	7	99
$Y^S X\cdot$	632		842	547	84	274	244	97	699	3	25	5	24	17	11	85
$X(\cdot Y^L)122d$		116		235	0	0	0	0	0	1	4	1	8	8	2	23
$Y^S X\cdot$	168		226	159	20	99	99	33	251	0	1	3	3	5	1	13

the deficiency of recovered y^+ centromere regions is a reflection of the inviability of only that class of strands which carry Y^S distally and Y^L proximally. The problem was to deconfound the contributions of inviability and nonrandomness to the inequality of reciprocal classes.

It is possible by tetrad analysis to solve for the frequency of no-exchange tetrads, E_0, one exchange tetrads, E_1, two exchange tetrads, E_2, the coefficient of nonrandomness, c, and the relative viability of the $Y^SX(\cdot Y^L)$ crossover type. Novitski has listed the expressions for the various recovered types in terms of E_0, E_1, E_2, and c as follows:

$$\text{non}^- = \tfrac{1}{2}E_0 + \tfrac{1}{2}cE_1 + \tfrac{1}{8}cE_2 + \tfrac{1}{16}E_2$$

$$\text{non}^+ = \tfrac{1}{2}E_0 + \tfrac{1}{2}(1-c)E_1 + \tfrac{1}{8}(1-c)E_2 + \tfrac{1}{16}E_2$$

$$\text{non} = E_0 - \tfrac{1}{2}E_1 - \tfrac{1}{4}E_2$$

$$\text{sgl}^- = \tfrac{1}{2}cE_1 + \tfrac{1}{4}cE_2 + \tfrac{1}{8}E_2$$

$$\text{sgl}^+ = \tfrac{1}{2}(1-c)E_1 + \tfrac{1}{4}(1-c)E_2 + \tfrac{1}{8}E_2$$

$$\text{sgl} = \tfrac{1}{2}E_1 + \tfrac{1}{2}E_2$$

$$\text{dbl}^- = \tfrac{1}{16}E_2 + \tfrac{1}{8}cE_2$$

$$\text{dbl}^+ = \tfrac{1}{16}E_2 + \tfrac{1}{8}(1-c)E_2$$

$$\text{dbl} = \tfrac{1}{4}E_2$$

where a plus sign indicates the presence of terminal Y^S and a minus sign its absence. According to the hypothesis espoused recovery of the normal centromere would be expected every time it goes into the functional egg nucleus; consequently, the best estimate of the total number of males expected is twice the number of y males recovered. This corrected total was used in calculating the observed frequencies. Since the only class expected to be affected by the inviability is sgl^+, this class alone is multiplied by the relative viability factor x:

$$\text{sgl}^+ = x[\tfrac{1}{2}(1-c)E_1 + \tfrac{1}{4}(1-c)E_2 + \tfrac{1}{8}E_2]$$

The solutions to these equations for the eight detachments tested are given in the first five columns of Table 23. The consistency in the values from cross to cross for E_0, E_1, E_2, and c are rather remarkable, x being the only factor showing considerable variation. If it is assumed that all of the discrepancy between the observed number and the expected number of males is accounted for by inviability of the one single-crossover class, this discrepancy can be added to the crossover classes and the map distance between w^a and the centromere (y^+) calculated. The values thus obtained, shown in the last column of Table 23, are in good agreement with the standard map distance of about 0.60.

These findings are considered to be in good agreement with the explanation originally postulated to explain the results. The cause of the inviability must be heterochromatic for two reasons: first, the detachments are euchromatically identical, and second, the same discrepancy of recovered crossover classes is observed for all

TABLE 23. RESULTS OF TETRAD ANALYSIS ON DATA PRESENTED IN TABLE 22

Detachment	E_0	E_1	E_2	c	x	w^a-sfa
3a	0.052	0.668	0.280	0.59	0.60	0.613
3b	0.138	0.616	0.246	0.70	0.89	0.546
3c	0.145	0.706	0.149	0.60	0.029	0.500
118a	0.078	0.704	0.218	0.64	0.002	0.572
122a	0.060	0.722	0.218	0.58	0	0.582
122b	0.230	0.592	0.278	0.60	0.033	0.567
122c	0.084	0.642	0.274	0.59	0.046	0.584
122d	0.018	0.812	0.170	0.62	0	0.577

marked regions. It can further be shown that the heterochromatic constitution of the chromosome rather than of the zygote is the important factor in this inviability. The four males of the constitution Y^S *y cv v f*$(\cdot Y^L)y^+/0$ recovered from detachment 122c shown in Table 22 were crossed to *y* females. The progenies of these crosses consisted of 239 *y*/0 males and 8 Y^S *y cv v f*$(\cdot Y^L)y^+/y$ females. Structurally, these females are Y^SXY^L/X, but the Y^SXY^L chromosome was derived from females of exactly the same heterochromatic content, Y^SX/XY^L, but in which the apportionment of the heterochromatin between the X chromosomes was different. The former females are extremely inviable and highly infertile, whereas the latter ones are normal in both respects. The progeny data from the eight Y^S *y cv v f*$(\cdot Y^L)y^+/y$ females by Y^S *y B*$\cdot Y^L/0$ males, although meager, are in agreement with the other data reported here: there were 17 *y* noncrossovers and no y^+ noncrossovers, and there were 2 y^+ single crossovers.

Kinetic Activity of Centromeres Associated with Y^L

D. L. Lindsley E. S. Von Halle

Single exchange within the inverted region of an inversion heterozygote produces a single first-anaphase bridge which is selectively eliminated from the egg nucleus. Line drawings of the consequences of double exchange show that double-crossover strands are recovered from two- and three-strand doubles. Four-strand doubles produce double first-anaphase bridges; if these bridges are excluded from the egg nucleus, a nullisomic nucleus results. Expectations among the progeny of females heterozygous for a sex-linked inversion on this basis are three recombinants: two patroclinous males resulting from fertilization of a nullo-X egg by an X-bearing sperm. When each end of the bridges produced is acrocentric, these expectations are fulfilled.[48] Novitski[49] has since shown that, if one end of the bridge is acrocentric and the other end metacentric, the frequency of patroclinous males is halved. Since the presumptive egg nucleus is at one end of a linear quarter of meiotic nuclei, this change in results has been attributed to dominant lethality of one of the orientations of the asymmetrical double bridges with respect to the egg nucleus. The analogy of these results to a tug of war between the two ends of the bridge demanded that the experiment in which both ends of the bridges were metacentric be performed. This situation results in the virtual elimination of the patroclinous male class. The hypothetical tug-of-war model can be fully described as follows: The strength of a terminal centromere or, more specifically, the force applied by the attached spindle fibers of a terminal centromere is less than the tensile strength of the chromatin bridge which is, in turn, less than the force applied by the spindle fibers attached to a subterminal centromere. Consequently, double first-anaphase bridges with equally matched weak centromeres will hold and always give rise to a nullisomic egg, whereas bridges with equally matched strong centromeres should invariably fragment, giving rise to egg nuclei with dominant lethal broken chromosomes. Unequally matched opposed centromeres, where the force applied to one is less than tensile strength of the chromatin strand, should result in the passage of the intact bridge to one pole where it should behave as a dominant lethal, leaving the other pole nullisomic; the egg nucleus will, therefore, be nullisomic in one-half the cases.

It was hypothesized that the contribution of a chromosome to spindle fiber formation or contraction is greater when paired heterochromatic regions exist in the neighborhood of the centromere than otherwise;[50] the kinetic strength of subterminal centromeres might then be a function of pairing of the heterochromatin on either side of the centromere. Novitski and Lindsley (unpublished data) have attempted to influence the kinetic behavior of centromeres by adding homologous heterochromatin to the system. This has been accomplished either by adding free heterochromatic duplications or by altering the heterochromatic constitution of the acentric fragments formed as a concommitant of double-bridge formation. In no case did the presence of a duplication or the constitution of the acentric fragment influence the kinetic behavior of a centromere. All subterminal centromeres tested in the past have not been strong, but all strong centromeres have been subterminal. The subterminal centromere, which has consistently proved

[48] A. H. Sturtevant and G. W. Beadle, *Genetics* 21, 554–604 (1936).

[49] E. Novitski, *Genetics* 37, 270–287 (1952).

[50] E. Novitski, *J. Cellular Comp. Physiol.* 45, Suppl. 2, 151–170 (1955).

to be strong, has been X·Y^L, where the center point represents the centromere. The present experiments were designed to test whether Y^L, rather than the subterminal nature of the centromere, could be responsible for kinetic strength in this case. Chromosomes with presumably terminal centromeres and with Y^L (XY^L·) were obtained as detachments of XY^L·X chromosomes, as shown in Fig. 17, where the y^+ duplication carries a centromere derived from the X chromosome. Two of the detachments were discovered to be linked with chromosome 4; consequently, these are metacentric detachments with chromosome 4 present as a second arm. Detachment 122-19 is *bb* in the absence of a Y and is probably the result of exchange in region c in Fig. 17; detachment 179-8 is bb^+ in the absence of a Y and is, therefore, the result of exchange in either region a or d. Detachments 164-9 and 174-13 do not carry bb^+ since they were derived from duplications 164 and 174 which do not carry bb^+, and detachments 3-18 and 118-12b carry bb^+ and were derived from duplications 3 and 118 which also carry bb^+.

Each detachment studied was tested against a known weak and a known strong centromere; the results are summarized in Table 24. They show that a subterminal centromere is not a *sine qua non* of strong kinetic behavior, since XY^L·/3-18 and 118-12b have strong centromeres. XY^L· chromosomes can behave as if they had strong or weak centromeres and, in the four cases studied, this behavior is correlated with the presence or absence respectively of bb^+. Furthermore, X(·Y^L)4 (order of elements in parentheses unknown) chromosomes can behave as if they had strong or weak centromeres, again in the two cases studied, correlated with the presence or absence of bb^+. It is tempting to speculate that there is some specific heterochromatic element, linked with bb^+ in the chromosomes studied here, which contributes in some way to spindle fiber formation or spindle fiber contraction.

Regional Inhibition of Crossing Over in the X Chromosome in Response to the Presence of an Autosomal Inversion

W. J. Welshons

It was reported earlier[51] that the presence of an autosomal inversion could both inhibit and stimulate crossing over in attached-X chromosomes, and that the region of inhibition was contiguous with the region of stimulation. Since the deficit of exchanges tended to be canceled by the excess, the effect was impossible to detect unless the marked regions of the chromosomes were very small. In the attached-X experiments, the inhibited region was bounded by the marker genes *vermillion* and *miniature* (*v–m*), and the stimulated region by *miniature* and *garnet* (*m–g*). Table 25 lists the crossover percentages obtained for attached-X females with no known autosomal inversion and for attached-X females which were heterozygous for the third chromosome Dichaete inversion, *Dcxf*. It can be seen that the crossover percentage for the inhibited region *v–m* dropped from 4.5 to 3.4, whereas the region *m–g* increased from 6.7 to 9.8.

Table 26 presents a summary of a homogeneity test which tests the hypothesis that the distribution of exchanges over the various regions is the same whether or not an autosomal inversion is present. Additional data have been added to that body of data reported earlier, but the results are essentially the same. The χ^2 value of 17.65 is highly significant, with the major portion being contributed by the regions *v–m* and *m–g*. When the inversion is present there are too few exchanges in region *v–m* and too many exchanges in region *m–g*.

In a similar experiment with free-X chromosomes and the same genetic markers, no inhibition of crossing over was detected when the parents possessed the inversion. The crossover percentages of Table 25 indicate an increased rate of crossing over for each region. The homogeneity test summarized in Table 26 indicates that the distribution of exchanges over the marked regions is the same whether or not the inversion is present. Therefore, the inversion causes an increase in crossing over but the increase is distributed proportionately over the marked regions. A discrepancy in the early report for the region *scalloped* to *forked* (*sd–f*) resulted from a miscalculation.

An X chromosome carrying the markers *scute*(*sc*), *echinus*(*ec*), *crossveinless*(*cv*), *pentagon*(*ptg*), and *vermillion*(*v*) was synthesized. Another crossover experiment was performed with free-X chromosomes. The crossover percentages are listed in Table 25. When the inversion was present, the exchange frequency for the region *cv–ptg* dropped from 13.5 to 13.0, whereas crossing over increased in all the other regions tested. The homogeneity

[51] W. J. Welshons, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 40–45.

TABLE 24. GENETIC RESULTS FROM VARIOUS INVERSION HETEROZYGOTES CROSSED TO In(1)dl-49, y w B MALES

Region 1, *y–cv*; region 2, *cv–v*; region 3, *v–f*; region 4, *f–car*; progeny of each cross tabulated in two rows with classes carrying same structure as the chromosome in the corresponding row of the genotype of the mother

Maternal Composition		F_1 Males, Non- and Double Crossovers in Regions:							Totals		Ratio
Structural	Genetic	0	1&2	1&3	1&4	2&3	2&4	3&4	Co	Patro	as 3x
XY^L-(3-18)	$y^2\ w^a$	2402	5	19	16	58	60	26	298	101	3:1.0
sc^8	$f\ v\ cv\ sc^8$	1510	5	20	20	24	36	9			
XY^L-(3-18)	$y^2\ w^a$	1317	8	26	7	45	23	10	240	14	3:0.2
$sc^8 \cdot Y^L$	$car\ f\ v\ cv$	1212	12	35	7	33	19	4			
XY^L-(11b-12b)	$y^2\ w^a$	2438	5	15	10	39	27	13	202	71	3:1.1
sc^8	$fv\ cv\ sc^8$	1542	8	22	11	28	18	6			
XY^L-(11b-12b)	$y^2\ w^a$	1037	1	11	15	22	6	1	92	9	3:0.3
$sc^8 \cdot Y^L$	$car\ fv\ cv$	891	6	14	13	18	4	0			
XY^L-(164-9)	$y^2\ w^a$	417	0	1	2	9	7	5	43	31	3:2.1
sc^8	$f\ v\ cv\ sc^8$	300	0	1	3	6	6	3			
XY^L-(164-9)	$y^2\ w^a$	577	3	14	5	27	18	13	162	62	3:1.1
$sc^8 \cdot Y^L$	$car\ f\ v\ cv$	574	4	23	10	17	24	4			
XY^L-(174-13)	$y^2\ w^a$	1015	0	6	5	19	27	15	128	69	3:1.6
sc^8	$fv\ cv\ sc^8$	716	3	5	8	14	20	6			
XY^L-(174-13)	$y^2\ w^a$	507	2	16	9	19	26	5	154	37	3:0.7
$sc^8 \cdot Y^L$	$car\ f\ v\ cv$	404	3	22	15	14	18	5			
XY^L-4(122-19)	$y^2\ w^a$	1940	4	17	4	25	39	21	175	115	3:2.0
sc^8	$f\ v\ cv\ sc^8$	1184	1	17	7	16	18	6			
XY^L-4(122-19)	$y^2\ w^a$	728	1	8	6	21	17	3	132	43	3:1.0
$sc^8 \cdot Y^L$	$car\ f\ v\ cv$	713	10	15	8	20	17	6			
$X(-Y^L)4(179-8)$	$y^2\ w^a$	859	2	5	1	22	20	9	99	40	3:1.2
sc^8	$f\ v\ cv\ sc^8$	489	0	11	5	10	15	3			
$X(-Y^L)4(179-8)$	$y^2\ w^a$	1035	6	13	10	27	18	10	168	11	3:0.2
$sc^8 \cdot Y^L$	$car\ f\ v\ cv$	650	9	28	5	27	12	4			

TABLE 25. REGIONAL DISTRIBUTION OF CROSSOVER PERCENTAGES IN THE X CHROMOSOME OF *DROSOPHILA*

	Crossover Region								
	ptg-v	*v-m*	*m-g*	*g-sd*	*sd-f*	*sc-ec*	*ec-cv*	*cv-ptg*	*ptg-v*
Attached-X									
Noninversion	10.8	4.5	6.7	5.4	5.1				
Inversion	12.2	3.4	9.8	8.1	4.4				
Free-X									
Noninversion	7.5	2.8	6.6	4.8	4.1	2.8	5.8	13.5	7.9
Inversion	10.4	4.1	9.8	7.4	5.3	3.5	8.6	13.0	10.1

TABLE 26. HOMOGENEITY TESTS OF CROSSOVER DISTRIBUTIONS

	Region of Exchange										
	ptg-v	*v-m*	*m-g*	*g-sd*	*sd-f*	Total	*sc-ec*	*ec-cv*	*cv-ptg*	*ptg-v*	Total
Attached-X											
Noninversion	315	129	194	165	149	952					
Inversion	190	51	162	108	76	587					
χ^2	0.60	7.37	8.17	0.24	1.81	17.65					
Free-X											
Noninversion	180	68	159	116	99	622	49	101	236	139	525
Inversion	217	86	203	154	110	770	55	136	205	159	555
χ^2	0.07	0.02	0.09	0.32	0.61	1.11	0.10	3.41	4.28	0.45	8.24

test summarized in Table 26 is significant at the 5% level. Again the major portion of this chi-square is contributed by two contiguous regions. The region *cv-ptg* has too few exchanges when the inversion is present, and the region *ec-cv* has too many exchanges. This, then, is the same phenomenon first observed in the attached-X experiments.

It was pointed out in the previous report that Hannah[52] has summarized much work on the salivary map localization of intercalary heterchromatin, and that the *v-m* region which in attached-X studies responded to the presence of an autosomal inversion with a decreased crossover frequency, was a region which appeared to be free of heterochromatin. It is interesting to note that the salivary region within the markers *cv-ptg* is also one of these regions which supposedly contains little or no heterchromatin throughout its length.

The indication from two separate experiments that crossing over in the X chromosome may be inhibited by the presence of an autosomal inversion, allows a confident statement that the interchromosomal effect of an inversion on crossing over may be inhibitory as well as stimulatory. This phenomenon has not been reported before. Since crossing over in the *v-m* region, under the influence of an inversion, was decreased in the attached-X studies and increased in free-X studies, regions free of heterochromatin may respond with an increase or decrease in crossover frequency. All heterochromatic regions tested so far have responded with an increased crossover frequency.

[52] A. H. Hannah, *Advances Genet.* 4, 87–125 (1951).

Since the region *cv-ptg* showed an inhibitory response to the presence of an inversion, an X chromosome will be synthesized which contains the marker *carmine*(*cm*) located between *crossveinless* and *pentagon*. With this marked chromosome it will be possible to determine if the inhibitory response is localized mainly in the small regions *cv-cm* or *cm-ptg*, or if the inhibitory response is spread over the entire regions *cv-ptg*.

Chromosomal Interference in Attached-X Females

W. J. Welshons

Shult and Lindegren[53] have emphasized the action of chromatid interference on the process of crossing over, and have indicated that what is frequently measured as chromosomal or chiasma interference may be the reflection of nonrandom chromatid exchanges. They concluded that the phenomenon of chromosomal interference has neither been demonstrated nor proved. It will be shown that the observation of chromosomal interference found in an attached-X study of crossing over cannot be explained as a reflection of chromatid interference.

From a section of chromosome which contains three or more closely linked markers, the crossover percentages for each region may be calculated from the recovery of single strands. The product of the crossover frequencies in two regions gives the expected frequency of single chromatids which have crossed over in both regions. The discrepancy between observed and expected frequencies of double crossover chromatids is a measure of chromosomal interference; it may be positive or negative, depending on whether too many or too few double-exchange chromatids are recovered. Chromatid interference exists when two nonsister chromatids which have crossed over at one level have a greater or lesser probability than the nonexchange strands of crossing over at a second level. Positive chromatid interference would result in an excess of four strand double-exchange tetrads and a deficiency of single chromatids of the double crossover type. Negative chromatid interference would increase the number of two-strand double-exchange tetrads and would provide too many chromatids of the double crossover type. Hence, positive chromatid interference could be responsible for positive chromosomal interference, and negative chromatid interference could be responsible for negative chromosomal interference.

In *Neurospora*, where all products of a tetrad are recovered singly and in order, the two types of interference can be distinguished.[54] A study of crossing over in attached-X chromosomes of *Drosophila melanogaster* will also permit measurement of chromatid and chromosome interference. Certain products of double exchanges are recognizable as such in the progeny of an attached-X female. These products can be used to determine the presence or absence of chromatid interference. Chromosomal interference can be calculated in the usual way when the two chromatids of the attached-X females have been classified as noncrossover, single crossover, and double crossover types.

The results of an attached-X study[55] indicate that two-, three-, and four-strand double-exchange tetrads occur at random with the possible exception that the two-strand double type is in excess when the levels of exchange are very close together. The results on the latter point are not convincing and additional data are being accumulated. Therefore, there is no indication of positive chromatid interference in these data, and for the moment the negative chromatid interference which may be present under the conditions specified can be ignored.

All tested chromatids were recovered in attached-X females with a wild phenotype; crossover and noncrossover chromatids were identified by progeny testing these females. This population of tested chromosomes has been used to calculate crossover values and the expected number of double-crossover chromatids. The results of these calculations and the coincidence values are listed in Table 27. There is little doubt that consistent departures from a coincidence value of unity indicates the action of chiasma interference. If the observation of negative chromatid interference is substantiated, then positive chiasma interference for the smaller regions of double exchange is even stronger than calculated.

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[53] E. E. Shult and C. C. Lindegren, *Nature* 175, 507 (1955).

[54] C. C. Lindegren and G. Lindegren, *Genetics* 27, 1–24 (1942).

[55] W. J. Welshons, *Genetics*, in press.

TABLE 27. MAP DISTANCES AND COINCIDENCE VALUES

Map Distances*					
ptg–v	*v–m*	*m–g*	*g–sd*	*sd–f*	Total
11.1	4.3	6.5	5.7	4.9	32.5

Regions**	Double Exchanges		Coincidence
	Expected*	Observed	
ptg–v, sd–f	10.8	7	0.63
ptg–v, g–sd	12.6	5	0.39
ptg–v, m–g	14.4	2	0.14
ptg–v, v–m	9.6	0	0.00
v–m, sd–f	4.2	3	0.71
v–m, g–sd	5.0	2	0.40
v–m, m–g	5.6	1	0.18
m–g, sd–f	6.4	0	0.00
m–g, g–sd	7.4	1	0.13
g–sd, sd–f	5.6	0	0.00

*All calculations are based on 2002 tested chromosomes.

**According to standard symbols for X chromosome mutants of *Drosophila melanogaster*.

MICROBIAL PROTECTION AND RECOVERY

RADIATION PROTECTION AND RECOVERY IN BACTERIA

A. Hollaender

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A. J. Sbarra	A. Henley
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Protein and Nucleic Acid Synthesis During Recovery of Bacteria

G. E. Stapleton D. H. Woodbury

As part of the investigation of the mechanism of recovery in *Escherichia coli* B/r[3,4,5] a study was made of the net synthesis of protein, RNA, and DNA under conditions where cells can show maximal recovery in yeast extract at 18°C, and of minimal recovery in salts-glucose medium at 37°C. Survival studies have shown that after 20 kr of γ rays, about 1% survival is obtained when cells are held on the simple inorganic salts-glucose medium, whereas about 30% survival is obtained when similar aliquots of irradiated cells are held on the same medium plus yeast extract (20 mg/ml). The experiment was performed in liquid medium with sufficient numbers of cells that aliquots could be taken at various times during the incubation period; some chemical analyses of the cells were carried out during the process. Aliquots of cells were removed from the suspensions, harvested by centrifugation at 0°C, washed and extracted with cold 10% trichloroacetic acid (TCA) for 30 min at 0°C. Nucleic acids were determined by a modified Schmidt-Thannhauser technique and protein was estimated on the hot TCA extracts by determining Kjeldahl nitrogen.

Nonirradiated cells show net synthesis of nucleic acids and protein on both types of media. The rate of synthesis is greater on the yeast extract fortified media for all fractions analyzed except the DNA (Table 28).

Since it was necessary to incubate cells in basal medium at 37°C and in basal plus yeast extract at 18°C to obtain maximal difference in survival, it seemed necessary to determine the rates of synthesis of these same fractions for nonirradiated cells at the two temperatures on the same medium.

As would be expected (Table 29) the rates of synthesis of all components studied are reduced by a factor of 3 to 4 by reduction of the temperature from 37 to 18°C. An outstanding difference is found for the acid-soluble fraction, which apparently does not pile up when cells are incubated at the lower temperature. This lack of buildup of this fraction before first division (~120 min at 37°C and ~600 min at 18°C) is reflected in a greater net synthesis of the acid insoluble component.

The results of experiments in which irradiated cells were incubated under the conditions which

[1]Terminated at close of this period.

[2]Temporary employee, summer 1955.

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

[4]G. E. Stapleton, A. J. Sbarra, and L. W. Bacon, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 35–36.

[5]G. E. Stapleton, A. J. Sbarra, and D. H. Woodbury, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 45–46.

TABLE 28. RELATIVE CONCENTRATION* OF NUCLEIC ACIDS AND PROTEIN IN NONIRRADIATED CELLS INCUBATED AT 37°C

Incubation Time (min)	Acid-Soluble RNA		RNA		DNA		Protein	
	Basal	Yeast Extr**	Basal	Yeast Extr	Basal	Yeast Extr	Basal	Yeast Extr
30	1.0	1.2	1.87	2.5	1.12	1.1	1.25	1.40
60	1.25	2.5	3.12	4.3	1.35	1.35	1.87	2.80
90	2.25	4.25	6.0	6.0	1.67	1.78	3.0	3.75

*Relative concentration = ratio of concentration at time t as compared to time zero t_0.

**Basal medium plus 20 mg/ml of Difco yeast extract.

TABLE 29. RELATIVE CONCENTRATION* OF NUCLEIC ACIDS AND PROTEIN BY NONIRRADIATED CELLS INCUBATED ON YEAST EXTRACT AT 18 AND 37°C

Incubation Time (min)	Acid-Soluble RNA		RNA		DNA		Protein	
	37°C	18°C	37°C	18°C	37°C	18°C	37°C	18°C
30	1.25		2.50		1.1		1.25	
60	2.5		4.30		1.35		1.87	
90	4.25		6.0		1.78		3.0	
120		1.25		1.67		1.25		1.37
240		1.50		3.50		1.62		1.87
360		1.50		7.50		2.25		2.75
480		1.37		10.0		4.0		4.10

*See Table 28.

TABLE 30. RELATIVE CONCENTRATION* OF NUCLEIC ACIDS AND PROTEIN BY IRRADIATED CELLS UNDER CONDITIONS OF MINIMAL AND MAXIMAL RECOVERY

Incubation Time (min)	Acid-Soluble RNA		RNA		DNA		Protein	
	Basal (37°C)	Yeast Extr (18°C)	Basal (37°C)	Yeast Extr (18°C)	Basal (37°C)	Yeast Extr (18°C)	Basal (37°C)	Yeast Extr (18°C)
30	1.2		1.2		1.1		1.2	
60	0.9	1.50	1.3	1.60	1.1	1.10	1.2	1.20
120	1.1	2.00	1.55	1.90	0.88	1.20	1.4	1.40
180		2.20		2.10		1.30		1.67
240	1.9**	2.40	4.0**	2.50	1.05**	1.37	2.5**	1.80
360		2.50		2.80		1.55		2.20

*See Table 28.

**The high values for concentration of all components at 240 min incubation in basal medium at 37°C represents growth of the surviving cells and are not to be compared with other reported experiments. All values for concentration of cells incubated in yeast extract at 18°C are for nondividing cells.

bring about minimal and maximal survival after γ irradiation are shown in Table 30.

It appears from these data that irradiated *E. coli* B/r show no net synthesis of acid-soluble RNA or DNA in the basal medium at 37°C, however the presence of yeast extract at 18°C stimulates synthesis of all components investigated. In fact, the rate of synthesis of RNA and DNA by irradiated cells is greater in yeast extract at 18°C than in basal medium at 37°C. How these findings are correlated with the recovery process remains to be demonstrated by further experiments in which the incorporation of labeled nutritional factors can be studied.

On the Relation Between X-Ray Protection and Induced Mutagenesis in *Escherichia coli* Auxotrophs

D. Billen H. K. Sherwood

In studies on the induction of reversions in *Escherichia coli* auxotrophs by X rays it has been shown that the number of reversions found appeared

to be related to the survival level rather than the dose received.[6,7,8] Thus when survival was increased by either a pre- or postirradiation treatment, there was a comparable decrease in the number of reversions. The reason for this relation is now obscure but several possible explanations are now being investigated.

Since the number of residual divisions undergone by biochemically deficient cells has been shown by Ryan[9] and Demerec and Cahn[10] to be dependent on the number of viable organisms plated, being greater as the number of cells plated is decreased, and since residual divisions may be related to the number of mutants finally obtained, the influence of such growth on reversion rates was studied. The reversion rates of *E. coli* strain T83-8 (tyrosineless) obtained on M/10 agar plates supplemented with sufficient tyrosine to allow an additional 3.3 or more generations was compared to the reversion rates obtained on the unsupplemented medium. The presence of added tyrosine increased the reversion rates observed but not to the extent that it could account for the increase found when survival is reduced tenfold by irradiation (Fig. 18). Such a reduction in number of viable cells plated would allow an additional 3.3 generations of cells per plate and would be equivalent to the additional residual divisions obtained on the supplemented plates. Thus decreases in the magnitude of residual growth in the presence of larger number of viable cells could account for a portion of the decrease observed in reversion rates when viability is increased because of a pre- or postirradiation treatment.

Another aspect of the same problem is that of the influence of "population pressure" or crowding on revertibility. In studies with three auxotrophs, T83-8, P82/r (purineless), and 45A-25 (arginineless), it was found that the number of viable cells plated does influence the number of revertants

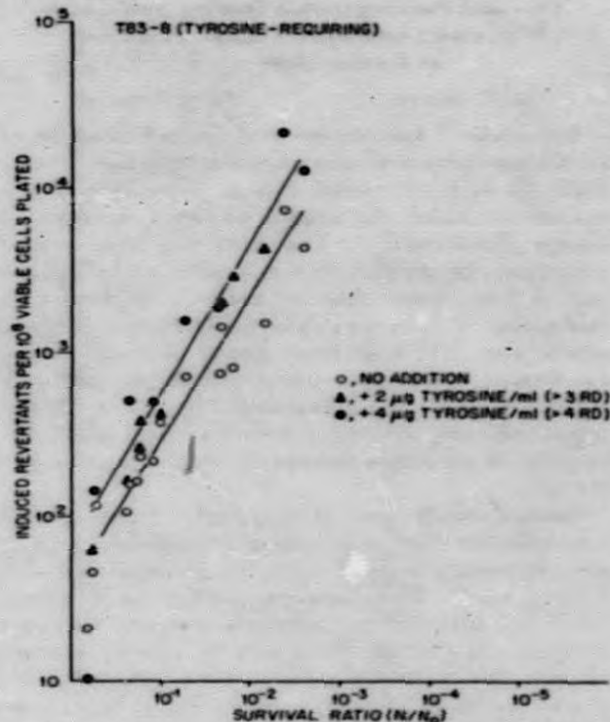

Fig. 18. Influence of "Residual Growth" on the Reversion Rates of X-irradiated *E. coli* Strain T83-8 (Tyrosine-Requiring). The cell suspensions were exposed in nitrogen to various doses of X rays. Only the surviving fractions are plotted.

observed. In the case of T83-8 this population effect is greater than can be attributed to residual growth alone. The results varied from a very marked population effect in T83-8 to relatively little influence (within certain limits of population) with 45A-25. The results of such studies are now being analyzed and will be reported in more detail at a later date. However, the present information does indicate that the relation between modification of X-ray killing and the comparable reduction in induced mutation rate requires further study and analysis before the reduction in mutations can be regarded as a true consequence of X-ray protection and recovery.

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

[7] D. Billen and R. W. Whittle, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 40–41.

[8] C. O. Doudney and A. Hollaender, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 46–47.

[9] F. J. Ryan and L. K. Schneider, *J. Bacteriol.* 58, 201–213 (1949).

[10] M. Demerec and E. Cahn, *J. Bacteriol.* 65, 27–36 (1953).

Pre- and Postirradiation Studies with Long-Ultraviolet and Short-Visible Radiations on *Escherichia coli* B/r

A. J. Sbarra · A. Hollaender

Hollaender[11] has shown that the lethal effect of long ultraviolet and short visible radiation (3500–4900 A) on *Escherichia coli* is dependent on the medium in which the organisms are suspended in during irradiation. The surviving fraction of organisms is greater if the irradiation is carried out in beef broth than in buffer. According to Hollaender,[11] two possible explanations for this effect are: (1) beef broth has a high absorption coefficient in the near-ultraviolet region and may protect the organisms suspended in it, and (2) the organisms are obtaining from the beef broth the material or materials necessary for the repairing of the cell.

Previous work from this group[3,4,5] has clearly demonstrated that a postirradiation recovery phenomena occurs when *E. coli* B/r is irradiated with X or γ rays. This recovery phenomena is stimulated by material or materials present in yeast extract. A similar effect may be operative when *E. coli* B/r is irradiated with long-ultraviolet and short-visible radiations. Experiments have been carried out to see if this is so.

An 18- to 20-hr aerated culture of *E. coli* B/r was used in all experiments. After washing twice, the organisms were irradiated in a salt solution[11] (NaCl, 3 g; KCl, 0.2 g; $CaCl_2$, 0.2 g; H_2O, 100 ml) at a concentration of about 2×10^6 organisms per milliliter. Exposures were made in a test tube which was slowly rotated by an electric motor. Both control and experimental tubes were held in a constant temperature water bath at 31°C. The light source used was a G-E AH-5 mercury lamp. Samples were removed from experimental and control tubes at various time intervals and were then plated on a glucose-inorganic salts medium (basal medium) and basal medium plus 2% yeast extract. The plates were then incubated at various temperatures for 72 hr and then counted.

Figure 19 shows a typical survival curve obtained when *E. coli* B/r is irradiated as previously described. It can be seen that the surviving fraction of cells on yeast extract is approximately 40 times as great as the surviving fraction on basal medium. This finding suggests strongly that a factor, or factors, present in yeast extract is responsible for the greater survival. Colonies picked from the yeast extract plates and plated on basal and basal plus yeast extract media grow equally well on both media, indicating that the phenomenon is not a mutation but a recovery process. This group has been able to substitute for yeast extract in promoting recovery from X or γ rays a synthetic medium containing glutamic acid, guanine, uracil, and inorganic salts. This synthetic medium was also effective in substituting for yeast extract in promoting recovery in cells irradiated with long-ultraviolet and short-visible radiations. Since a considerable portion of the radiation emitted from the light source is 3650 A and since this wave length destroys riboflavin and pyridoxine very effectively, it seemed worth while to supplement the synthetic medium with these two vitamins. No added activity was found; in fact, a slight decrease in activity was noted. These results can be seen in Table 31.

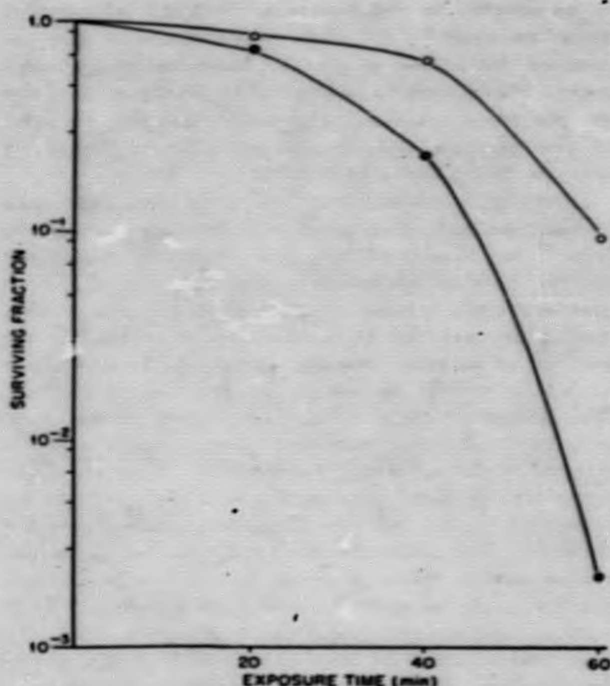

Fig. 19. Surviving Fraction of *E. coli* B/r as a Function of Dose. O, Basal medium + yeast extract; ●, basal medium.

[11] A. Hollaender, *J. Bacteriol.* 46, 531–541 (1943).

TABLE 31. RELATIVE ACTIVITY OF VARIOUS MEDIA COMPARED TO YEAST EXTRACT

Medium	Percentage
Basal	1
Basal + 2% yeast extract	100
Synthetic*	100
Synthetic + riboflavin	34
Synthetic + pyrodoxine	80
Synthetic + riboflavin + pyrodoxine	35

*See ref. 4.

The recovery process with X or γ rays is temperature dependent. A somewhat similar finding has been made with long-ultraviolet and short-visible radiations. At this survival (~15%), the temperature dependence seems to be more pronounced if the irradiated organisms are plated on a basal medium instead of basal medium plus yeast extract; the same was observed with X or γ rays. As with X or γ rays, maximal survival on basal medium is obtained at 18°C. These results can be seen in Fig. 20.

Studies now in progress indicate that as with ionizing radiations, oxygen-saturated suspensions are more sensitive than oxygen-free suspensions. This work will be more fully reported in a later publication.

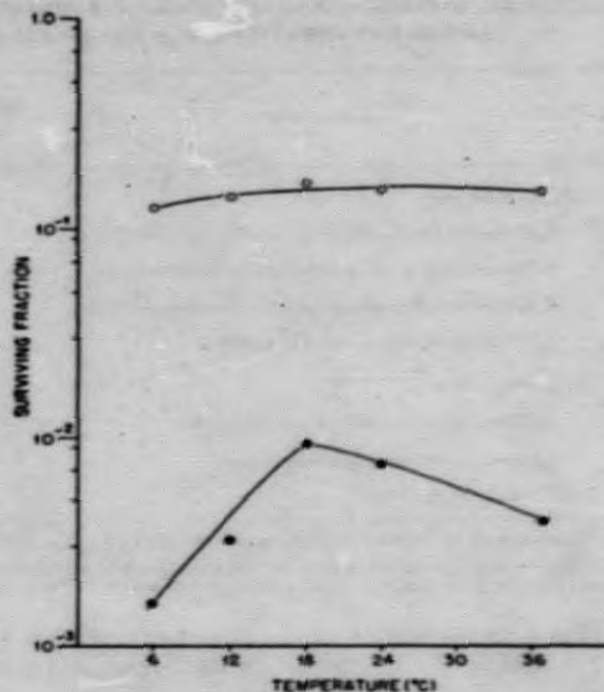

Fig. 20. Comparison of Viability of *E. coli* B/r on Basal Medium and Basal Medium + Yeast Extract as a Function of Postirradiation Incubation Temperature. O, Basal medium + yeast extract; ●, basal medium.

Radiation Protection by S,β-Aminoethylisothiuronium-Br-HBr and Related Compounds

W. T. Burnett, Jr.

D. G. Doherty[12] R. Shapiro[12]

The two most effective compounds in providing mice with protection against X radiation,[13] S,β-aminoethylisothiuronium-Br-HBr (AET) and S,γ-aminopropylisothiuronium-Br-HBr (APT), have been extensively studied to establish their relative effectiveness and therapeutic index. Such factors as the effect of divided dose, time of administration, pH of the thiuronium solution, and strain of mouse will be considered in this report. In addition, an effort was made to improve the testing technique through the use of streptomycin posttreatments.

The previous examination of the protective activities of about 60 nitrogen- and sulfur-containing compounds[13,14,15] has allowed the formulation of a working hypothesis as to the protective action of certain derivatives of β-mercaptoethylamine (MEA). On the basis of the hypothesis described elsewhere in this report,[16] several new derivatives have been prepared and examined for protective activity by a sequential method of analysis.[17] The results of these tests are shown

[12] Biochemistry Section.

[13] W. T. Burnett, Jr., D. G. Doherty, and A. W. Kimball, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 48-52.

[14] D. G. Doherty and W. T. Burnett, Jr., *Proc. Soc. Exptl. Biol. Med.* 89, 312–314 (1955).

[15] D. G. Doherty, W. T. Burnett, Jr., R. Shapiro, and E. Eavenson, *ibid.*, p 93–94.

[16] See Doherty, Burnett, Shapiro, and Eavenson, Biochemistry, this report.

[17] W. T. Burnett, Jr., and A. W. Kimball, Demonstration, Radiation Research Soc., New York City, May 1955. *Radiation Research*, in press (Abstract).

TABLE 32. NITROGEN- AND SULFUR-CONTAINING COMPOUNDS; SEQUENTIAL ANALYSES, TOXICITY TESTS, AND 28-DAY SURVIVORS; C_3H MALE MICE GIVEN AN LD_{100} DOSE OF X RADIATION (800 r)

Compounds	Toxicity[a] (mg/kg)	Dose (mg/mouse)	$\Sigma ES_i - \Sigma EC_i$[b]	28-Day Survival (%)
Protective				
S,β-Aminoethylisothiuronium·diHBr (AET)	650	8.8	11^3	100
1-Thia-4,6,9-triazaspiro(4,4')nonane·diHBr	200	3.8	11^3	13
1,6-Dithia-4,9-diazaspiro(4,4')nonane·diHBr	400	5.5	7^3	0
S,β-Isothiuroniumpropylamine·HBr	>700	15.0	8^3	0
Nonprotective				
S,β-Aminobutylisothiuronium·diHBr	400	6.8	1^3	0
S,β-Aminoethylethylenethiourea	60	1.2	-4^3	0
Propylene sulfide	450	7.0	4^3	0

[a]Estimated by inspection of data. Number of mice too few for satisfactory statistical analysis.

[b]Survivors in treated group minus survivors in saline group. Superscripts show number of trials made in sequential test before a compound was considered protective (see ref. 17).

TABLE 33. NITROGEN- AND SULFUR-CONTAINING COMPOUNDS; SEQUENTIAL ANALYSES, TOXICITY TESTS, AND 28-DAY SURVIVORS; SWISS ALBINO MALE MICE GIVEN AN LD_{100} DOSE OF X RADIATION (800 r)

Compounds	Toxicity[a] (mg/kg)	Dose (mg/mouse)	$\Sigma ES_i - \Sigma EC_i$[b]	28-Day Survival (%)
Protective				
S,β-Aminoethylisothiuronium-Br-HBr (AET)	650	8.8	10^3	93
2-Aminothiazoline	160	2.4	10^3	87
1-Thia-4,6,9-triazaspiro(4,4')nonane·diHBr	200	3.8	7^3	33
2-Amino-1,3-diisothiuroniumpropane·triHBr	>700	10.0	3^2	20
Ethyleneisothiuronium-β-ethylamine·diHBr	60	5.0[c]		20
N'-Allylisothiuronium-β-ethylamine·diHBr	160	4.5[d]		20
Nonprotective				
2-Aminothiazole·HBr	300	5.0	1^3	0
N'-Phenylisothiuronium-S,β-ethylamine	100	1.9	5^3	7
2-Aminothiazoline-4-carboxylate	>600	8.3	2^2	0
S,β-Isothiuroniumpropylamine·HBr	>700	10.0	1^2	0

[a]Estimated by inspection of data. Number of mice too few for statistical analysis.

[b]Survivors in treated group minus survivors in saline group. Superscripts show number of trials made in sequential test before a compound was considered protective (see ref. 17).

[c]Greater than LD_{50} dose.

[d]Approximately the LD_{50} dose.

in Tables 32 and 33. Five of these compounds provided protection against an LD_{100} radiation dose by both the sequential test and the survival at 28 days. The most effective, 2-aminothiazoline, is one of the chemical degradation products of AET. However, with the exception of S,β-isothiuroniumpropylamine, all the compounds were more toxic than AET or APT. The failure of S,δ-aminobutylisothiuronium-Br-HBr (ABT) to protect the mice offers support to the hypothesis that a cyclic intermediate is essential for activity in thiuronium compounds since, in this case, an unlikely seven-membered ring would have to be formed. The protective activity of 2-aminothiazoline is also in accord with this idea since it is a labile ring system containing a potential sulfhydryl group.

Effect of Streptomycin on Survival. – One hundred each of male and female $C_3H \times 101$ mice 10–14 weeks old were irradiated in ten 10-mouse groups with 650–1325 r in steps of 75 r, according to previously described procedures.[18] One-half the mice were given daily subcutaneous injections of 5 mg of streptomycin, beginning on the second day and continuing until the eleventh day postirradiation. In both cases, the radiation doses selected were too high for a good estimation of the LD_{50}; however, the results obtained suggest that the LD_{50} is less than 700 r with or without the supplementary treatment of streptomycin. The streptomycin treatment did not materially increase the number of 28-day survivors. Nevertheless, it would appear that the survival times of the streptomycin-treated mice were slightly greater than those of the nontreated animals.

Protective Effectiveness of AET and MEA by Use of $C_3H \times 101$ Mice. – Table 34 shows the effect of AET and MEA, in near toxic doses, on the 28-day survival of $C_3H \times 101$ mice exposed to graded doses of X radiation. Inspection of the data indicates that the LD_{50} of the AET-treated mice is in the neighborhood of 1250 r, in contrast to an LD_{50} of ~1150 r for the MEA-treated mice. In the range of doses 1400–1700 r, a few more AET-treated mice that were given the streptomycin posttreatment survived than did mice in the groups

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

TABLE 34. EFFECT OF S,β-AMINOETHYLISOTHIURONIUM-Br-HBr AND β-MERCAPTOETHYLAMINE ON THE SURVIVAL OF $C_3H \times 101$ MICE[a] EXPOSED TO X RADIATION

X-Ray Dose (r)	AET[b]				MEA[c]			
	Female		Male		Female		Male	
	*	**	*	**	*	**	*	**
1100	5/5		4/5	9	5/5		4/5	12
1175	4/5	18	3/5	12	1/5	10	1/5	12
1250	3/5	13	4/5	16	2/5	12	0/5	12
1325	2/5	10	4/5	7	1/5	13	1/5	11
1400	0/5	10	0/5	12	0/5	12	0/5	12
1475	0/5	8	0/5	10	0/5	9	0/5	11
1550	0/5	11	0/5	7	0/5	9	0/5	10
1625	0/5	9	0/5	12	0/5	6	0/5	9
1700	0/5	8	0/5	5				

*Number of 28-day survivors/number irradiated.

**Mean death time (days) for nonsurvivors.

[a] Daily subcutaneous injections of 5 mg of streptomycin from second to eleventh day following X irradiation. Housed in individual cages.

[b] 8.8 mg, intraperitoneally, 10 min before X irradiation.

[c] 2.6 mg, intraperitoneally, 10 min before X irradiation.

that were not treated with the antibiotic.

The relative merits of AET and MEA, on an equimolar basis, at six dose levels each are illustrated in Table 35. Both compounds provided good protection against 800 r of X rays at the lowest dose levels tried – 2.2 mg of AET and 0.9 mg of MEA. However, against 1100 r of X rays, AET appears to be superior to MEA since the lowest doses of MEA and AET that provide protection to 50% or more of the mice were 2.7 and 3.8 mg, respectively. Very few survivors were obtained at doses smaller than 2.7 mg of MEA. In contrast, three of ten mice that were given 2.2 mg of AET survived. This dose is approximately one-fifth or one-sixth the LD_{50} dose of AET.[13] Some protection against 1100 r of X rays might be achieved with even lower doses by the intraperitoneal route.

Experiments with C_3H Mice Treated with AET and APT. – *Since AET may exist in a number of tautomeric forms, depending on the pH of the solution, male C_3H mice were X-irradiated (800 and 1100 r) in groups of ten mice each 10 min after the intraperitoneal injection of AET solutions (8.8 mg/0.3 ml) which had been adjusted to a series of pH's from 4.0 to 9.0. Equivalent protection (28-day survival) was obtained over the pH range of 5.0–8.0 with 20–30% drop in activity at both pH 4.0 and 9.0.*

Earlier screening tests have indicated that APT in near-toxic doses,[13,14] is about as effective as AET as a radiation-protective agent. This finding, together with the lack of effectiveness of the butyl derivative (ABT), has important implications with respect to the relation of structure of MEA derivatives to protective activity.[16] Although the propyl

TABLE 35. EFFECT OF GRADED DOSES OF S,β-AMINOETHYLISOTHIURONIUM-Br-HBr AND β-MERCAPTOETHYLAMINE ON THE SURVIVAL OF $C_3H \times 101$ MICE[a] EXPOSED TO TOTAL-BODY X RADIATION

Compound Dose (mg)	X-Ray Dose, 800 r				X-Ray Dose, 1100 r			
	Females		Males		Females		Males	
	*	**	*	**	*	**	*	**
S,β-Aminoethylisothiuronium-Br-HBr								
2.2	5/5		5/5		2/5	13	1/5	12
2.9	5/5		4/5	10	4/5	15	0/5	10
3.8	5/5		5/5		4/5	15	5/5	
5.0	5/5		5/5		5/5		5/5	
6.6	5/5		5/5		3/5	13	4/5	18
8.8	4/5	8	5/5		5/5		4/5	9
β-Mercaptoethylamine[b]								
0.9	3/5	18	5/5		0/5	11	0/5	13
1.2	5/5		5/5		0/5	13	1/5	11
1.6	5/5		5/5		1/5	12	1/5	14
2.0	5/5		4/5	14	0/5	18	2/5	14
2.7	5/5		5/5		5/5		4/5	14
3.6	5/5		5/5		5/5		4/5	12

*Number of 28-day survivors/number irradiated.

**Mean death time (days) for nonsurvivors.

[a]Mice housed in individual cages following irradiation. Daily subcutaneous injections of 5 mg of streptomycin from the third to the thirteenth day following irradiation.

[b]Dose levels of MEA equimolar to those of AET indicated in the first half of the table.

derivative is slightly more toxic than the ethyl derivative,[14] its protective properties were investigated over a range of equivalent dose levels. Table 36 shows the effect of graded doses of AET and APT on the survival and survival times of C_3H mice exposed to 800 and 1100 r of total-body X radiation. The radioresistance of nonprotected C_3H mice appears to be less than that of nonprotected $C_3H \times 101$ mice; it was therefore not unexpected that the number of 28-day survivors in the AET-treated groups (Table 36) would be less than in similarly treated groups of the hybrid (Table 35). However, on an equimolar basis over the range of doses tested, the protection offered by APT against both 800 and 1100 r of X rays was about the same as that offered by AET. This seems to be the case even at toxic and near-toxic levels of AET and APT.

TABLE 36. EFFECT OF GRADED DOSES OF S,β-AMINOETHYLISOTHIURONIUM-Br-HBr AND S,γ-AMINOPROPYLISOTHIURONIUM-Br-HBr ON THE SURVIVAL OF C_3H MICE[a] EXPOSED TO TOTAL-BODY X RADIATION

Compound Dose (mg)	X-Ray Dose, 800 r				X-Ray Dose, 1100 r			
	Females		Males		Females		Males	
	*	**	*	**	*	**	*	**
S,β-Aminoethylisothiuronium-Br-HBr								
2.2	1/5	13	4/5	12	0/5	7	0/5	9
2.9	2/5	18	0/5	13	0/5	10	0/5	11
3.8	4/5	8	5/5		0/5	14	0/5	12
5.0	4/5	16	2/5	15	0/5	12	1/5	10
6.6	3/5	18	5/5		0/5	13	2/5	8
8.8	3/4	16	5/5		3/5	14	0/5	9
S,γ-Aminopropylisothiuronium-Br-HBr								
2.3	4/5	19	5/5		1/5	12	0/5	11
3.1	4/5	19	5/5		0/5	15	0/5	12
4.0	3/5	16	2/5	19	0/5	14	2/5	16
5.3	4/5	15	5/5		2/5	11	3/5	14
6.9	4/4[b]		5/5	11	1/5	15	1/5	16
9.3	0/0[b]		4/5	8	0/2[b]	14	1/3[b]	14
Saline								
	0/5	9	0/5	11				

*Number of 28-day survivors/number irradiated.

**Mean death time (days) for nonsurvivors.

[a]Mice housed in individual cages following irradiation. Daily subcutaneous injections of 5 mg of streptomycin from the third to the thirteenth day following irradiation.

[b]The deaths in group (5 mice) attributed to toxic effect of AET and APT not included in this summary.

MODIFICATION OF RADIATION INJURY IN MICE

C. C. Congdon

L. H. Smith P. Urso
T. Makinodan I. Urso

Recovery of X-irradiated Mice Injected with Whole Leukemoid Blood or with Leukocyte-Platelet Suspensions

L. H. Smith C. C. Congdon
B. Anderson[1] T. T. Odell, Jr.[1]

It has been reported[2] that blood from mice carrying a transplantable squamous cell carcinoma injected intravenously into normal mice previously exposed to 750 r whole body X rays results in 30-day survival of 10–70%. The blood from mice carrying this tumor shows an extreme granulocytosis (leukemoid reaction). Since there is evidence[3] that the granulocyte is important in recovery of animals exposed to radiation, advantage was taken of this granulocytosis to learn more about the role of these leukocytes in recovery from whole body X rays. In these experiments, the first step was the isolation of leukocytes from the blood of BALB/c mice bearing the squamous cell carcinoma. The isolation technique used was one in which fibrinogen, added to whole blood, caused the agglomeration and rapid sedimentation of erythrocytes, thereby leaving a plasma-leukocyte-platelet supernatant. A 6% fibrinogen solution (saline) was employed to agglomerate erythrocytes according to a method described by Skoog and Beck.[4] Mice bearing the tumor were injected intravenously with a mixture of heparin, nembutal, and saline (1:1:10). Blood, withdrawn from the heart with a heparinized syringe, was placed in a 5-cc siliconed test tube, and an equal volume of 6% fibrinogen solution was added. The tube was inverted 15 times to ensure good mixing, and was placed in a rack to allow the erythrocytes to agglomerate and sediment. After about 1 hr, the overlying plasma-fibrinogen suspension containing leukocytes and platelets was removed with an elongated medicine dropper. This suspension was centrifuged at about [illegible]0 × *g* for 20 min; the clear supernatant was removed, and the packed cells were resuspended in buffered saline (pH 7.4). This procedure was repeated twice and erythrocyte and leukocyte counts were made of the final suspension.

A 1-hr sedimentation time, longer than that recommended by Skoog and Beck, was used to reduce the erythrocyte contamination to a minimum. As a result of extending the time, the leukocyte yield was only about 80% rather than 100% as reported by these workers. Although a systematic study of the erythrocyte contamination has not been conducted here, preliminary results indicate that no more than 10% of the total erythrocyte-leukocyte population in the supernatant are erythrocytes. Furthermore, the leukocyte yield was increased slightly by the addition of more fibrinogen to the sedimented fraction and allowing the erythrocytes to agglomerate again. The overlying suspension thereby obtained was treated as originally, and the final saline suspension was combined with the first preparation.

The results of using whole leukemoid blood or the leukocyte-platelet suspension, prepared in the manner described, to promote recovery of mice exposed to 750 r whole-body X rays (LD_{100}) are summarized in Table 37. Male and female BALB/c mice were used in all experiments as both donors and recipients. Mice were injected intravenously into the tail vein with 0.5 ml of whole leukemoid blood diluted 1:3 with Tyrode's solution or with the leukocyte-platelet suspension obtained from leukemoid blood. Mice of groups I, II, and III (Table 37) were given a single injection postirradiation, usually within several hours after exposure. In group IV, the leukocyte-platelet suspension was injected 1 hr preirradiation.

In group I, 65% of the mice injected with diluted whole leukemoid blood were alive 30 days postirradiation. This survival figure represents the average of the results from three separate experiments, in which the individual 30 day survivals were 42, 66, and 100%. These results confirm those reported by Congdon *et al.*[2] who obtained

[1] Pathology and Physiology Section.

[2] C. C. Congdon, T. W. McKinley, H. Sutton, and P. Urso, *Radiation Research*, in press. (Abstract of paper at Radiation Research Soc., New York City, May 1955.)

[3] E. P. Cronkite and G. Brecher, *Naval Medical Research Institute Rep.* NMRI-54-4, 1954, p 187–208.

[4] W. A. Skoog and W. S. Beck, *Univ. of Calif. Rep.* UCLA-333 (1955).

TABLE 37. EFFECTS OF INTRAVENOUSLY INJECTED LEUKEMOID BLOOD OR LEUKOCYTE-PLATELET SUSPENSIONS SEPARATED FROM LEUKEMOID BLOOD ON SURVIVAL OF MICE EXPOSED TO 750 r OF WHOLE-BODY X RAYS

Group		Number of Mice	Treatment	30-Day Survival (%)
I	Controls	34	X ray	0
	Experimentals	26	X ray + whole leukemoid blood (2.6×10^7)*	65
II	Controls	8	X ray	0
	Experimentals	7	X ray + WBC-platelet suspension (10^8)	43
III	Controls	12	X ray	0
	Experimentals	12	X ray + WBC-platelet suspension (1.3×10^8)	100
IV	Controls	12	X ray	0
	Experimentals	12	WBC-platelet suspension + X ray (8.7×10^7)	0

*Figures in parentheses are the approximate numbers of leukocytes injected per mouse.

30-day survivals of 10–70%. In groups II and III, 43 and 100%, respectively, of the mice which received an intravenous injection of leukocyte-platelet suspensions were alive 30 days postirradiation.

Although it is evident that the injection of either whole leukemoid blood or the leukocyte-platelet suspension prepared from leukemoid blood promotes recovery in X-irradiated mice, the question arose whether the suspension, if injected into mice prior to irradiation, would afford protection. None of the mice injected with the leukocyte-platelet suspension before irradiation survived (group IV, Table 37).

The results of these experiments support the concept that the leukocyte is an important factor in promoting recovery of X-rayed mice. It is not possible at this time to implicate conclusively the leukocytes in the recovery process, because the injected suspensions were contaminated with erythrocytes and platelets. Congdon *et al.* (unpublished observations), however, observed that erythrocytes from normal mouse blood did not promote recovery when injected into mice following X irradiation. Furthermore, it has been shown[5] that injected platelet suspensions, although controlling postirradiation hemorrhage, do not increase significantly the survival time of dogs exposed to 400–600 r of X rays.

Implication of cells of the granulocyte series in the recovery process is evidenced by two factors: (1) an increase in granulocytes is primarily responsible for the leukocytosis in the blood of mice carrying the squamous cell carcinoma, and (2) intraperitoneally injected lymphocytes do not afford recovery in X-irradiated rats.[6]

[5] E. P. Cronkite, G. Brecher, and K. M. Wilbur, *The Military Surgeon* 114, 359–365 (1954).

[6] L. L. Campbell and M. H. Ross, Topical Rep. ORNL-1193 (1952).

Relation of Lymphatic Tissue Regeneration to Survival of Bone Marrow-Treated Irradiated Mice

C. C. Congdon I. Urso

The striking regeneration of lymphatic tissue in spleen- and thigh-shielded irradiated mice and in bone marrow-treated irradiated mice has proved to be of primary importance in determining whether irradiated mice subsequently develop lymphocytic neoplasms.[7] In acute lethal total body irradiation, lymphatic tissue regeneration occurs in surviving mice treated with isologous bone marrow (also in spleen and thigh shielding) but it is not clear whether the lymphatic tissue regeneration is essential for survival as is the bone marrow regeneration and that of the red pulp of the spleen.

An additional related problem was raised by the demonstration that heterologous and homologous bone marrow did not cause lymphatic tissue regeneration (determined by thymic weight measurement) in the leukemogenic irradiation situation,[7] whereas isologous bone marrow and thigh shielding are associated with lymphatic tissue regeneration. In this study, LAF_1 mice were given acute lethal total-body irradiation. Thirty-day survival and thymic weight determinations were made on surviving mice that received bone marrow from one femoral bone shaft of isologous donor mice or the same amount of bone marrow from homologous donor mice. Table 38 shows the data obtained at the present time. The effect of larger amounts of isologous, homologous, and heterologous (rat) donor bone marrow on survival and thymus weight will be determined.

Bone Marrow Response of X-irradiated Mice Receiving Varying Amounts of Isologous Bone Marrow Suspensions

P. Urso C. C. Congdon

Experience in studying the mechanism of action of suspensions of bone marrow cells and related tissues on the recovery of lethally irradiated mice has demonstrated a need for detailed and reproducible quantitative and qualitative biological data on several aspects of the recovery phenomenon.

[7] M. B. Brown, B. B. Hirsch, C. S. Hagemeda, S. K. Hochstetler, W. G. Faughan, P. Tuch, and H. S. Kaplan, *J. Natl. Cancer Inst.* 15, 949–973 (1955).

For example, information may be needed on how varying the amount of bone marrow cells injected into the irradiated animal at one exposure level influences, not only 30-day survival, but also the actual response of the irradiated host animal's bone marrow at varying intervals after exposure. Techniques for counting the number of bone marrow cells injected and for counting the number of bone marrow cells in the irradiated host's femoral bone shaft have been developed. Determination of the weight and volume (micro hematocrit procedure) of bone marrow in the femoral bone shaft has also been recorded. Additional data taken on the sacrificed irradiated host animal for further correlations include body weight, thymus weight, the opposite femoral shaft bone marrow for histology (quantitative and qualitative study), and peripheral blood leukocyte count, and differential blood smear. With each experimental run a separate group of animals is used for survival data.

Figure 21 shows the femoral shaft bone marrow response of irradiated male mice to two different amounts of injected bone marrow cells. Figure 22 shows the peripheral blood leukocyte counts for the same male mice at the time of sacrifice.

By this same type of experimental study it is planned to determine several types of dose-response relations by use of different response end points including survival, peripheral blood leukocyte counts, bone marrow response, and thymus weight response.

Preliminary Data from Studies of Delayed Effects in Mice Surviving Total-Body Exposures to Massive Doses of X Radiation

C. C. Congdon D. G. Doherty[8]
A. C. Upton[1] A. W. Kimball[9]

The demonstration by Burnett and Doherty that mice receiving 2000–2600 r of total-body γ rays can survive beyond a 30-day observation period,[10] provided they receive S,β-aminoethylisothiuronium-Br-HBr (AET) preirradiation and bone marrow-streptomycin treatment postirradiation makes it

[8] Biochemistry Section.

[9] Mathematics Panel.

[10] W. T. Burnett, Jr., and D. G. Doherty, *Radiation Research*, in press. (Abstract of paper at Radiation Research Soc., New York City, May 1955.)

TABLE 38. EFFECT OF ISOLOGOUS AND HOMOLOGOUS BONE MARROW ON SURVIVAL AND ORGAN WEIGHTS OF X-IRRADIATED (900 r) LAF_1 MICE*

	Expt No.	Number and Sex of Mice	Age of Irradiation (weeks)	30-Day Survival (%)	Mean Day of Death for Nonsurvivors	30-Day Survivors: Mean Thymus Weight (g)	30-Day Survivors: Mean Spleen Weight (g)	30-Day Survivors: Mean Body Weight (g)
Marrow from LAF_1 mice	1	15 ♂	11–12	87	14.5	42.5	175.3	22.5
	2	15 ♂	12	80	19.3	28.1	226.7	20.9
	3	15 ♂	12–16	87	11.0	27.4	173.1	23.6
	4	15 ♀	15–16	93	7.0	39.9	174.9	20.5
	5	15 ♀	11–12	67	12.0	38.7	133.7	21.8
	6	15 ♀	11–12	100		43.0	119.4	23.1
Marrow from C_3H X $101F_1$ mice	1	14 ♂	11–12	0	19.2			
	2	15 ♂	12	7	18.3	16.0	69.8	17.3
	3	15 ♂	12–16	7	16.6	3.7	117.5	18.0
	4	13 ♀	15–16	7	13.1	7.6	165.4	15.8
	5	14 ♀	11–12	43	14.1	26.7	142.4	20.2
	6	15 ♀	11–12	20	20.3	11.5	127.1	17.1
Age control, *no X ray*	1	10 ♀	11–12			46.5	186.1	21.3
	2	10 ♀	12			47.7	155.9	21.9
	3	11 ♂	16			33.3	131.4	27.2
	4	10 ♂	15–16			33.7	132.0	29.2
	5	5 ♂	11–12			38.7	108.8	28.5
	6	5 ♂	11–12			38.1	116.6	28.5

*59 X-ray control mice all died within the 30-day period. Mean day of death was 8.2.

desirable to obtain data on life span, tumor incidence, cataract induction, and other delayed effects resulting from exposure to radiation in a range not capable of being studied in the past.

In the preliminary planning it was decided to investigate the delayed effects at about the LD_{50}/30-day exposure level for mice with no treatment, those pretreated with AET, those posttreated with isologous bone marrow, and those receiving combination pre- and postirradiation treatment. Determination of the LD_{50}/30-day exposure values for these four situations is now partially completed.

The LD_{50}/30-day X-radiation point estimates for C_3H X $101F_1$ mice were: untreated mice, 692 r with a 95% confidence interval of 649–753 r; AET-pretreated mice, 1148 r with a 95% confidence interval of 1071–1212 r; isologous bone-marrow-posttreated mice, 1292 r with a 95% confidence interval of 1212–1382 r. This point estimate for combined treatment of X-irradiated mice of this strain has not been determined. However, in one experiment, good survival was obtained at 1700 r and in another small experiment all mice receiving 2000 r or more died; thus the LD_{50}/30-day value may lie in this range.

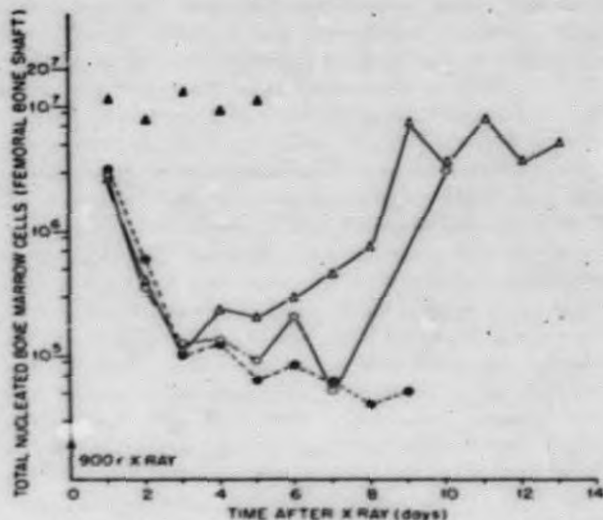

Fig. 21. Femoral Shaft Bone Marrow Counts of Irradiated (900 r) Male CAF$_1$ Mice Receiving Two Different Amounts of Isologous Bone Marrow Cells Intravenously. ▲, Normal CAF$_1$ mice; ●, X-ray only; O, X-rayed mice receiving 4.5×10^5 to 7.5×10^5 bone marrow cells; △, X-rayed mice receiving 7.4×10^5 to 1.2×10^6 bone marrow cells.

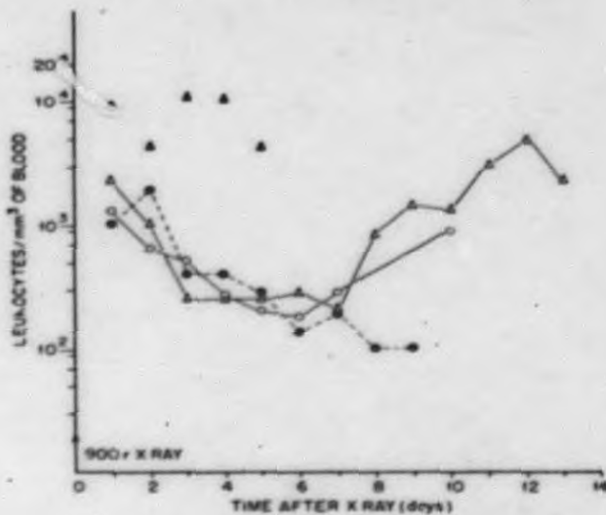

Fig. 22. Peripheral Blood Leukocyte Counts of the Same Mice Shown in Fig. 21.

Screening of Tissues for New Recovery Agents

C. C. Congdon I. Urso

The search for tissues, other than those of the hematopoietic system, which will promote recovery from lethal total-body X irradiation has demonstrated the failure of intraperitoneal transplants of *urinary bladder and jejunum* to alter the 30-day mortality following a 900 r exposure. LAF$_1$ mice were exposed to 900 r of irradiation and shortly thereafter were anesthesized. Through a laparotomy incision each animal in one group received a urinary bladder, cut into small pieces, from a normal LAF$_1$ mouse and each animal in a second group received about ½ in. of jejunum, cut into small pieces, from a normal LAF$_1$ mouse. The mean day of death closely approximated that of the control X-rayed mice. The rationale for testing urinary bladder depends on the old observation that urinary bladder mucosa will stimulate bone formation on transplantation.[11] The testing of intestinal tissue follows from the recent work of Goldwasser and White indicating a recovery effect from noncellular extracts of hog intestinal mucosa when injected into irradiated mice.[12]

Harderian Gland Test for Transplantation of Homologous Bone Marrow Cells Following Total-Body X Irradiation

C. C. Congdon
R. M. Merwin[13] T. W. McKinley[13]

A technique developed by Merwin and Hill[14] was used as an immunological test for the type of bone marrow present in irradiated LAF$_1$ mice that received C$_3$Hf bone marrow cells intravenously following the irradiation exposure. The test animals were normal LAF$_1$ mice carrying tiny, nonvascularized grafts of Harderian gland from young C$_3$H mice in a transparent window. C$_3$H tissue injected subcutaneously into the test animals will cause the graft to disappear; LAF$_1$ tissue would not cause it to disappear.

[11] G. J. Loewi, *J. Pathol. and Bacteriol.* 68, 419–422 (1954).

[12] E. Goldwasser and W. F. White, *Radiation Research*, in press. (Abstract of paper at Radiation Research Soc., New York City, May 1955.)

[13] National Cancer Institute.

[14] R. M. Merwin and E. L. Hill, *J. Natl. Cancer Inst.* 14, 819–839 (1954).

In two series of experiments, each LAF_1 mouse exposed to 900 r of X rays received the bone marrow cells from one femoral shaft of a C_3Hf mouse. The irradiated recipients (LAF_1) were sacrificed at different intervals following the exposure and the bone marrow from one femoral shaft was injected subcutaneously into a test animal (normal LAF_1 mouse carrying nonvascularized C_3H Harderian gland graft). The graft in the test animal was then observed through the transparent window for continued viability of the graft or its necrosis and disappearance. On day zero following irradiation two tests (the grafts did not disappear) failed to demonstrate presence of C_3Hf cells. Six tests on day 1 showed presence of C_3Hf cells in three and absence of these cells in three. On day 2, three tests showed presence of C_3Hf cells in two and absence in one. On day 3, two tests showed a positive (presence of C_3Hf cells) and a negative (absence) result. On day 4, four of five tests were positive, one was negative. From day 7 through 180 all nine tests were positive indicating the presence of C_3Hf cells in the LAF_1 mice.

The present interpretation of these results is that, between day zero and day 7 after C_3Hf bone marrow cells were injected into an irradiated LAF_1 mouse, sufficient C_3Hf cells "take" and multiply in the bone marrow of the femoral shaft of the LAF_1 mouse to change the test from negative (absence of C_3Hf cells) to positive (presence of "sufficient" C_3Hf cells). This is further interpreted to indicate that homologous transplantation of C_3Hf cells occurs in this experiment. Total repopulation of LAF_1 femoral bone marrow by C_3Hf cells would be a special case of transplantation, and this evaluation has not been made. The role of the cells that do transplant in determining survival remains to be studied. Evidently transplantation is an early step in the mechanism of recovery. These results are in line with those of Main and Prehn on skin homografts after irradiation and bone marrow injection.[15] In their work the transplantation of hybrid bone marrow cells had far-reaching immunological consequences but the interpretation of total cellular repopulation of irradiated bone marrow by the hybrid cells cannot be made at this time.

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[15] J. M. Main and R. T. Prehn, *J. Natl. Cancer Inst.* 15, 1023–1029 (1955).

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell, Section Chief

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

W. St. Amand

Radiation-Induced Mutations in the Mouse

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

Investigations on radiation-induced mutation rates in mice,[3] and a comparison of the results with as nearly similar data as were available for *Drosophila*, showed a higher mean rate in the mouse, and led to the conclusion "that estimates of human hazards based on *Drosophila* mutation rates may be too low." It was recognized that the *Drosophila* data that were available were not ideally suited for comparison with the mouse data. Thus the mouse mutation rates were determined for irradiated spermatogonia, seven specific autosomal loci being used, and there was no information on specific-loci rates for irradiated spermatogonia in *Drosophila*, although there was information on specific-loci rates for irradiated spermatozoa. This was considered a serious lack. Therefore, a study was suggested and supported in this laboratory by Alexander[4] (1954) which was designed specifically to provide *Drosophila* data more suitable for comparison with the mouse. In this investigation, the mutation rates were determined: (1) at specific loci, (2) on autosomes, (3) for irradiated spermatogonia as well as spermatozoa, (4) with essentially the same genetic technique and method of scoring the mutations as had been used on the mice, and (5) with the same X-ray machine and method of dosimetry as that used in the mouse experiment. Since there is a suddenly increased interest in genetic hazards of radiation, it seems desirable at this time to discuss the species comparison again, the *Drosophila* data of Alexander and new *Drosophila* data of other workers being used.

Alexander's data on *Drosophila* spermatogonia provide the most closely similar results for comparison with the mouse data. The mouse mutation rate may be calculated on the basis of the tested mutations reported in 1951 publication[3] plus 10 mutations that had been only partially tested at that time and 12 presumed mutations that had not yet been tested. These additional 22 have since been fully tested and all of them have proved to be mutations at the specific loci. The six mouse "mutants" that died before testing are excluded for purposes of comparison with Alexander's data, which include only tested mutants. This reduces the published figure for the mouse from 25×10^{-8} to 22×10^{-8}/r/locus. Comparison of this with 1.5×10^{-8}/r/locus for the *Drosophila* spermatogonia mutation rate gives an estimate of 15 as the ratio of the mouse rate to the *Drosophila* rate.

In this comparison there is no question of a statistically significant difference between the mean mutation rates of the two sets of loci. Statistical tests of high sensitivity are not available for the more important question of whether the data indicate a significant difference in overall mutation rates between the two species, if the two sets of loci are assumed to be, at least, random samples of comparable kinds of loci. Nevertheless, the application of a nonparametric test, namely, Pitman's (1937), does show a difference of this kind which is significant at the 3.9% level.[5] The following semiquantitative considerations are also instructive. Five of the seven loci tested in the mouse spermatogonia gave induced mutation rates higher than the highest of the rates obtained by Alexander for the eight loci in *Drosophila* spermatogonia and also higher than the highest of the eight rates obtained by Alexander for *Drosophila* spermatozoa, even without adjusting for the fourfold greater mean rate in her spermatozoa results.

[1]Consultant.

[2]Research Associate.

[3]W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* 16, 327–336 (1951).

[4]M. L. Alexander, *Genetics* 39, 409–428 (1954).

[5]This test was suggested and computed by A. W. Kimball of the Mathematics Panel.

Since the *Drosophila* spermatogonia mutation rate for specific loci is based on only ten mutations, it is still of interest to compare the mouse rate with the *Drosophila* spermatozoa rate, particularly the new and independent sets of information on specific loci in autosomes. Alexander's rate for 71 tested mutations in spermatozoa is 5.98×10^{-8}/r/locus. Mickey's[6] (1954) rate for 32 mutations at the same eight loci is 3.35×10^{-8}. Patterson's rate (quoted by Alexander) for a total of 70 mutations at seven of the same loci and one other locus is 4.39×10^{-8}. In estimating spermatogonia rates from these spermatozoa rates, a step necessary before comparison with the mouse data, it would defeat the purpose of getting estimates independent of the limited information on specific loci rates in *Drosophila* spermatogonia if Alexander's estimate of the ratio of spermatozoa to spermatogonia rates were used. The information that comes closest to providing a satisfactory independent estimate of this ratio is that of Auerbach[7] (1954). Table 1 in her publication gives the rate of mutation to recessive lethals on the second chromosome in broods of *Drosophila* coming from irradiated males mated for successive periods of three days each. Data from the last brood are presumed to be from irradiated spermatogonia. In Mickey's experiment, the males were mated for six days following irradiation. His data may, therefore, be taken as being roughly equivalent to the first two broods of Auerbach's data. The ratio of the weighted mean mutation rate for these two broods compared with the rate in the last brood is 4.6. Mickey's rate divided by this, gives 0.73×10^{-8}/r/locus, as an estimate of the spermatogonia rate in *Drosophila*. Dividing the mouse rate by this adjusted *Drosophila* rate gives a ratio of mouse to *Drosophila* rates of 30. In Alexander's experiment the males were mated for four days following irradiation. If this is taken as corresponding to Auerbach's first brood and one-third of her second brood, 3.7 is the figure with which to divide Alexander's spermatozoa rate. This gives an estimate of 1.6×10^{-8}/r/locus, for the spermatogonia rate, and 14 as the ratio of the mouse to the *Drosophila* rate. These last figures are in excellent agreement with those obtained directly from Alexander's spermatogonia data. It may be noted that Alexander's data, when compared with the mouse results, give a more conservative estimate of the difference between mouse and *Drosophila* mutation rates than Mickey's data.

Comparisons between species as different as *Drosophila* and mice are difficult. There are many complicating factors which make it unwise to generalize about the relative rates of radiation-induced mutation in *Drosophila* and mice. However, because of the human hazards, there is a pressing need to draw the best possible conclusion. The new *Drosophila* data indicate that the induced mutation rate per locus in the mouse may be about one order of magnitude higher than that in *Drosophila*. This reinforces the conclusion reached in 1951[3] that "from the point of view of those concerned with the immediate problems of protection in man, it would be risky to ignore the indication that estimates of human hazards based on *Drosophila* mutation rates may be too low."

[6] G. H. Mickey, *Am. Naturalist* **88**, 209–314 (1954).

[7] C. Auerbach, *Z. indukt. Abstammungs-u. Vererbungslehre* **86**, 113–125 (1954).

Gamma-Ray Sensitivity of Spermatogonia of the Mouse

E. F. Oakberg R. L. DiMinno

In order to confirm the sensitivity curve previously published for spermatogonia,[8] hybrid (101 × C_3H) male mice were given γ-ray doses of 5, 8, 12, 17, 23, 38, 47, 57, 68, 80, and 100 r at a dose rate of 6 r/min, and killed 72 hr after exposure. Techniques of fixation, sectioning, staining, and scoring were the same as described previously.[8,9] Data for the four control mice and the four mice at each dose level have been combined in Tables 39 and 40.

With the recent estimate[10] of the duration of spermatogenesis available, the stages at which cells are irradiated and subsequently scored can be determined with greater accuracy. Thus comparison of sensitivities of spermatogonia at different stages of their developmental sequence is facilitated. The 72-hr interval between irradiation and observation was chosen for comparisons based on counts of newly formed spermatocytes at stages

[8] E. F. Oakberg, *Radiation Research* **2**, 369–391 (1955).

[9] E. F. Oakberg, *J. Morphol.* **97**, in press.

[10] E. F. Oakberg and R. L. DiMinno, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 59–61.

TABLE 39. GAMMA-RAY SENSITIVITY OF DIVIDING TYPE A, INTERMEDIATE, AND TYPE B SPERMATOGONIA

Dose (r)	Type A in Division + Early Intermediate		Intermediate + Type B	
	Total Cells	Fraction of Control	Total Cells	Fraction of Control
0	1376*		1489**	
5	1301	0.945	1372	0.921
8	1161	0.844	1162	0.780
12	1020	0.741	1091	0.733
17	911	0.662	1018	0.684
23	780	0.567	774	0.520
30	479	0.348	546	0.367
38	406	0.295	306	0.206
47	253	0.184	254	0.171
57	193	0.140	268	0.180
68	43	0.031	41	0.028
80	11	0.008	42	0.028
100	5	0.004	8	0.005

*Scored as resting primary spermatocytes at stage VII.

**Scored at beginning of leptotene at stage VIII.

TABLE 40. GAMMA-RAY SENSITIVITY OF TYPE A SPERMATOGONIA

Dose (r)	All Stages Combined		Irradiated at Stages VIII-X[a]; XI[a]		Irradiated at Stages VI[b]; VII[b]	
	Total Cells	Fraction of Control	Total Cells	Fraction of Control	Total Cells	Fraction of Control
0	1593		387		258	
5	1555	0.974	346	0.894	281	1.089
8	1284	0.805	311	0.804	192	0.744
12	1320	0.827	303	0.783	182	0.705
17	1095	0.686	251	0.649	160	0.620
23	924	0.579	217	0.561	129	0.500
30	881	0.552	198	0.512	143	0.554
38	730	0.457	153	0.395	122	0.473
47	628	0.393	120	0.310	100	0.388
57	633	0.397	99	0.256	99	0.384
68	557	0.349	101	0.261	79	0.306
80	522	0.327	80	0.207	74	0.287
100	496	0.311	82	0.212	88	0.341

[a]Mitotic peaks at IX and XI at mid-point of irradiation to scoring time.

[b]Cells in "dormant" stage during irradiation.

VII and VIII, but is not optimum for all spermatogonial stages. Some sensitivity curves for type A spermatogonia, however, can be determined.

Since stage VII occupies about 20.6 hr of the total spermatogenic cycle, the count of resting primary spermatocytes is drawn from a similar time range 72 hr earlier. This would correspond to irradiation of spermatogonia at tubule stages II, during the third division of type A cells; and at tubule stage III, when transition from A to the intermediate type is occurring. The mid-point falls late in stage II, at the peak of spermatogonial division. The count of spermatocytes about to enter leptotene at stage VIII represents a range of cells irradiated from stages III to IV, with the mid-point at stage IV, when intermediate spermatogonia divide to form cells of type B. The two survival curves (Table 39) are remarkably similar, with the LD_{50} between 23 and 30 r in both cases. These results are in excellent agreement with the sensitivity of intermediate and type B spermatogonia published earlier.[8]

The survival curve obtained by combining the counts of all type A cells for all 12 stages of the cycle is given in column 2 of Table 40. The surviving fraction agrees well with the data of Table 39 at doses of 5, 8, 12, 17, and 23 r, but at doses of 30–100 r the type A curve flattens noticeably, with the "fraction of control" being almost uniform at 47–100 r. This has been interpreted as an indication of a heterogeneity of sensitivities within the type A population, and the suggestion has been made that "dormant" type A cells are most resistant.[8] In order to test this hypothesis, number of spermatogonia at stages I (representing cells irradiated at stages VIII–X, with the mid-point at IX) and III (representing irradiation at stage XI) were used for estimation of sensitivity during the mitotic peaks at stages IX and XI (table 40, column 4). Sensitivity of cells irradiated in the "dormant" stage was estimated by spermatogonial counts at stages X and XI, which represent cells irradiated at stages V, VI, and VII (Table 2, column 6). These cells have gone through one division at stage IX, and some are undergoing a second division at stage XI, providing the opportunity for expression of radiation damage. The survival curves given in columns 4 and 6, Table 2, are not markedly different except at doses of 57, 68, 80, and 100 r, where irradiation at the mitotic peaks gives a slightly lower survival. That the difference is not greater is not surprising, however, for even during the mitotic peaks most cells are in interphase. The more interesting observation is the rapid decrease in number of type A spermatogonia at low doses even when irradiated in the "dormant" state. With the exception of the 8- and 12-r doses, survival of type A cells irradiated at tubule stages V–VII does not differ from the general response of all type A spermatogonia. Thus the existence of differences of sensitivity is indicated even for the mitotically inactive "dormant" type A spermatogonia.

PATHOLOGY AND PHYSIOLOGY

PATHOLOGIC AND PHYSIOLOGIC EFFECTS OF RADIATION

A. C. Upton, Section Chief

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W. H. Benedict[1]	F. G. Tausche
K. W. Christenberry[1]	B. Anderson
G. S. Melville[2]	P. Ledford
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Radiation-induced Myeloid Leukemia

F. F. Wolff P. Ledford
W. D. Gude

The induction of myelogenous leukemia in mice of the RF strain by ionizing radiation has been reported previously.[5] Because of the resemblance of this disease to radiation-induced myelogenous leukemia in man and because of the paucity of experimental data concerning its pathogenesis, an extensive investigation of this condition, begun in collaboration with J. Furth, is in progress.

Mice of the RF strain are being exposed to whole-body X radiation at 5–6 weeks of age; the radiation factors are as follows: 250 kvp, 30 ma, TSD 97.3 cm, rate 70–80 r/min in air with scatter, filtration 3 mm of Al (hvl 0.55 mm of Cu). Doses were administered in a single exposure unless otherwise indicated. In order to study the role of certain physiologic factors in leukemia induction, various organs were removed one week before irradiation. Because of the large numbers of mice required for the experiment, the individual treatment groups are being filled sequentially, littermates divided among the various groups concomitantly. The mice are being observed throughout life under standard laboratory conditions and submitted to postmortem examination promptly after death. As reported earlier, there is a marked increase in the incidence of myeloid leukemia after a single exposure to only 150 r, the death rate from this disease being maximal in mice 8–15 months of age (Table 41). As the dose is elevated from 150 to 300 r, the incidence rises and the onset is hastened; however, with further elevation of the dose to 450 r, the frequency declines. Because it was thought that the decreased incidence of myeloid leukemia accompanying the increase in dose from 300 to 450 r might result from the intercurrent death of mice with thymic lymphomas, a group of mice were thymectomized preirradiation; although as yet inconclusive, the preliminary data suggest that thymectomy has not significantly affected the incidence of myeloid leukemia; it is noteworthy, however, that in the irradiated, thymectomized mice, an increased frequency of nonthymic lymphoid tumors has been observed within the first 12 months of life. With fractionation of 450 r into three exposures of 150 r each, with a five-day interval between treatments, there appears to have been an enhanced induction of thymic lymphoma, as noted by Kaplan;[6] by contrast, the incidence of myeloid leukemia appears to have been reduced as a result of dividing the dose. Castration has resulted in a leukemia response in the male more like that of the female, i.e., a greater number of thymic lymphomas and fewer myeloid leukemias; similarly, gonadectomy of the female has brought about a leukemia-induction pattern more like that of the male; however, both the male and the female retain to some extent after gonadectomy the disease response characteristic of their sex, suggesting the existence of sex-specific leukemia-modifying factors operative in the absence of the gonads.

Mechanism of Radiation Recovery by Marrow Transfusion – Evidence of Repopulation

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It has been demonstrated that recovery from lethal doses of X radiation is promoted by the injection of unirradiated bone marrow cells, but the mode of action of the bone marrow is not presently known. It has been postulated on one

[1]Consultant.

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[4]Joined the section in July 1955.

[5]A. C. Upton, J. Furth, and K. W. Christenberry, *Cancer Research* 14, 682–690 (1954).

[6]H. S. Kaplan and M. B. Brown, *J. Natl. Cancer Inst.* 13, 185–208 (1952).

[7]*Drosophila* Genetics Group.

TABLE 41. INCIDENCE OF LEUKEMIA IN RF MICE GIVEN WHOLE-BODY X IRRADIATION AT 5–6 WEEKS OF AGE

X-Ray Dose (r)	Operation	Total Mice/Total Dead, and Sex	Number Dead of Leukemia in Indicated Period (months)								
			Myeloid Leukemia			Thymic Lymphoma			Other Leukemias		
			0–7	8–14	15–22	0–7	8–14	15–22	0–7	8–14	15–22
0		78/19 ♂	0	0	0	0	0	1	0	1	10
150		104/52 ♂	1	16	4	3	3	1	0	2	12
300		95/65 ♂	8	25	6	6	7	2	0	2	3
450		86/62 ♂	4	13	3	10	10	1	0	5	3
450	Thymectomy	101/44 ♂	5	14	0	1	1	0	2	10	1
450*		57/24 ♂	2	3	0	6	8	0	0	0	0
450**		93/42 ♂	3	3	0	20	7	0	1	1	0
300	Castration	113/85 ♂	0	23	6	12	14	3	0	3	3
0	Castration	101/32 ♂	0	0	0	2	2	6	0	0	7
0		99/74 ♀	0	1	2	0	4	12	0	2	21
0	Castration	121/15 ♀	0	0	0	1	3	0	1	2	0
300		94/84 ♀	1	8	1	20	20	5	0	1	5
300	Castration	90/44 ♀	0	6	1	16	8	0	1	5	0

Protocol: 250-kvp X rays; 30 ma; TSD, 97.3 cm; rate, 70–80 r/min in air with scatter; filtration, 3 mm of Al (hvl, 0.55 mm of Cu).

*Three exposures of 150 r each at intervals of two days.

**Three exposures of 150 r each at intervals of five days.

hand that the introduced marrow cells proliferate and are responsible for recovery by cell repopulation and on the other hand that they elaborate some humoral substance which elicits a recovery response of the indigenous marrow; a tiding-over of the host by the injected cells until the irradiated marrow can recover has also been suggested. It is plausible, of course, that a combination of these actions may be responsible for recovery. Information in the literature concerning the transplantation of tumor tissue after irradiation and/or cortisone administration, and the known inhibiting effect of irradiation on the immune reaction make it seem likely that the introduced marrow might repopulate the acellular marrow space of the irradiated animals. Because the use of a strain of rats having a pair of known red cell antigens seemed an ideal tool for testing the repopulation hypothesis, the following experiments were designed to carry out this test.

The rats used have the cellular antigens C and D, which are controlled by a single pair of alleles; erythrocytes of the two homozygotes, C/C and D/D, carry antigens C and D, respectively, while those of the heterozygote, C/D, carry both antigens C and D. It is possible, therefore, to

detect cells of either type C or type D in a mixture of both by the specific agglutination of each with its homologous antiserum. (Animals segregating for these genes, as well as agglutination reagents specific against each antigen, were kindly supplied by R. D. Owen of the California Institute of Technology.) Rats bearing C or D antigen were irradiated and subsequently injected intravenously with bone marrow cells, in the initial experiments with type CD bone marrow (since it alone was available) and in later experiments with marrow from rats of the opposite (D or C) blood type (Table 42). At various intervals after injection the red blood cells of the recipients were typed serologically; when agglutination was not observed with the unaided eye, samples of the resuspended mixture were examined microscopically in a hemocytometer. Two classes of controls were used – one was an irradiation control in which rats were subjected to the same dose of X rays as the experimentals and injected with saline only; the other was an implantation control in which unirradiated rats were injected with bone marrow (Table 42).

The results of the experiments are presented in Table 43. By the end of the second week nearly all the irradiated animals surviving had erythrocytes of the implanted type in their peripheral blood; however, the injected but unirradiated animals showed no sign of the implanted cells at this time or any subsequent time. The presence of cells of the implanted type was often recognized first only upon microscopic examination of the agglutination test mixture, later becoming apparent upon visual observation of the reaction tube. At the present time, one mosaic animal from experiment 2 is living 90 days after implantation, and 14 mosaic animals from the third experiment 35 days after implantation without any evidence of regression of the implant. Preliminary quantitative tests on several animals indicate that cells of the implanted type make up from 10–90% of the circulating red cells of the recipients.

The results are interpreted to indicate a repopulation of the bone marrow of irradiated recipient animals with marrow from the donors. (The phenomenon of "transformation" found in bacteria is thought to be a very unlikely explanation for the observed genetic change in the red blood cell population.) These experiments do not prove that implantation of the marrow is the only, or even the most important, mechanism involved in the recovery from radiation injury. Furthermore, it should also be noted that it can as yet only be inferred that the myeloid elements repopulate, which is important since several lines of investigation now tend to implicate the myeloid elements of the blood and bone marrow as recovery agents.

TABLE 42. TREATMENT SCHEDULE OF RATS INJECTED WITH ANTIGEN-LABELED MARROW

Experiment No.	Number of Rats	Dose (r)	Type of: Host	Type of: Implant	Time of Marrow Injection Postirradiation (hr)
1 a	10	900	C or D	CD	0–3
b	10	900	CD		
c	10	0	C or D	CD	0–3
2 a	11	750	C or D	CD	18–24
b	7	750	CD		
3 a	23	750	C or D	D or C	18–24
b	11	750	CD		
c	7	0	C or D	D or C	18–24

TABLE 43. RESULTS OF BONE MARROW ADMINISTRATION FOLLOWING TOTAL BODY IRRADIATION

Experiment No.	Treatment	Time After Irradiation (days)*											
		0	6	8	10	13	14	18	20	22	28	35	46
1 a	Experimental	–/10	0/5	–/3	–/3		1/1	1/1	–/0				
b	X-ray control	–/10	–/8	–/5									
c	Implant control	–/10	–/10	–/10	–/10	0/10							
2 a	Experimental	–/11	–/11	0/4	–/4		3/3	3/3	3/3	2/2	2/2	2/2	1/1**
b	X-ray control	–/7	–/7	–/6	–/6		–/5	–/5	–/5	–/4	–/4	–/3	–/3
3 a	Experimental	–/23	–/23	–/21	2/19		13/18	–/18	14/16	–/16	14/16	14/16	
b	X-ray control	–/11	–/11	–/10	–/8		–/5	–/5	–/5	–/4	–/4	–/4	
c	Implant control	–/7	–/7	–/7	0/7		0/7	–/7	0/7	–/7	0/7	0/7	

*Number of animals showing erythrocytes in peripheral blood of implanted antigenic type/number of surviving animals. On days when no blood typing was done, no numerator is given.

**This rat continues positive at 91 days. (When typed quantitatively at this time, only about 10% of its circulating red cells were of the animal's original genetic type.)

Effects of Radiation on Megakaryocytes and Platelets – Uptake of S^{35}-labeled Sulfate

T. T. Odell, Jr. F. G. Tausche

From studies of the uptake of $S^{35}O_4$ by megakaryocytes and platelets following whole-body irradiation,[8] it is apparent that the total radioactivity of blood platelets from rats injected with S^{35}-labeled sulfate during recovery from an $LD_{50}/30$ days dose of X rays (750 r) is much higher than the activity of platelets from nonirradiated controls (all sulfate injections were made approximately 24 hr prior to sacrifice of the rats by exsanguination). The platelet activity has been observed to be highest 14 days postirradiation, the first time during the recovery phase when sufficient platelets are obtainable for activity measurements; from a value several times normal it then declines to less than twice the control value at 24 days (Table 44). Since the platelets are believed to be labeled, at least to a major extent, during their production in the bone marrow, the increased radioactivity is probably associated with the younger composition of the platelet population during its buildup in the recovery period. The proportion of new platelets in the circulating population presumably decreases as the platelet count returns to normal.

[8] T. T. Odell, Jr., W. D. Gude, and F. G. Tausche, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 76–78.

TABLE 44. EFFECTS OF IRRADIATION ON THE UPTAKE OF S^{35}-LABELED SULFATE BY PLATELETS

Day Postirradiation[a]	Radioactivity of Platelets (counts/min × 10^7)	Number of Rats
14	57.9	1[c]
16	40.1 ± 7.65[b]	2[c]
18	26.7 ± 3.03	5
20	31.2 ± 4.80	2
22	22.4 ± 4.61	5
24	16.8 ± 1.61	4
Control	11.0 ± 1.53	11

[a] Rats received 750 r in a single exposure and were injected with 300 μc of $Na_2S^{35}O_4$ 24 hr before sacrifice.

[b] Standard deviation, ±1.

[c] Platelets from two animals were combined in order to obtain a sufficient number for an activity measurement.

In addition to study of the uptake of $S^{35}O_4$ by the platelets, observations were made on the number of megakaryocytes in the bone marrow and on the peripheral platelet counts during the first 24 days postirradiation (Fig. 23). The number of megakaryocytes begins to drop three days after irradiation and decreases almost to zero at six days; at 12 days, the cells appear to be returning,

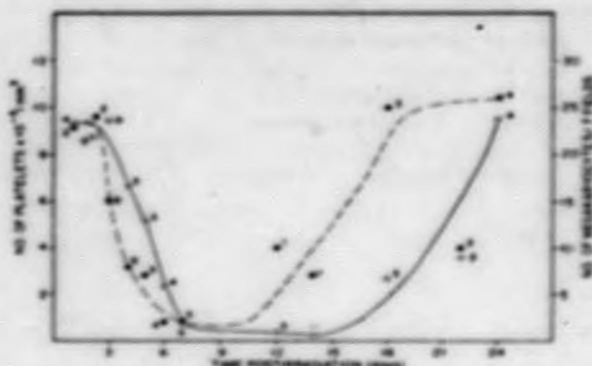

Fig. 23. Numbers of Megakaryocytes (---●) in the Bone Marrow and Platelets (——O) Circulating in the Blood in Relation to Time Postirradiation. Numerals beside points show numbers of animals used.

and at 18 days, they are back to normal numbers. The peripheral platelet count, taken just before sacrifice, remains normal for the first three days and then begins to drop rapidly; at seven days it is less than 50,000 platelets/mm^3. It begins to rise again in the survivors between 14 and 18 days, and at 24 days is back in the normal range. The numerical counts of megakaryocytes and platelets show that the platelet drop lags about one day behind the megakaryocyte decrease, and the megakaryocyte rise precedes the platelet increase by five or six days.

Transplanted Chloromyeloid Leukemia in Rats

F. F. Wolff

A chloromyeloid leukemia, encountered originally at Mound Laboratory in an actinium-injected Sprague-Dawley rat,[9] has been transferred in serial passage through four transplant generations in this laboratory. The disease has been transmitted by intraperitoneal, intravenous, or subcutaneous injection of whole blood or of leukemic leukocytes obtained from sites of leukemic infiltration; the latter appear to be more effective than whole blood, presumably because of the greater number of leukemic cells present. Thus far, with a single inoculation of 0.1–0.5 ml of leukemic cell suspension, it has not been possible to secure takes in all adult hosts; however, newborn recipients have developed the disease in nearly every instance, dying 4–8 weeks after injection. Viewed grossly, the leukemic infiltrations are green; microscopically, they are seen to consist of myelocytes and promyelocytes. Infiltration of the liver, spleen, lymphatics, and bone marrow has been noted terminally, but splenomegaly has not been encountered, even in rats inoculated intravenously. The peripheral blood has been observed to contain variable numbers of immature myeloid cells and to exhibit varying degrees of leukocytosis in the final stages of the disease.

Attempts are being made to develop a method whereby transmission of this leukemia can be effected uniformly in adult hosts, in the hope that this disease will then furnish a useful tool for the study of certain aspects of myeloid leukemia.

Binding of S^{35}-labeled Sulfate in Rat Serum

L. H. Smith
B. Anderson T. T. Odell, Jr.

It has been shown with the use of filter paper electrophoresis that a major fraction of injected S^{35}-labeled sulfate becomes bound to a constituent of rat serum which migrates to the α_1-globulin region;[4] furthermore, this region stains intensely with the PAS staining routine, suggesting that the constituent in question is associated with a carbohydrate. Additional experiments have been conducted to extend knowledge of this substance.

Results from the analysis of nine serum samples confirm those previously reported[10] (Table 45). Sixty per cent of the whole serum activity was recovered on the strip, 40% of it concentrated in a peak.[11] The remainder of the S^{35} activity was distributed over the rest of the area occupied by the serum proteins.

In one experiment (strip 159, Table 45) the serum was dialyzed in the cold (2°C) against three portions of the barbiturate buffer (pH 8.6) during the 24 hr prior to electrophoresis. The failure of this procedure to alter the pattern of distribution or the magnitude of the sulfate activity

[9] We are indebted to D. M. Anthony and H. Miller for kindly providing the leukemic rats, and for assistance in initial transplantations.

[10] L. H. Smith and B. Anderson, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 66–68.

[11] The S^{35} activity peak includes the activity present between the maximum point of the albumin curve and the low point anodal to the α_2-globulin region.

TABLE 45. DISTRIBUTION OF S^{35} ACTIVITY AFTER ELECTROPHORESIS ON FILTER PAPER OF SERUM FROM NORMAL RATS INJECTED INTRAVENOUSLY WITH $Na_2S^{35}O_4$

Animal No.	Strip No.	Time Postinjection (hr)	Whole Serum [(counts/min)/25 μl]	Activity* (counts/min)		Percentage Total Counts Recovered		Percentage of Recovered Counts Found in Peak
				Whole Strip	Peak	On Strip	In Peak	
640	91	24	3987	2134	1382	53.5	34.7	64.8
	106	24	3987	2370	1599	59.4	40.1	67.5
	161	24	3987	2270	1510	56.9	37.9	66.5
	159**	24	3987	2426	1596	60.8	40.0	65.8
641	92	24	4468	2384	1671	53.4	37.4	70.1
	100	24	4468	2925	2111	65.5	47.2	72.2
654	143	48	2581	1451	1006	56.2	39.0	69.3
655	144	48	2110	1345	914	63.7	43.3	68.0
645	118	48	1910	1359	844	71.2	44.2	62.1
				Mean:		60.0 ± 1.8	40.5 ± 1.3	67.6 ± 1.0

*Corrected for self-absorption.

**This sample was dialyzed for 24 hr in cold against three changes of barbiturate buffer prior to electrophoresis.

on the strip is evidence that free sulfate ions were not responsible for the activity peak. It was also noted that the S^{35} activity distribution in the serum was qualitatively the same 24 and 48 hr postinjection.

Whereas a salt linkage between a protein and the sulfate ion could explain the present observations, results of the following experiments do not support such an hypothesis. After dialysis of S^{35} sulfate-labeled plasma against a buffer of pH 12.0, 38% of the whole activity remained in the plasma, essentially the same amount (40%) as was found in the α_1-globulin region following electrophoresis of undialyzed plasma at pH 8.6. At pH 12.0, few if any positively charged groups of a protein would be available for anion binding through a salt linkage. Even at pH 8.6, the number of positively charged groups of a protein would be small.

There are three forms in which S^{35} may be found in the serum: as –S–S or –SH groups of amino acids, as the sulfate ion bound to a protein through a salt linkage, and as sulfuric acid esters. It is known that sulfur administered to rats in the form of sulfate becomes incorporated into amino acids in negligible amounts.[12,13] It seems, therefore, that the S^{35} activity peak reflects the presence of a sulfuric acid ester. Winzler[14] described the isolation from plasma of a mucoprotein. This mucoprotein has the electrophoretic mobility of an α_1-globulin at pH 8.4,[15] and contains approximately 30% carbohydrate.[16] In

[12] H. Boström and E. Jorpes, *Experientia* 10, 392–396 (1954).

[13] D. C. Dziewiatkowski, *J. Biol. Chem.* 207, 181–186 (1954).

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

[15] J. W. Mehl, J. Humphrey, and R. J. Winzler, *Proc. Soc. Exptl. Biol. Med.* 72, 106–109 (1949).

[16] H. E. Weimer, J. W. Mehl, and R. J. Winzler, *J. Biol. Chem.* 185, 561–568 (1950).

the present series of studies it was observed that the S^{35} sulfate activity peak corresponded to an area on the strip which contained PAS positive constituents. Since the S^{35} sulfate activity peak and the PAS positive region appear to coincide electrophoretically with Winzler's mucoprotein (Mehl *et al.*[15]), it is suggested that the carbohydrate moiety of this mucoprotein is esterified with sulfuric acid. A similar interpretation was previously suggested by Mehl *et al.*[15] to account for the finding that sulfur in excess of that required by methionine and cystine was present in a mucoprotein of rat plasma.

Effects of X Radiation on the Distribution of $Na_2S^{35}O_4$ in Rat Plasma

L. H. Smith
B. Anderson T. T. Odell, Jr.

It was reported[6] that the level of labeled sulfate in whole plasma was usually much lower in rats on the third day following whole-body exposure to 750 r of X radiation than in the plasma of unirradiated controls. In order to investigate this difference, the plasma of irradiated rats was subjected to electrophoresis on filter paper, and S^{35} activity determinations were made on segments of the filter paper as previously described. The pattern of S^{35} distribution and the magnitude of the activity on the paper strips were essentially the same for the irradiated animals as found for plasma of unirradiated controls. Accordingly, the difference between the whole plasma levels of S^{35} in X-rayed and control rats cannot be accounted for by changes in the S^{35} fraction which is bound to a plasma constituent having the electrophoretic mobility of an α_1-globulin.

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

F. F. Wolff

In an attempt to investigate the factors influencing the growth of leukemic cells and the resistance of the leukemic host, the effect of hypothyroidism on the survival of rats bearing a transplanted lymphoid leukemia has been examined.

Inbred male and female rats of a Wistar subline were divided into two experimental groups at six weeks of age; one group was given propylthiouracil in the drinking water (0.025%), and the other group received tap water. The animals were caged individually, weighed regularly, and pair-fed to ensure uniform body weight throughout both groups. Approximately nine weeks after beginning the administration of propylthiouracil, all rats were injected intraperitoneally with 0.1 ml of a suspension of isologous lymphoid leukemia cells in physiological saline solution (the suspension was prepared by mincing leukemic lymph nodes in saline and aspirating the cells through a cotton filter). On the day after inoculation and every three days thereafter, half the rats in each group received a subcutaneous injection of thyroxine (crystals, Squibb), 1.0 mg in each of the first two injections, and 0.5 mg/dose subsequently.

The survival patterns are summarized in Table 46. The propylthiouracil-treated rats survived longer than the controls ($p, 0.5\%$); this was not observed, however, in the females receiving thyroxine concomitantly. The differences were more marked in females than in males.

The results of this experiment tend to confirm those observed in radiothyroidectomized AKR mice[17] and further strengthen the hypothesis that the survival of lymphoid leukemia-bearing hosts is enhanced by depression of thyroid function.

Effects of Radiation on the Plasma Iron Concentration in Intact and Splenectomized Rats

G. S. Melville, Jr. F. P. Conte

Elevation of serum iron concentrations in rats[18] and of plasma iron levels in burros[19] and sheep[19] has been observed following acute irradiation in the lethal range. The effect of removal of the spleen on plasma iron levels in rats has also been noted.[20] The following experiments were undertaken to determine the response of plasma iron concentrations in intact and splenectomized rats after exposure to X-ray doses in the sublethal range.

Groups of male, albino, 250-g, Sprague-Dawley rats were exposed to a single whole-body dose

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[17] D. M. Morris and F. F. Wolff, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 65–66.

[18] A. Chanutin and S. Ludewig, *Am. J. Physiol.* **166**, 380–383 (1951).

[19] G. S. Melville, Jr., and B. F. Trum, unpublished data.

[20] A. Chanutin, E. A. Lentz, and S. Ludewig, *Am. J. Physiol.* **173**, 474–480 (1953).

TABLE 46. EFFECT OF HYPOTHYROIDISM ON THE SURVIVAL OF RATS BEARING A TRANSPLANTED LYMPHOID LEUKEMIA

Animal No.	Treatment	Survival (days)	Mean Survival (days)
2156 ♂	Propylthiouracil	29	25.7
2157 ♂		30	
2158 ♂		18	
2159 ♀	Propylthiouracil	22	25.3
2160 ♀		26	
2161 ♀		28	
2162 ♂	Propylthiouracil + thyroid	25	24.5
2164 ♂		24	
2165 ♀	Propylthiouracil + thyroid	14	18.3
2166 ♀		14	
2167 ♀		27	
2168 ♂	Controls	17	18.7
2169 ♂		24	
2170 ♂		15	
2171 ♀	Controls	16	17.3
2172 ♀		15	
2173 ♀		21	
2174 ♂	Controls + thyroid	15	18.0
2175 ♂		24	
2176 ♂		15	
2177 ♀	Controls + thyroid	13	13.7
2178 ♀		13	
2179 ♀		15	

of 75, 150, or 225 r of 250-kvp X radiation. Iron determinations were performed by a microspectrophotometric method adapted from the analytical procedure of Ramsay.[21] Blood samples were obtained from the aorta of anesthetized rats, three experimental animals and three controls being sacrificed to obtain each point, and duplicate analyses were performed on each plasma sample. In the splenectomized group, the splenectomy was carried out four weeks before irradiation.

Following exposure to 75–225 r, there was elevation of the plasma iron level (Fig. 24). The differences in iron concentration between irradiated rats and concurrently sampled controls (Table 47) were subjected to an analysis of variance[22] and found to be significant at the 5–10% level. The hyperferremia occurred in two phases (Fig. 25), the first during the initial 24 hr postirradiation and the second 8–15 days after exposure, simulating the pattern noted in acutely irradiated burros and sheep.[15] Splenectomy appeared to curtail the early elevation of the plasma iron produced by exposure to 75 r (Fig. 26).

[21] W. N. M. Ramsay, *Biochem. J.* 57, xvii (1954).

[22] Statistical treatment of the data was performed by A. W. Kimball and G. J. Atta, Mathematics Panel.

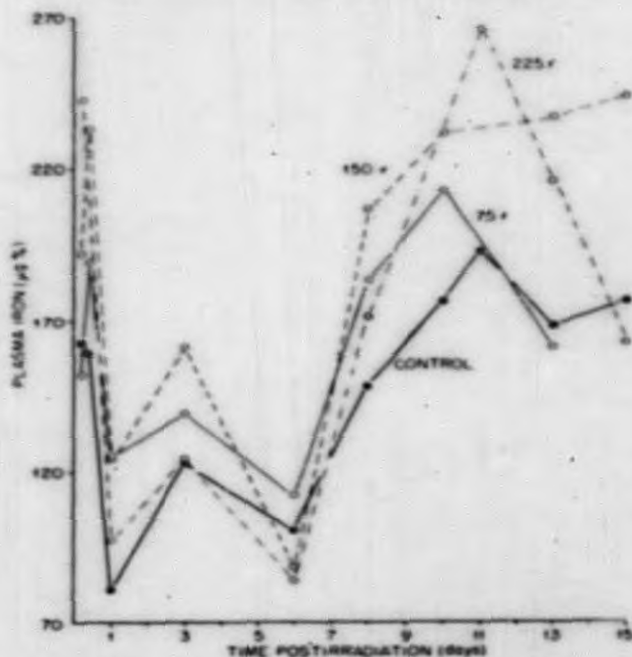


Fig. 24. Plasma Iron Concentrations in Irradiated Rats. Each point represents the mean iron concentration of three animals, sacrificed concomitantly.

TABLE 47. COMPARISON OF THE PLASMA IRON CONCENTRATION IN IRRADIATED RATS WITH THAT IN CONCURRENT NONIRRADIATED CONTROLS

Time Postirradiation	X-Ray Dose, Whole-Body (r)		
	75*	150*	225*
5 hr	37.2 ± 27	29.0 ± 22	80.2 ± 22
10 hr	75.7 ± 27	73.9 ± 22	
24 hr	45.0 ± 22	43.2 ± 22	16.5 ± 22
3 days	−1.2 ± 22	20.0 ± 22	21.0 ± 22
6 days	11.7 ± 22	−12.7 ± 22	−16.7 ± 22
8 days	39.0 ± 16	72.2 ± 17	7.2 ± 16
10 days	36.7 ± 22	55.8 ± 22	73.1 ± 22
13 days	13.4 ± 22	89.4 ± 22	26.9 ± 22
15 days		60.7 ± 22	−8.3 ± 22

*Mean difference in plasma iron concentration (in µg%) between irradiated rats and concurrent controls.

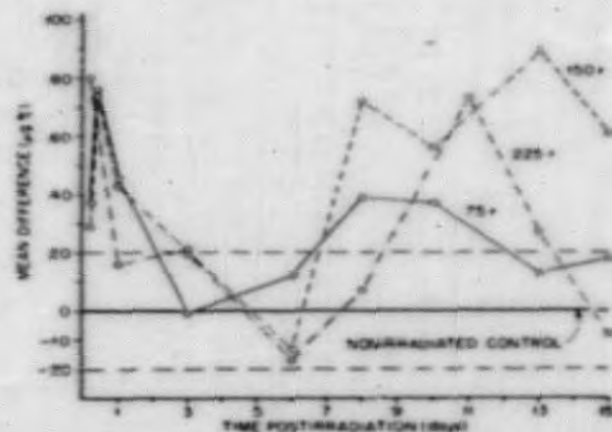


Fig. 25. Net Differences in Plasma Iron Concentration Between Irradiated Rats and Their Concurrent Nonirradiated Controls.

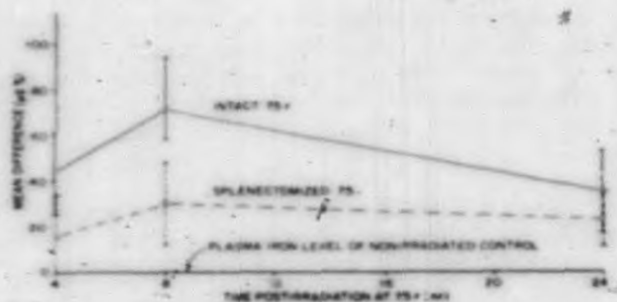


Fig. 26. Effects of Splenectomy on the Response of the Plasma Iron in Irradiated Rats. The irradiated rats, intact or splenectomized, are compared with their respective intact or splenectomized sham-irradiated controls.

The mechanism of these alterations is under study; plasma iron changes are being correlated with reticulocyte counts and bone marrow cytology. The significance of these observations in relation to radiation-induced depression of erythropoiesis, as measured by Fe^{59} uptake in the red blood cells, is being explored.

MICROBIOLOGY

TRACER STUDIES IN INTERMEDIARY METABOLISM

S. F. Carson E. F. Phares
M. I. Dolin M. V. Long

Succinic Acid Decarboxylase System

S. F. Carson E. F. Phares

A previous report[1] on succinic acid decarboxylase studies described an enzyme system, from combined cell extracts of *Propionibacterium pentosaceum* and *Veillonella gazogenes*, which fixed a C_1 fragment (from the decarboxylation of succinyl-CoA) into malate. This transfer was dependent on the presence of DPNH (reduced diphosphopyridine nucleotide) and pyruvate. It was proposed that the C_1 fragment was combining with pyruvate to form OAA (oxalacetate) by a unique carboxylation mechanism, and that this product (OAA) was converted to malate by the malic dehydrogenase system.

[1]S. F. Carson and E. F. Phares, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 84–88.

Additional support for this hypothesis was obtained from tracer experiments employing succinyl-1-2-3-4-C^{14}-CoA as a substrate, performed in the presence of malonate to inhibit the direct conversion of succinyl-CoA to malate through fumarate. The data presented in Table 48 demonstrate the inhibition of CO_2 (but not propionate) production by addition of pyruvate and DPNH, and the accompanying increased labeling of the malate carboxyl. Only negligible amounts of labeled lactate were formed.

In addition, it has been observed that catalytic amounts of malate will replace the requirement for succinyl-CoA during DPNH oxidation with this system. Figure 27 presents spectrophotometric

TABLE 48. EFFECT OF PYRUVATE PLUS DPNH ON LABELING OF MALATE AND CO_2

Compounds Isolated / Tracer →	Control		Pyruvate and DPNH Added		
	Succinate-C^{14}		Succinate-C^{14}		$C^{14}O_2$
	Radioactivity (counts/sec)	Ratio – Outside : Inside	Radioactivity (counts/sec)	Ratio – Outside : Inside	Radioactivity (counts/sec)
Succinate	2800	1.9	2800	1.9	2.8
Propionate	75	1.9	73	1.9	0.2
Malate	2	1.8	16	15.8	13.0
CO_2	46		34		7000

Protocol: Materials used (amounts in micromoles unless otherwise stated): 50, potassium phosphate (pH, 6.5); 0.32, cysteine HCl; 150, sodium malonate (pH, 7.0); 3, DPNH; 3, potassium pyruvate; 0.75, succinyl-CoA; 10, potassium succinate (in presence of $C^{14}O_2$); 10, potassium succinate-1,2-C^{14} (2800 counts/sec, C-1 : C-2 ratio of 1.84 : 1, used in absence of $C^{14}O_2$); 1, $BaC^{14}O_3$ (7000 counts/sec; converted to $C^{14}O_2$ by addition of phosphoric acid from Siamese sidearm); cell-free extracts (centrifuged at 100,000 × *g* for 1 hr) representing 1 mg of protein from each organism, *P. pentosaceum* and *V. gazogenes*; total fluid volume, 0.65 ml.

Gas phase, 5% CO_2 in helium, freed from O_2 by treatment with chromous sulfate. Incubated in Warburg vessels (5 ml total volume) for 15 min at 30°C. Acids isolated and separated at end of experiment by partition chromatography,[2] and degraded to determine ratios of outside to inside carbons (C-1 : C-2).[3–5]

[2]E. F. Phares, E. H. Mosbach, F. W. Denison, Jr., and S. F. Carson, *Anal. Chem.* 24, 660–662 (1952).
[3]J. W. Foster and S. F. Carson, *Proc. Natl. Acad. Sci. U.S.* 36, 219–229 (1950).
[4]E. F. Phares and M. V. Long, *J. Am. Chem. Soc.* 77, 2556–2557 (1954).
[5]E. F. Phares, *Arch. Biochem. and Biophys.* 33, 173–178 (1951).

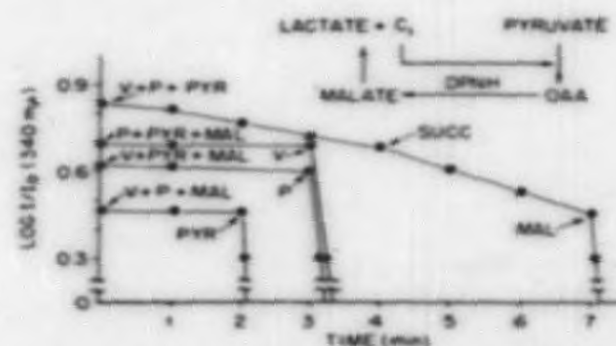


Fig. 27. Dependence of DPNH Oxidation on Malate, Pyruvate, and Cell-free Extracts of *Propionibacterium* and *Veillonella*. Each microcuvette contained 20 μmoles of potassium phosphate (pH 7.0) and about 0.03 μmole of DPNH. In addition, as indicated, cell-free extracts (centrifuged at 100,000 × g for 1 hr) of *P. pentosaceum* (P) and *V. gazogenes* (V), representing 0.02 mg of protein; 1 μmole of potassium malate (Mal), 1 μmole of potassium pyruvate (Pyr), and 1 μmole of potassium succinate (Succ). Final volume, 0.25 ml; 24°C.

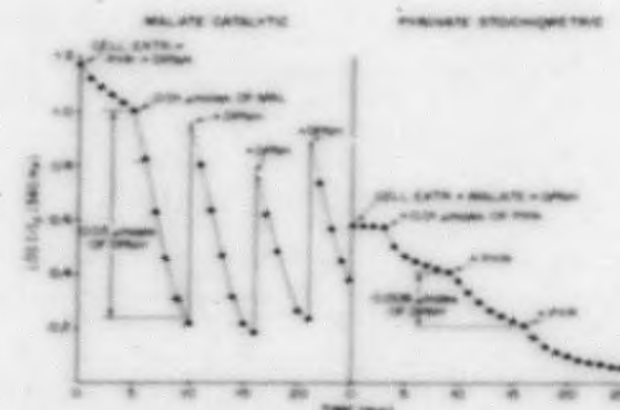


Fig. 28. Requirements for Catalytic Amounts of Malate and Stoichiometric Amounts of Pyruvate During DPNH Oxidation by Cell-free Extracts of *Propionibacterium* and *Veillonella*. Conditions and additions are the same as for Fig. 27, except where indicated.

data showing the dependence of DPNH oxidation on each of the components of the system: malate, pyruvate, *Propionibacterium* and *Veillonella* cell-free extracts. Figure 28 demonstrates the catalytic nature of the dependency for malate, and the stoichiometric dependency for pyruvate.

Tracer experiments with the same system (Table 49) show that pyruvate-2-C^{14} is converted to lactate in the presence of catalytic amounts of malate and substrate quantities of DPNH; no lactate is formed in the absence of malate; trace amounts of labeled OAA are found in both cases. With substrate amounts of malate, DPNH oxidation is faster (measured by spectrophotometry) and lactate is formed from labeled pyruvate; but the recovered malate has very low specific activity. Without DPNH, some labeled lactate and larger amounts of unlabeled OAA (than in the presence of DPNH) are produced.

The spectrophotometric and tracer experiments can probably be interpreted most simply as indicating that with DPNH present, pyruvate is converted to lactate on a pathway through OAA and malate; catalytic amounts of the C_1 fragment from malate decarboxylation furnish the (carboxyl) carbon required to initiate the cyclic process.

Further experiments in which the cell-free extracts were used with added purified condensing enzyme and acetyl-CoA demonstrate that incorporation of pyruvate-2-C^{14} into citrate is dependent on the presence of malate (Table 50). Pyruvate incorporation into citrate depends on the intermediate formation of OAA; malate is the donor of the carbon fragment, which is necessary for the conversion of pyruvate to OAA. Carbon dioxide cannot act as the carbon donor. The proof of the existence of the pyruvate to OAA step (and its requirement for malate) establishes the link for the malate-dependent conversion of pyruvate to lactate through OAA.

Figure 29 summarizes reactions involving the C_1 fragment derived from succinyl-CoA and malate decarboxylations. The operation is clockwise, for the C_1 cycle or portions thereof, from pyruvate when a C_1 source, succinyl-CoA or malate, is present together with DPNH or condensing enzyme. The DPNH probably drives OAA reduction, rather than pyruvate reduction, because of the limiting amounts of lactic dehydrogenase compared with the overwhelming amounts of malic dehydrogenase in the *Propionibacterium* extract. The condensing enzyme pulls the reactions in the same direction because the equilibrium strongly favors citrate

TABLE 49. EFFECT OF DPNH AND MALATE ON LACTATE FORMATION FROM PYRUVATE-C^{14}

Compound Isolated	Additions: DPNH → Malate → Time →	None 3 μmoles 20 min			3 μmoles 3 μmoles 6 min			4 μmoles 0.05 μmole 20 min			4 μmoles None 20 min		
		cts/sec	μmoles	(cts/sec) per μmole	cts/sec	μmoles	(cts/sec) per μmole	cts/sec	μmoles	(cts/sec) per μmole	cts/sec	μmoles	(cts/sec) per μmole
Pyruvate		34	1.5	22.7	17	0.8	21	25	1	25	82	2.5	33
Malate		12.0	2.4	5.0	2	1.2	1.7		Negligible			Negligible	
OAA		2	0.5	4	4	0.1	40	14	0.25	55	10	0.25	60
Lactate		24	0.8	30	60	2.0	30	90	2.2	41		None	

Samples were incubated in microcuvettes containing 20 μmoles of potassium phosphate (pH 7.0), 0.01 ml of centrifuged (100,000 × g for 1 hr) and dialyzed [illegible] and [illegible] cell-free extracts (containing 0.2 mg of protein of each extract), 3 μmoles of pyruvate-2-C^{14} [60 (cts/sec)/μmole], and malate and DPNH as indicated; final volume, 0.3 ml. Subsequent to times indicated, 0.08 ml of concentrated HCl with excess 2,4-dinitrophenylhydrazine (1–1.25 mg) were added, and the mixture allowed to stand for 1 hr. The acids were separated as in Table 48. Carbon-14 assays were made on aliquots plated (infinitely thin layers) on watch glasses.

TABLE 50. EFFECT OF MALATE ON PYRUVATE INCORPORATION INTO CITRATE

Additions	C^{14} Incorporation (counts/sec)	Pyruvate Incorporation ([illegible])
Pyruvate-2-C^{14} + CO_2	2.5	1.5
Pyruvate-2-C^{14} + CO_2 + malate	18.0	10.0
Pyruvate + $C^{14}O_2$ + malate	0.2*	0.1**

*Corrected for differences in original specific activity of CO_2 and pyruvate.

**Based on amounts of $C^{14}O_2$ fixed.

Protocol: Samples were incubated in 5-ml (total volume) Warburg vessels containing 100 μmoles of potassium phosphate (pH 7.4), 2.3 μmoles of acetyl-CoA, 3.0 μmoles of potassium pyruvate or (as indicated) potassium pyruvate-2-C^{14} (180 counts/sec), 3.0 μmoles of potassium malate, 1.5 μmoles of $BaC^{14}O_3$ (10,000 counts/sec; converted to $C^{14}O_2$ by addition of phosphoric acid from Siamese sidearm), centrifuged (100,000 × *g* for 1 hr) and dialyzed *P. pentosaceum* and *V. gazogenes* cell-free extracts (containing 0.5 mg of protein of each extract), and purified condensing enzyme. Final volume, 1.5 ml; gas phase, 5% CO_2 in air; incubated 40 min at 24°C. The acids were isolated and separated, and assayed for C^{14} as in Table 49.

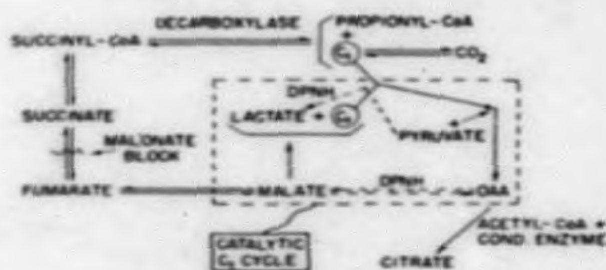


Fig. 29. Reactions Involving "C_1" Derived from Succinyl-CoA and Malate Decarboxylations.

formation. The clockwise reaction with DPNH stops at malate if succinyl-CoA is present, because the malate ⟶ lactate + C_1 step apparently needs "pulling" by removal of the C_1. It appears to be this pulling effect that keeps labeled malate (derived from OAA) from equilibrating with an unlabeled pool. When DPNH and condensing enzyme system are absent, but with malate present, the cycle runs counterclockwise; this is possible because of the coupling of pyruvate reduction with malate oxidation to regenerate DPNH from the catalytic amounts of DPN remaining in the extracts after dialysis.

[6]H. R. Mahler and D. G. Elowe, *J. Biol. Chem.* **210**, 165–179 (1954).

DPNH-Flavoprotein Complex of DPNH Peroxidase

M. I. Dolin

The complex formed between DPNH and DPNH peroxidase flavoprotein, is being investigated further since the system offers a tool for determining one of the mechanisms by which pyridine nucleotides are bound to enzymes. Any successful hypothesis regarding the structure of such a complex must explain the following features of the system: (1) electron transfer between the coenzymes, (2) the blocking of the hydrosulfite reaction by DPN or DPNH, (3) DPN is not bound to the enzyme or, at least, if bound, is not bound at the same site as DPNH (a twentyfold excess of DPN does not inhibit the oxidation of DPNH by peroxide, nor is there any evidence for complex formation between DPN and oxidized flavoprotein), and (4) the acid pH optimum of the reaction.

A structure embodying these characteristics is shown in Fig. 30. It is an adaptation of Mahler's hypothesis[6] regarding the prosthetic group of DPNH cytochrome c reductase, in which an iron-flavin chelate is bound to the protein. In the present system, the unshared electrons on the ternary nitrogen of DPNH are used to form a coordinate covalent link with the metal. On the addition of peroxide to the chelate, DPNH is oxidized to DPN. One of the resonance forms of DPN has a quarternary nitrogen which cannot

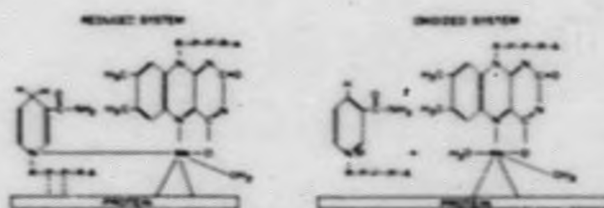


Fig. 30. Proposed Structure for the DPNH-Flavoprotein Complex of DPNH Peroxidase.

therefore, form a covalent linkage. DPN should dissociate from the complex. In such a structure, hydroxyl ions would compete with the ternary nitrogen of DPN for orbitals of the metal; thus a low pH optimum might be expected. This ternary nitrogen should not bind protons in the optimum pH range of the enzyme (5–5.5). Scale models show that there are several ways of orienting the coenzymes by using an octahedral metal structure, without encountering hindrance.

Several features of model reactions lend support to this hypothesis, but there is no definitive evidence for the existence of such a structure in DPNH peroxidase.

There is as yet no conclusive evidence for the presence of a metal in the purified enzyme. Indirect evidence that suggests the presence of a metal constituent is shown in Table 51. Direct spectrographic examination of the purified enzyme indicates that there may be 1 atom of iron present per 2–3 bound flavins; however, owing to the small amount of highly purified enzyme available, exhaustive analysis for trace metals has not been possible.

TABLE 51. EFFECT OF METAL COMPLEXING AGENTS ON DPNH PEROXIDASE

Inhibitor	Molarity	pH	Inhibition (%)
Potassium pyrophosphate	0.05	6.1	37
		7.2	62
Potassium citrate	0.05	6.1	0
	0.09	7.2	46
o-Phenanthroline	3.3×10^{-3}	5.4	18
		7.0	32

DPNH peroxidation was followed in the standard system[7] by using a purified enzyme of specific activity of 10,000 in the presence of the inhibitors as shown. The activities were compared with controls run in 0.05 *M* potassium phosphate at the appropriate pH. At pH 5.4, the buffer was 0.033 *M* sodium acetate.

Further characterization of the complex is being carried out by determining which structural features of the substrate are necessary for the formation of a complex with flavoprotein. It appears that the pyridinium linkage in itself is insufficient to allow complex formation. It may be possible, by pursuing this particular approach, to correlate enzyme activity with spectral properties of the complex and structural properties of the oxidizable substrate.

[7] M. I. Dolin, *Arch. Biochem. and Biophys.* 55, 415–435 (1955).

STUDIES ON NUCLEIC ACIDS, ENZYMES, AND RADIATION-PROTECTIVE COMPOUNDS

W. E. Cohn[1]

L. Astrachan, E. Eavenson, D. G. Doherty, M. H. Jones, J. H. Khym, A. B. Ottinger, R. Shapiro, D. J. Cavanaugh[2], E. Volkin, J. F. Christman[2]

Nucleotide Sequences in Deoxyribonucleic Acid

W. E. Cohn, E. Volkin

In the double helix structure of deoxyribonucleic acid (DNA) postulated by Crick and Watson, pyrimidines in one chain are complemented by purines in the other, specifically cytosine by guanine, and thymine by adenine. Therefore, a sequence of pyrimidines, for example, cytidylic, thymidylic, cytidylic indicates a sequence in the complementary chain of guanylic, adenylic, guanylic. Sequences of pyrimidines may be obtained from DNA by hydrolysis with normal HCl for periods of 30 min or longer and these sequences are isolable on ion-exchange columns. Thus it should be possible to describe the numbers and kinds of pyrimidine sequences obtained from a given DNA, and simultaneously to describe the purine sequences from an analysis of the acid digest. Different DNA's could be compared statistically.

In preliminary experiments, thymus DNA has been degraded by boiling with 1 *N* HCl for 1 hr and 4 hr. These times were chosen because of the plateaus reached in the curve of liberation of inorganic phosphate at these times (33 and 45%, respectively). The resultant digests have been chromatographed and the various fractions analyzed for composition. Although many isomeric tri- and tetranucleotides remain to be separated and characterized, the pattern of elution is sufficiently well defined to be used analytically on DNA's from different sources. There is little evidence for pyrimidine sequences of more than four in length and even this degree of complexity is seldom encountered. On the other hand, polythymidylic acids seem to be more prevalent than polycytidylic acids.

[1]On leave of absence – Guggenheim Fellowship.

[2]Research participant.

Catalytic Reduction of Pyrimidine Nucleotides

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

The study of catalytic reduction of pyrimidine compounds has been extended to include nucleotides and nucleosides of both the ribose and deoxyribose series. All can be reduced, all show the previously described alkali lability in the 1.6 bond (although to varying degrees), and all are subsequently very easily hydrolyzed by dilute acid to yield the corresponding sugar or sugar phosphate. Proof of the postulated mechanism of alkali splitting lies in the isolation and identification of β-ureidopropionic acid from dihydrouridylic acid, after treatment with alkali and acid.

Nucleic Acid Turnover in Cell-free Extracts of *Escherichia coli*

E. Volkin
L. Astrachan, M. E. Jones

In an effort to understand more completely the differences in pathways of nucleic acid synthesis in normal *Escherichia coli* and the phage-infected system, various methods of disrupting the cells to obtain active cell-free extracts were tried. These included alumina grinding, sonic vibration, and Virtess homogenizing, a variety of resuspending media having been used in order to retain some stable, active system. After removal of essentially all the remaining viable cells, the extracts were incubated with inorganic $P^{32}O_4$ in a Dubnoff shaker, and incorporation of the isotope into the phosphorus-containing fractions followed. Although most procedures yielded almost totally inactive extracts with respect to the nucleic acids, one of the methods yielded workable extracts, though the extent of incorporation of P^{32} was discouragingly small. This method involved grinding log-stage cells with alumina and resuspension in Manson's[3] SMA medium, fortified with 0.88 *M* sucrose and 0.01 KCl. The method of fractionation described by Tyner *et al.*[4] in which relatively undegraded nucleic acids were obtained, gave much less spurious results than hydrolytic

[3]L. A. Manson, *J. Bacteriol.* 69, 104–105 (1955).

[4]E. P. Tyner, C. Heidelberger, and G. A. LePage, *Cancer Research* 13, 186–203 (1953).

methods such as described by Schneider.[5] In all such experiments, it was necessary to eliminate a large amount of tightly bound (adsorbed?) radioactivity from the nucleic acids by extended dialysis against phosphate buffer, before the truly incorporated isotope could be assayed.

In addition, proof of incorporation into the RNA was accomplished by ion-exchange separation and assay of the mononucleotides obtained on alkaline hydrolysis. The results of one such experiment with a log-phase *E. coli* extract are seen in Table 52. It is worth noting that the degree of incorporation represents some 500- to 1000-fold more than could be accounted for on assumption of complete turnover of all the phosphorus in those few viable cells remaining in the extract.

Nucleotide Sequences in Bacteriophage Deoxyribonucleic Acid

E. Volkin
L. Astrachan M. H. Jones

Further information has accumulated with respect to the hydroxymethylcytosine (HMC) polynucleotides derived from enzymically degraded phage DNA's. Purified DNA's from bacteriophages T2r+, T4r+, and T6r+ were degraded by deoxyribonuclease and whole snake venom (phosphodiesterase + phosphomonoesterase) and the digests separated by ion exchange, the gradient elution scheme being used. Thus it was possible accurately to compare the elution profiles of all the polynucleotide fractions from these DNA's by direct observation of the superimposed patterns. Such a comparison indicated that the relative amounts of some of these products differ among the phage sources; this, in turn arises probably as a function of nucleotide sequential differences in the intact nucleic acids. Much further work characterizing these products remains; however, it appears that these internucleotidic sequence differences may be a chemical reflection of the genetic differences among these phages.

[5] W. C. Schneider, *J. Biol. Chem.* 164, 747–751 (1946).

Stability of Sugars in the Presence of Alkaline Borate

J. X. Khym D. A. Mandan
L. P. Zill A. B. Ottinger

In the development of the borate method for the separation of sugars by ion exchange[6,7] little attention was given to the possibility that the weakly alkaline (pH ~9.0) sodium or potassium tetraborate eluting solutions, or the borate exchanger, could cause chemical changes in the sugars tested. However, even in weakly alkaline solution, some sugars may undergo radical changes. A series of experiments was therefore arranged to determine the extent of any degradations or transformations of various types of sugars subjected to the borate procedure.

The following sugars were tested: fructose, mannose, galactose, glucose, maltose, melibiose, turanose, sucrose, lactose, ribose, arabinose, and xylose. Each sugar (concentration, 20 mg/100 ml) was allowed to stand at room temperature in

[6] J. X. Khym and L. P. Zill, *J. Am. Chem. Soc.* 74, 2090–2092 (1952).

[7] L. P. Zill, J. X. Khym, and G. M. Cheniae, *J. Am. Chem. Soc.* 75, 1339–1342 (1953).

TABLE 52. TOTAL COUNTS PER SECOND INCORPORATED IN PHOSPHORUS-CONTAINING CELL FRACTIONS

Cell Fraction	Incubation at 36°C (min)				
	0	15	45	90	180
Acid-soluble	900,000	900,000	900,000	900,000	900,000
Lipid	45	1,110	1,545	2,550	2,540
RNA	60	410	450	700	910
DNA			(Too low to be significant)		
Protein	62	428	476	508	755

0.001 *M* and 0.1 *M* $K_2B_4O_7$ for 1, 3, and 5–7 days. At the end of these intervals, the reducing power was checked colorimetrically by comparing the experimental sugar against a standard dissolved in water only and kept in the cold. The borate was then removed and the sugar solutions were analyzed by paper chromatography to determine the extent of any transformation that might have taken place through the Lobry de Bruyn mechanism. The sugars were also allowed to remain in contact with the borate exchanger for 1, 3, and 5–7 days. Three columns were employed (1 cm^2 × 4 cm) and on each column 20 mg of a test sugar was absorbed. At the end of a given interval, the sugars were eluted with 0.1 *M* $Na_2K_4O_7$ and, after the solution was checked for reducing power, the borate was removed and then the sugars were subjected to analysis by paper chromatography, as before.

No measurable changes were observed in any of the 12 sugars which were dissolved in the two concentrations of tetraborate and which had been allowed to stand at room temperature for periods up to seven days. Definite changes were observed in some of the sugars after they had remained in contact with the borate exchanger. Mannose, fructose, and glucose, although showing no loss in reducing power, underwent chemical change as determined by paper chromatography. The extent of the change was slight (<5%) but was detected at the end of the first day with no further noticeable increase occurring up to six days. The three monosaccharides involved apparently underwent 2-epimerization, which allowed the interconversion of all three. Ribose and turanose were the only sugars which showed considerable loss of reducing power after being removed from the borate exchanger. Essentially no change was observed up to three days, but at the end of six days the reducing power of both these sugars was about 60% of their initial values. The alkalinity of the borate exchanger presumably caused maltose and turanose to hydrolyze partially to their constituent monosaccharides. At the end of one day, the extent of hydrolysis was estimated at greater than 10%.

These experiments clearly demonstrate that chemical changes can occur in some sugars if they are allowed to remain in contact with the borate exchanger for prolonged periods of time. The use of smaller columns which allow most analytical runs to be carried out in less than 24 hr, or the use of neutral sodium or potassium borate to condition and elute a column, should eliminate the difficulties which have been discussed.

Enzyme Substrate Binding

D. G. Doherty
E. Eavenson R. Shapira

A survey of the specificity requirements of other proteolytic enzymes was made in an effort to extend the scope of carbon-carbon bond scission[8] to systems other than α-chymotrypsin. Trypsin seemed to be nearly identical to α-chymotrypsin in that the specific binding of the substrate is at the groups behind the point of hydrolysis whereas the group being removed has no effect on the observed rates.[9] Thus trypsin requires for maximum binding a carbonyl at site I, an acylamino group at site II, and a basic group four carbons out at site III (Fig. 31). The hydrolyzable group R has been restricted so far to an amide and a wide variety of esters.

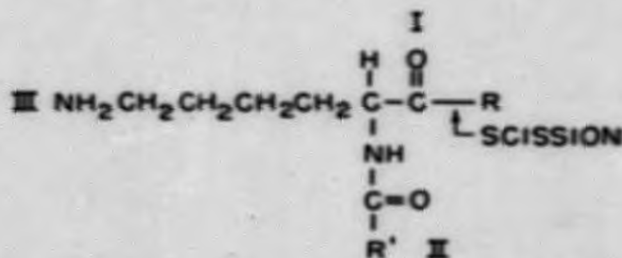


Fig. 31. Basic Structure of a Typical Trypsin Substrate.

Since the synthetic work for the preparations of β-keto esters would be considerably easier if the acyl amino group at site II could be omitted without loss of enzyme activity, the susceptibility of the esters of ε-aminocaproic acid to trypsin action was investigated. Contrary to previous reports[9] both the ethyl and methyl esters were rapidly hydrolyzed by trypsin to the free acid. The reaction was zero order over an eighteenfold

[8] D. G. Doherty and E. Eavenson, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 74.

[9] H. Neurath and G. W. Schwert, *Chem. Revs.* 46, 69–153 (1950).

range of enzyme concentration, the equilibrium constants being 0.0127 (mole/min)/(mg of protein N/ml). The effect of pH on reaction rates was found to be similar to other ester substrates of trypsin (Fig. 32), with a broad maximum in the range pH 7.5–10.0. The effect of chain length on the reaction rate was tested by subjecting ethyl Δ amino valerate and ethyl γ-amino butyrate to trypsin action. The valerate ester was hydrolyzed at a rate one-half that of the caproate whereas the butyrate was not hydrolyzed at all, indicating that the positively charged amino group has to be four to five carbon atoms out from the sensitive linkage.

Since the enzyme results with substrates lacking an α-acyl amino group were promising, synthesis of the corresponding ethyl 8-amino-3-keto octanoate was attempted. ε-Aminocaproic acid was converted first to ε-carbobenzoxyaminocaproic acid and then to the acid chloride and coupled to

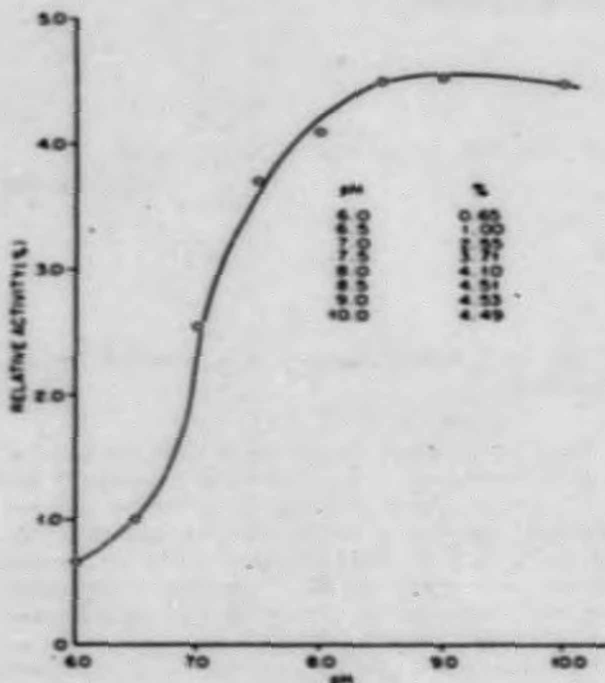


Fig. 32. pH Activity Curve with Trypsin. Percentage relative activity – percentage hydrolysis per minute (measured at 10 min).

acetoacetic ester[9] via the magnesium complex. The coupling product ethyl 8-carbobenzoxyamino-3-keto-2 acetyloctanoate was isolated in fair yield as the crystalline copper complex. Experiments are in progress on the purification of the complex and the removal of the acetyl and carbobenzoxy groups in order to obtain the desired β-keto ester in a pure condition for enzymic experiments.

Preliminary Studies on the Metabolism of S,β-Aminoethylisothiuronium-Br-HBr (AET) with S^{35}-labeled Compound

D. G. Doherty — R. Shapiro
W. T. Burnett, Jr.[10] — C. C. Congdon[11]

S^{35}, β-Aminoethylisothiuronium-Br-HBr (AET) was prepared from 0.52 mmole of thiourea containing 3 mc of S^{35}. The radioactive AET was administered by three routes to four mice, and after a period of time the mice were sacrificed, dissected, and the individual organs were homogenized. A small sample of each homogenate was plated, dried, weighed, and counted. The resulting counts per minute per milligram of dry weight were converted arbitrarily for comparison purposes to a heart count of 10, as shown in Table 53.

In general, the label was most concentrated in the urine and the blood plasma. No organ examined was completely void of labeled material. At the present time, it is not possible to tell whether the labeled material is all in the forms of AET or exists partly as degradation products. The high concentration in the blood plasma suggests that it is bound to plasma proteins and that its primary route of transport is via the circulatory system.

Structure and Activity in Radiation-protective Sulfhydryl Compounds

D. G. Doherty — R. Shapiro
W. T. Burnett, Jr.[10] — E. Eavenson

The study of structure and radiation-protective activity of compounds previously reported[12] can be summarized with a theory of action which correlates structures of compounds with their activities.

[10] Microbiological Protection and Recovery Section.

[11] Mammalian Recovery Section.

[12] D. G. Doherty, W. T. Burnett, Jr., R. Shapiro, and E. Eavenson, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 93–94.

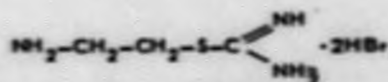
TABLE 53. S^{35} SPECIFIC ACTIVITY* IN MICE TREATED WITH AET

Organ	Time Between Treatment and Sacrifice (hr) →	Type of Administration			
		Intraperitoneal	Intravenous	Oral	Oral
		$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$2\frac{1}{2}$
Kidney		15	16	22	
Thymus		19	16	5	3
Spleen		14	10	9	14
Liver		11	10	15	
Heart muscle		10	10	10	10
Plasma		8	40	42	
Lungs		6	10	22	18
Blood		5	3	5	9
Eyes		3	6	5	
Brain		2.1	1.7	6	
Femoral bone marrow		1.5	12	6	
Thigh muscle		0.5	3	5	7
Testes				2.8	8
Urine and urinary bladder				160	
Pancreas				7	12
Lymph nodes				3	10
Red blood cells				1.1	

*Converted to a heart count of 10.

S,β-Aminoethylisothiuronium-Br-HBr (AET) has been reported by Doherty and Burnett[13] to have the profound effect of protecting against total-body X irradiation if administered prior to irradiation. This effect is similar to that observed with β-mercaptoethylamine.

Structure of AET



AET is an isothiuronium compound with several exceptional properties in addition to its protective nature. Normal isothiuronium compounds are very stable at pH 7 and are hydrolyzed only by strong base above pH 9. Alkyl mercaptans and urea are the primary products. AET, on the other hand, appears to be readily hydrolyzed at pH 7, as indicated by a positive nitroprusside –SH test. Further studies showed that all the compounds which protect also give a positive –SH test at pH 7. The best explanation of this phenomenon is that AET exists in multiple forms, and the equilibrium between these forms can be changed by modifying the pH (Fig. 33). When AET is boiled briefly in water, 2-aminothiazoline hydrobromide *d* is obtained. This indicates that, as soon as AET is placed in water, the equilibrium *a* ⇌ *b* takes place. Addition of bicarbonate solution displaces the equilibrium in the direction of *c*, as shown by the –SH test.

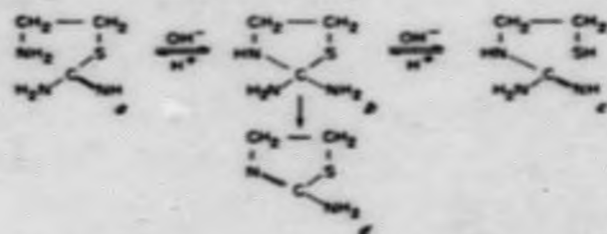


Fig. 33. Multiple Equilibrium Structures of AET.

[13]D. G. Doherty and W. T. Burnett, Jr., *Proc. Soc. Exptl. Biol. Med.* 89, 312–314 (1955).

All the compounds that are active in protecting mice are capable of establishing an intermediate of type *b* and give a positive –SH test in neutral solution. Compounds which do not give a positive –SH test at neutral pH have no protective activity. Some of the compounds tested are shown in Table 54 in their *a* and *b* forms. Several of the active compounds are too toxic to be tested on an equimolar basis with AET; however, when they are given in smaller quantities, partial protection is observed. These results are given elsewhere in this report.[14]

Table 54 illustrates the apparent essentiality of an active compound to undergo a thiazolidine or a thiazane ring closure of type *b*. In over 100 compounds tested, no exceptions to this theory have been found. Thiazoline and hexthiazene rings act similarly to their saturated forms.

In summary, it is impossible to think of AET or related compounds as being of one structure but rather as a mixture of three tautomeric forms. In this respect, it is similar in nature to coenzyme A[15] and glutathione,[16] both of which exhibit multiple forms. Also it is concluded that two basic ring systems (Fig. 34) may be substituted with excellent probability of protective activity.

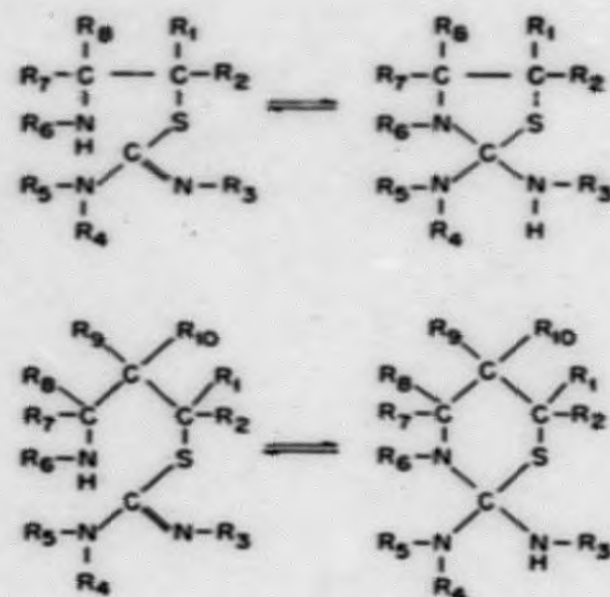


Fig. 34. Possible Substituted Isothiuronium Structures with Protective Activity.

Microbial Utilization of Heparin

D. G. Doherty J. F. Christman

It has been shown[17] that, by using heparin as a selective culture substrate, a wide variety of microorganisms that could utilize heparin as a substrate could be isolated from soil. In view of the evidence[18,19] that heparin has a strong bacteriostatic action on certain bacteria in minimal media, an examination of various microorganisms to determine the extent of this inhibitory action seemed in order. Accordingly, a wide variety of bacteria and yeasts were examined for their ability to grow in various media with and without added heparin. The organisms used and their sources are given in Table 55.

Three media were prepared. The composition of medium I is shown in Table 56. Medium II was identical to medium I, except that the vitamin-free, enzymically hydrolyzed casein was omitted. Medium III consisted of 10 g each of yeast extract and tryptone, and 5 g each of glucose and KH_2PO_4.

These media were adjusted to pH 6.5, and tubed in 4-ml portions. To one-half the tubes of each medium, 1 ml of heparin was added prior to sterilization; the final concentrations of heparin were then 1, 0.1, 0.01, 0.001, 0.0001, and 0.00001%. The other half of each medium, in tubes, was sterilized and the appropriate filter-sterilized heparin solutions were added to produce the equivalent concentrations. The latter group was identical to the first except that, in the first group, the heparin was autoclaved with the media.

The organisms were then inoculated into each of the three media, and serially subcultured through four transfers to establish the organism on the medium. Several of the organisms were unable to

[14] W. T. Burnett, Jr., D. G. Doherty, and R. Shapira, this report, Microbiological Protection and Recovery Section.

[15] R. E. Basford and F. M. Huennekens, *J. Am. Chem. Soc.* 77, 3878–3882 (1955).

[16] M. Calvin, in *Glutathione, A Symposium*, Academic Press (1954), p 21–26.

[17] D. G. Doherty and J. F. Christman, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 92–93.

[18] S. B. Stoker, *J. Physiol.* 110, 26P (1949).

[19] J. R. Warren and F. Graham, *J. Bacteriol.* 60, 171–174 (1950).

TABLE 54 MULTIPLE FORMS, NITROPRUSSIDE TESTS, AND ACTIVITY OF PROTECTIVE COMPOUNDS

INITIAL[a]	a	⇌	b	-SH TEST pH 7	PROTECTIVE ACTIVITY
AET	[illegible]	⇌	[illegible]	+	+
γ-APT	[illegible]	⇌	[illegible]	+	+
ABT	[illegible]	⇌	NOT FORMED, STRAINED RING	−	−
MAET	[illegible]	⇌	[illegible]	+	+
diMAET	[illegible]	⇌	NOT FORMED, NO HYDROGEN ON AMINE	−	−
diEAEAET	[illegible]	⇌	[illegible]	+	+
ATEA	[illegible]	⇌	[illegible]	+	+
diATEA	[illegible]	⇌	[illegible]	+	+
ETEA	[illegible]	⇌	[illegible]	+	+
MTEA	[illegible]	⇌	[illegible]	+	+
2-AMINO-THIAZOLINE			[illegible]	+	+
2-AMINO-THIAZOLE			[illegible]	−	−
diTPA	[illegible]	⇌	[illegible]	+	+
β-APT	[illegible]	⇌	[illegible]	+	+

[a]AET = S,β-aminoethylisothiuronium-Br-HBr
γ-APT = S,γ-aminopropylisothiuronium-Br-HBr
ABT = S,δ-aminobutylisothiuronium-Br-HBr
MAET = N-methyl-S,β-aminoethylisothiuronium-Cl-HCl
diMAET = β-dimethylaminoethylisothiuronium-Cl-HCl
diEAEAET = N,β-diethylaminoethyl-S,β-aminoethylisothiuronium-Br-HBr
ATEA = N-allylisothiuronium-β-ethylamine-diHBr
diATEA = N,N'-dialkylisothiuronium-β-ethylamine-diHBr
ETEA = ethyleneisothiuronium-β-ethylamine-diHBr
MTEA = S,2-mercaptothiazolinium-β-ethylamine-diHBr
—
—
diTPA = S,β,β'-diisothiuroniumisopropylamine-triHBr
β-APT = S,β-aminopropylisothiuronium-Br-HBr

TABLE 55. HEPARIN SENSITIVITY OF A VARIETY OF MICROORGANISMS IN VARIOUS MEDIA

Organism	Source or Strain No.	Maximum Percentage of Heparin in the Medium in Which Growth Will Occur					
		Medium I		Medium II		Medium III	
		A[a]	S[b]	A	S	A	S
Micrococcus citreus	Midwest cultures	1	1	NG[c]	NG	1	1
M. pyogenes var. *albus*	Midwest cultures	1	1	NG	NG	1	1
M. pyogenes var. *aureus*	Midwest cultures	1	1	NG	NG	1	1
Bacillus brevia	Univ. of Tenn.	1	1	0.1	0.1	1	1
B. laterosporus	Midwest cultures	1	1	0.1	0.1	1	1
B. subtilis	Midwest cultures	1	1	1	1	1	1
B. cereus	Univ. of Tenn.	1	1	NG	NG	1	1
B. megatherium	Univ. of Tenn.	1	1	NG	NG	1	1
Alkaligenes faecalis	Univ. of Tenn.	1	1	NG	NG	1	1
Streptococcus faecalis	Univ. of Tenn.	1	1	NG	NG	1	1
S. pyogenes	Univ. of Tenn.	1	1	NG	NG	1	1
Proteus vulgaris	Univ. of Tenn.	1	1	0.1[d]	0.01[d]	1	1
Paracolobactrum aerogenoides	ATCC 11604	1	1	1	1	1	1
P. coliforme	ATCC 11605	1	1	1	1	1	1
P. intermedium	ATCC 11606	1	1	1	1	1	1
Escherichia coli	ATCC 10795	1	1	1	1	1	1
E. coli	ATCC 8739	1	1	1	1	1	1
E. coli	ATCC 10586	1	1	1	1	1	1
E. coli	Strain B	1	1	1	1	1	1
Bacterium cadaveris	Gale	1	1	NG	NG	1	1
Aerobacter aerogenes	Univ. of Tenn.	1	1	NG	NG	1	1
Serratia marcesens	Univ. of Tenn.	1	1	1[e]	1[e]	1	1
Salmonella enteriditis	Univ. of Tenn.	1	1	1[f]	1[f]	1	1
Saccharomyces cerevisiae	ATCC 4110	1	1	1	1	1	1
S. cerevisiae	ATCC 4126	1	1	1	1	1	1
S. cerevisiae	ATCC 9896	1	1	1	1	1	1
S. cerevisiae	ATCC 4125	1	1	1	1	1	1
S. fragilis	ATCC 8644	1	1	1	1	1	1
S. intermedius	ATCC 2360	1	1	1	1	1	1
S. logos	ATCC 10630	1	1	1	1	1	1
S. thermantitonum	ATCC 563	1	1	1	1	1	1

[a]A, medium containing autoclaved heparin.

[b]S, medium containing heparin sterilized by filtration and added aseptically.

[c]NG, no growth in organisms serially subcultured on this medium.

[d]This organism grew on media containing 0.1% heparin after serially subculturing on media of gradually increasing concentrations of heparin.

[e]Organism nonpigmented when grown in this medium.

[f]Better growth of the organism in medium containing heparin than in absence of heparin.

TABLE 56. COMPOSITION OF MEDIUM I

Compound	g*	Compound	mg*	Compound	μg*
NaCl	5	$MgCl_2$	50	Na_2MoO_4	10
K_2HPO_4	2.5	$Na_2B_4O_7$	10	Thiamine HCl	200
$(NH_4)_2SO_4$	4.5	$FeCl_3$	10	Nicotinic acid	100
Glucose	2	$CoCl_2$	10	Calcium pantothenate	100
Casein (vitamin-free, enzymically hydrolyzed)	5	$MnCl_2$	1	Pyridoxine HCl	40
		Inositol	1	Biotin	0.5
				Choline chloride	500
				Riboflavin	5
				p-Aminobenzoic acid	10
				p-Hydroxybenzoic acid	10
				Vitamin B_{12}	0.05

*Amounts given are weights per 800 ml of medium before addition of heparin.

maintain continued growth on medium II, but all grew adequately in 48 hr on the other two media. The organisms were then inoculated dropwise into the corresponding heparin series. After 48 hr, the tube with growth in the highest concentration of heparin (usually 1%) was serially subcultured through three transfers and finally transferred to the original heparin-free medium. Following growth, the organism was examined microscopically for any change or possible contamination. The results of these experiments are given in Table 55.

It would appear that most of the organisms are completely insensitive, under the conditions tested, to relatively high concentrations of heparin. This observation is in disagreement with the observations of Warren and Graham,[19] who showed that in a synthetic medium, heparin was inhibitory in concentrations as low as 10 ppm. The single difference between the two media was the presence of the trace quantities of the B-vitamins. These materials were found to be essential in most of the organisms for continued growth in the absence of heparin.

ENZYMOLOGY AND PHOTOSYNTHESIS

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ENZYMOLOGY

Activity of Compounds Related to β-Mercaptoethylamine as Inhibitors of Enzymic Acetylation

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J. R. Totter

The relatively high degree of protection against radiation injury which has been obtained by treatment of mice with certain derivatives of β-mercaptoethylamine raises the possibility that metabolic effects may play some part in the function of these compounds as protective agents. Since β-mercaptoethylamine is a constituent of coenzyme A, the cocatalyst for acetylation, metabolic effects might be exerted through an influence on reactions which are CoA dependent. There is, of course, no evidence that an *in vivo* alteration of the rate of acetylation could affect survival after irradiation. It remains for future work to determine whether the *in vitro* effects demonstrated in this investigation do reflect *in vivo* changes and whether such changes might have an influence on survival rates.

A series of compounds which had previously been examined for toxicity and for relative ability to increase the survival of mice after irradiation[5,6] was tested for ability to inhibit the acetylation of sulfanilamide in the Kaplan and Lipman CoA assay test system.[7] Aside from interest in a possible correlation of these three tests, there is intrinsic interest in possible competitive inhibitors of CoA, since inhibitors of other vitamin derivatives have proved extremely useful in the treatment of disease and as tools for elucidation of biochemical reaction sequences.

The results of the assays for inhibition of acetylation are given in Table 57. It may be seen that the first three compounds listed are quite active. Of these, S,γ-aminopropylisothiuronium-Br-HBr and S,β-aminoethylisothiuronium-Br-HBr are excellent protective compounds, whereas the di-n-butyl compound was too toxic for satisfactory testing and is of uncertain activity. All the other compounds show less activity as inhibitors and have not been found to be very active in the mouse tests, although additional testing will be required to establish any correlation. β-Mercaptoethylamine itself does not appear to affect the acetylation reaction even though it is a very effective protective agent. Since it is a constituent of CoA, not an analog, it would not be expected to inhibit acetylation. Additional compounds will have to be investigated to establish whether a good correlation exists between the *in vitro* activity of the derivatives and their activity as protective agents. It should be borne in mind that such a correlation should not necessarily be expected for a protective effect which is exerted solely by competition for radicals produced in water by irradiation.

Fluorescence Polarization

J. W. Davis W. A. Arnold

In studies on the fluorescence polarization of methanolic extracts of green leaves and of purified chlorophyll, the material under examination was dissolved in castor oil in an optical cell having polarized light incident on one of its sides. The fluorescent light emitted from the material along a path at a right angle to the beam of incident light was analyzed for degree of polarization through a movable Nicol prism (Fig. 35). The relative intensity of fluorescence was detected by a photomultiplier tube, and recorded.

The polarization of the light by the material in the cell is given by:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

[1]Consultant.

[2]Research participant.

[3]Temporary employee, summer 1955.

[4]Biochemistry Section.

[5]W. T. Burnett, Jr., D. G. Doherty, and A. W. Kimball, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 48–52.

[6]W. T. Burnett, Jr., D. G. Doherty, and R. Shapira, this report, Microbiological Protection and Recovery Section.

[7]N. O. Kaplan and F. Lipman, *J. Biol. Chem.* 174, 37–44 (1948).

TABLE 57. ACTIVITY OF COMPOUNDS RELATED TO β-MERCAPTOETHYLAMINE AS INHIBITORS OF THE COENZYME A-DEPENDENT ACETYLATION OF SULFANILAMIDE

At pH 6.5–6.8 and CoA concentration of 0.0267 µmole/liter

Compound	Range of Concentration Tested (mmolar)	Concentration Required for 50% Inhibition of Acetylation (mmolar)
S,γ-Aminopropylisothiuronium-Br-HBr	1.41–28.2	2.8
N,N'-Di-*n*-butylisothiuronium-β-ethylamine-diHBr	1.06–21.2	3.8
S,β-Aminoethylisothiuronium-Br-HBr	0.59–17.7	4.0
N-Acetylaminoethylisothiuronium-HBr	1.83–36.6	6.0
N,N'-Diisopropylisothiuronium-β-ethylamine-diHBr	1.14–22.8	7.1
N-Methyl-S,β-aminoethylisothiuronium-Cl-HCl	2.02–40.4	10.5
1,6-Dithia-4,9-diazaspiro(4,4')nonane-diHBr	0.645–12.9	13.0
N,N'-Diethylisothiuronium-β-ethylamine-diHBr	1.24–24.8	13.4
Trimethylaminoethylisothiuronium-Br-HBr	1.28–25.6	19.2
Dimethylaminoethylisothiuronium-Cl-HCl	1.9–38.0	31.0
N,β-Bromoethylthiourea	4.65–46.5	41.0
2-Aminothiazoline	2.45–81.7	57.0
β-Mercaptoethylamine	10.8–108.0	>108
2-Aminothiazoline-4-carboxylate	0.57–11.4	>11.4

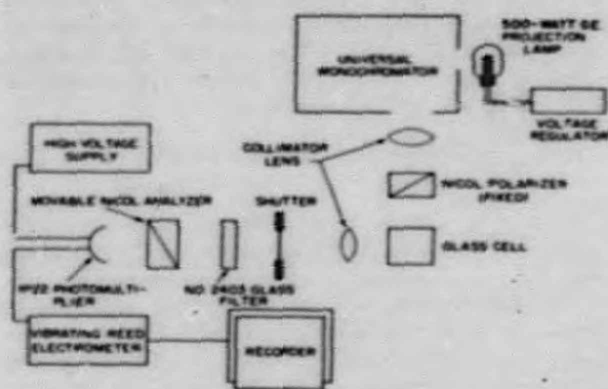

Fig. 35. Polarization Apparatus.

where $I_{\parallel}$ and $I_{\perp}$ are the intensities of fluorescence reaching the photomultiplier when the plane of the polarizer and analyzer are parallel and perpendicular to each other, respectively.

When degree of polarization was measured as a function of the wave length of the exciting light (Fig. 36) a low value occurred in the neighborhood of 440 mµ for both the crude plant extract and the chlorophylls partially purified by sucrose column chromatography. This depolarization of light by the plant extract can be accentuated by increasing its concentration in the castor oil.

The effect on polarization of adding back the extracted carotenoid fraction to each of the chlorophyll preparations was tested with the hope of finding evidence for a transfer of energy between these two molecules. Preliminary data indicate that carotenoids have no effect on the depolarization by chlorophyll *a* but a measurable effect on the depolarization by chlorophyll *b* (Fig. 37).

The occurrence of negative polarization of light by the plant extract material suggests either that two molecules involved in energy transfer have a fixed spatial relation to each other or that the chlorophyll alone is responsible for the depolarization through transfer from one electronic oscillator to another within the same molecule.

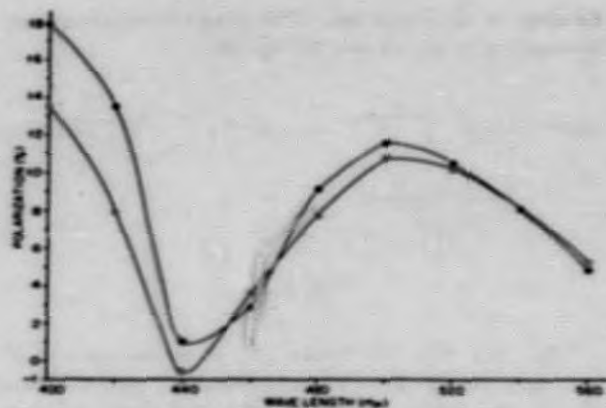

Fig. 36. Polarization of Plant Materials. O, Plant extract in castor oil; ●, purified chlorophyll a in castor oil.

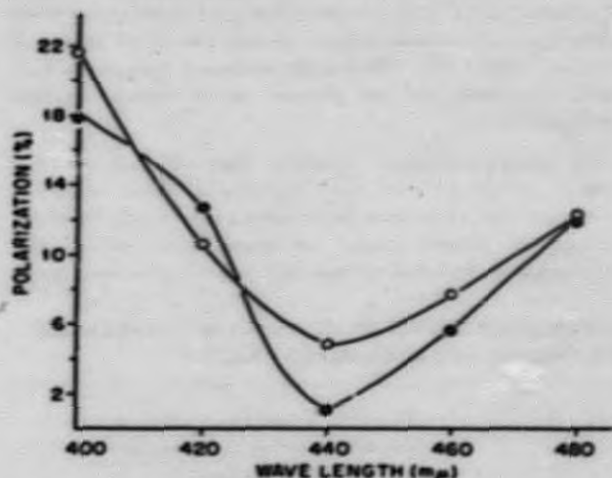

Fig. 37. Effect of Carotene on Polarization of Chlorophyll b. O, Purified chlorophyll b; ●, chlorophyll b with plant carotenes added.

Fluorescence polarization has also been applied in investigating the destruction of deoxyribonucleic acid (DNA) by various agents such as deoxyribonuclease, gamma radiation, and ultraviolet light. The kinetics of DNA degradation may be followed and the relative sizes of the particles formed by various methods of treatment may be estimated by this means.

A Requirement for Pyridoxal in the Interconversion of Glycine and Serine

J. V. Passonneau J. R. Totter

The interconversion of serine and glycine involves activation of a one-carbon intermediate which is also an intermediate in nucleotide synthesis. Since the serine-glycine interchange is more easily studied than nucleotide formation, the former reaction has been used as an experimental model.

The formation of serine has been investigated by Kisliuk and Sakami[8] and by Alexander and Greenberg.[9] The latter workers postulated a requirement for a pyridoxal derivative in their liver extract system on the basis of inhibition by deoxypyridoxine. However, they were unable to demonstrate directly a need for pyridoxal in the uninhibited reaction.

An investigation of water extracts of acetone-dried luminescent coccobacilli[10] indicated that the extracts contained an active system promoting the formation of serine from glycine. Treatment of the extracts with Dowex-1 inactivated the glycine → serine reaction. The activity could be fully restored by the addition of a boiled, untreated extract or by the addition of both tetrahydrofolic acid and pyridoxal or pyridoxal phosphate. Either of the latter compounds alone failed to restore the activity fully. Pyridoxine partially replaced pyridoxal but pyridoxamine was entirely inactive.

The test system consisted of treated extract, serine, glycine 2-C^{14}, and phosphate buffer at pH 7. Compounds to be tested were added to this mixture and after incubation for 3 hr the reaction was stopped with trichloroacetic acid. Serine carrier was added to the acid extract, precipitated with alcohol and pyridine, then repeatedly recrystallized and washed. The isolated carrier was shown to be free of C^{14}-glycine by paper chromatographic methods. The radioactivity of the serine increased linearly with time (Table 58) in the complete system. Table 59 gives the results of typical experiments with the Dowex-treated extract.

[8] R. L. Kisliuk and W. Sakami, *J. Biol. Chem.* 214, 47–57 (1955).

[9] N. Alexander and D. M. Greenberg, *J. Biol. Chem.* 214, 821–837 (1955).

[10] M. J. Cormier and B. L. Strehler, *J. Cellular Comp. Physiol.* 44, 277–289 (1954).

Reduction of Methemoglobin by Phenylhydrazine

H. H. Rostorfer

Several reducing substances which are known methemoglobin-forming agents are also methemoglobin-reducing agents. It is shown here that phenylhydrazine is a member of this group. The data presented indicate the nature and velocity of the reaction of methemoglobin with phenylhydrazine. The experiments, which were carried out under the most rigid anaerobic conditions possible, established the relationship of the nitrogen and benzene evolved with the amount of methemoglobin reduced in the reaction. The over-all reaction can be expressed as shown in Fig. 38.

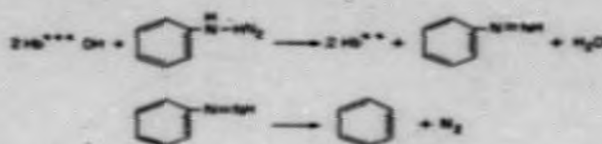

Fig. 38.

From the data of Table 60, it is evident that, for each volume of nitrogen liberated, two volumes of CO are taken up during the reduction of methemoglobin. Thus the reduction of 1 mole of methemoglobin requires the oxidation of 2 moles of phenylhydrazine. Also, the data clearly show that all of the nitrogen of the phenylhydrazine may be evolved when the amount of methemoglobin exceeds the amount of phenylhydrazine and sufficient time is allowed for completion of the reaction (lines 2 and 4, Table 60). The stoichiometry suggests that only benzene and not phenol could remain as one end product.

In spectrographic studies carried out on the vapor arising from the surface of the reaction medium, benzene was definitely identified. Furthermore, no phenol could be found after all of the nitrogen of the phenylhydrazine was evolved, even

TABLE 58. INFLUENCE OF TIME OF INCUBATION ON CONVERSION OF C^{14}-GLYCINE TO C^{14}-SERINE BY DOWEX-TREATED BACTERIAL EXTRACTS FORTIFIED WITH PYRIDOXAL PHOSPHATE AND TETRAHYDROFOLIC ACID

Time (min)	Total C^{14} Activity in Serine per Milliliter of Extract (counts/sec)
15	23
30	39
60	72
120	143
180	207

TABLE 59. EFFECT OF PYRIDOXINE DERIVATIVES AND TETRAHYDROFOLIC ACID ON THE CONVERSION OF C^{14}-GLYCINE TO C^{14}-SERINE ON DOWEX-TREATED BACTERIAL EXTRACTS

Compound Added (20 µg each)	Tetrahydrofolic Acid (225 µg)	Total C^{14} Activity in Serine per Milliliter of Test System (counts/sec)
None	–	10
None	+	38
Pyridoxamine	+	40
Pyridoxine	+	62
Pyridoxal	+	129
Pyridoxal phosphate	+	179
Pyridoxal phosphate	–	29
None*		195

*Untreated bacterial extract.

TABLE 60. RELATION BETWEEN NITROGEN EVOLVED AND CO UPTAKE DURING METHEMOGLOBIN REDUCTION

Methemoglobin Added (<> μl of CO)	Phenylhydrazine Added (<> μl of N_2)	Nitrogen Evolved (μl)	CO Uptake (μl)
262	157	103	214
262	91	83	160
91	154	42	93
91	40	46	91
76	77	34	67
76	154	39	74

with the sensitive phenol reagent of Folin and Ciocalteau.

A modification of this test[11] may be used as an analytical method for phenylhydrazine. The test is positive for phenylhydrazine and the color obeys Beer's Law. It was therefore used to quantitate the phenylhydrazine which disappeared during the methemoglobin reduction. Table 61 indicates the equality of the nitrogen evolved and the phenylhydrazine decomposed. When nearly all of the phenylhydrazine nitrogen was evolved, the "phenol" test indicated that practically no phenylhydrazine remained. Thus benzene is the only product formed in the reaction with methemoglobin, as it is in the reaction with oxyhemoglobin.

The velocity of the reduction of methemoglobin was measured by subtracting the amount of N_2 (μl) evolved in the absence of CO from the amount

[11]O. H. Lowry, H. J. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.* 193, 265–275 (1951).

TABLE 61. RELATION BETWEEN NITROGEN EVOLVED AND PHENYLHYDRAZINE DESTROYED EXPRESSED AS PERCENTAGE OF TOTAL PRESENT INITIALLY – THE MOLAR EQUIVALENCE OF METHEMOGLOBIN REDUCED AND PHENYLHYDRAZINE OXIDIZED (WITH 7 μmoles OF PHENYLHYDRAZINE ADDED)

Phenylhydrazine Oxidized			Hb_4^{+++} Reduced (μmoles)
Calculated from N_2		Analyzed % of Total	
Percentage of Total	Amount (μmoles)		
56	3.92	62	1.92
25	1.75	36	0.87
14	0.98	15	0.52
83	5.82	93	2.98

(μl) of change in volume in a companion flask containing CO. The algebraic sum is equivalent to the methemoglobin reduced in a given time. The rate of reaction may be expressed as a second-order velocity constant since, under proper conditions, the kinetics of a bimolecular reaction are satisfied. The average velocity constant in these experiments was about 0.10 (μmole-ml^{-1}-mm^{-1}).

The reduction of methemoglobin is only somewhat slower than the velocity of its formation in the reaction of phenylhydrazine with oxyhemoglobin. This high velocity makes possible a cyclic reaction between phenylhydrazine and oxyhemoglobin in the presence of atmospheric oxygen, in which relatively large amounts of phenylhydrazine can be decomposed to nitrogen and benzene.

PLANT BIOCHEMISTRY

N. E. Tolbert

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A. R. Kroll
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P. C. Kerr
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Effect of Ionizing and Ultraviolet Radiation on Photosynthesis in Plants

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

Further details of experiments on the effects of ionizing and ultraviolet radiation on photosynthesis, discussed in the previous semiannual report,[3] will be presented.

Green Thatcher wheat plants, seven days old, were exposed to γ radiation from a Co^{60} source delivering about 1515 r/min or to ultraviolet radiation from a 15-w G-E germicidal lamp emitting the major portion of its energy at 2537 A. Ultraviolet dose was determined solely as a function of time for the particular geometry used. Photosynthetic ability was determined by the amount of $C^{14}O_2$ fixed in a 10-min period from a standard amount of $C^{14}O_2$ available. Leaf samples were frozen, ground in liquid nitrogen, and extracted in boiling water. An aliquot of this extract was plated out on a glass planchet and counted in a gas-flow proportional counter to determine the total counts of $C^{14}O_2$ fixed. Paper chromatograms were prepared from another aliquot and the radioactive spots counted to determine the percentage distribution of compounds formed by the fixation of $C^{14}O_2$.

Figure 39 depicts the loss of photosynthetic CO_2 fixation with increasing dose of ultraviolet radiation. The initial rapid loss in photosynthetic rate to about 20% in the untreated plants was followed by a considerable period in which no further loss in ability was observed. This continuation of a certain fraction of normal CO_2 fixation at much higher ultraviolet doses is an interesting phenomenon which is being further investigated. It might be explained on the basis of an internal filtering of radiation by the leaf material itself but experimental evidence is lacking.

In a like manner, the effect of increasing doses of ionizing radiation (Fig. 40) was to decrease the rate of CO_2 fixation up to about 100,000 r. Above 100,000–300,000 r, the photosynthetic rate was not further decreased below the level of about 80% inhibition. In fact, there appeared to be a small increase in total $C^{14}O_2$ fixed during the 10-min test period. The extremely penetrating nature of the γ radiation excludes any explanation of the continuance of photosynthesis at higher doses on the basis of an internal filtering process.

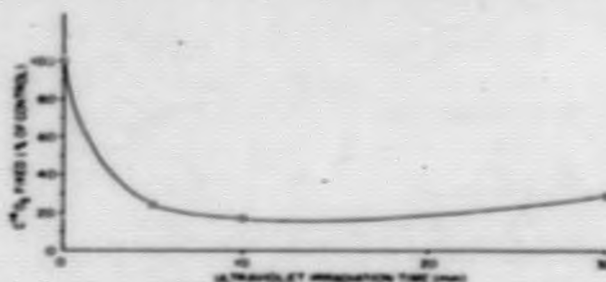


Fig. 39. Effect of Ultraviolet Radiation (2537 A) on $C^{14}O_2$ Fixation by Thatcher Wheat.

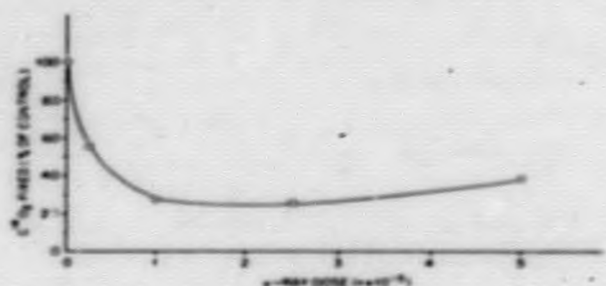


Fig. 40. Effect of Cobalt Gamma Radiation on $C^{14}O_2$ Fixation by Thatcher Wheat.

[1]Research participant.

[2]ORINS predoctoral fellow.

[3]L. P. Zill, N. E. Tolbert, D. A. Mondon, and P. C. Kerr, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 98.

There was no visible loss of chlorophyll after each of these ionizing radiation exposures.

An approximate percentage distribution of the products formed by the fixation of the labeled CO_2 was obtained by paper chromatography for each of the points in Figs. 39 and 40. Results for ultraviolet radiation are given in Table 62 and for γ radiation in Table 63. In no experiments were labeled compounds, other than those in the control, formed nor were any compounds lost which were present in the control. A certain amount of variation in the percentage distribution of the labeled compounds is to be noted, but not of sufficient magnitude to reflect the approximately 80% loss in photosynthetic ability. Indeed, these variations are more probably related to the differential radiation sensitivities of the enzymes, for formation or utilization of the various normal intermediates of CO_2 fixation. It thus appears that if CO_2 enters the photosynthetic cycle at all, after irradiation, it does so in a more or less normal fashion, and that the gross decrease in photosynthetic fixation can be attributed only to an effect on some other process than the path of carbon in photosynthesis. It might be postulated that the effect is connected with the utilization of light energy and very possibly with the derangement of the chlorophyll molecule. The inhibition may also be only a reflection of over-all cell lethargy after irradiation. A certain amount of support for the last argument is found in the similarity of response to both ionizing and nonionizing radiation since these are dissimilar in mode of absorption and mechanism of damage.

The reversibility of the damage to photosynthesis by ionizing radiation is shown in Fig. 41.

TABLE 62. DISTRIBUTION OF PRODUCTS FROM $C^{14}O_2$ FIXATION AFTER VARIOUS DOSES OF ULTRAVIOLET RADIATION

Compound or Area	Control	UV Exposure (min) → 5	10	30
		Percentage of Total Activity on Chromatogram		
Phosphate area	34	44	47	35
Sucrose	30	17	13	24
Glucose	Trace	Trace	Trace	Trace
Glycine and serine	24	26	28	30
Fructose	2	Trace	Trace	Trace
Alanine	1	Trace	Trace	Trace
Malic acid	9	10	7	3

TABLE 63. DISTRIBUTION OF PRODUCTS FROM $C^{14}O_2$ FIXATION AFTER VARIOUS DOSES OF GAMMA RADIATION

Compound or Area	Control	Gamma-Ray Dose (r) → 25,000	100,000	250,000	500,000
		Percentage of Total Activity on Chromatogram			
Phosphate area	34	35	38	26	27
Sucrose	30	23	23	23	22
Glucose	Trace	1	1	Trace	Trace
Glycine and serine	24	32	30	38	42
Fructose	2	1	2	1	1
Alanine	1	0	1	2	2
Malic acid	9	7	5	4	4

About 77% of the rate of photosynthesis in nonirradiated controls was regained in a 24-hr period after irradiation. Table 64 presents the distribution of labeled products formed at various times after irradiation. After 24 hr, approximately normal distribution of products is observed. Whether this recovery is caused by the reversibility of the of the damaged material or process or by the production of new material is not known.

These data are summarized as follows: (1) Large doses of ultraviolet or γ radiation (100,000 r) were required to produce an 80% inhibition of photosynthesis. (2) This inhibition was temporary and the photosynthesis rate recovered substantially in 24 hr. (3) Gamma-ray doses even as high as 500,000 r did not destroy a residual capacity of the leaf to photosynthesize at about 20% of the nonirradiated control. (4) During inhibition of photosynthesis or after recovery, there were no gross changes in the distribution of $C^{14}O_2$ fixed into the products of the path of carbon in photosynthesis. (5) The main reason for inhibition of photosynthesis by ionizing radiation is not known.

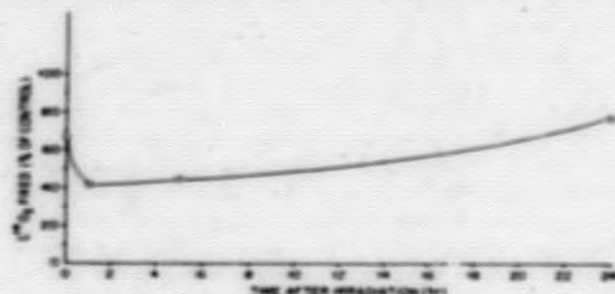


Fig. 41. Recovery of Ability to Fix $C^{14}O_2$ at Various Times After Irradiation. Four leaves of Thatcher wheat were irradiated with 100,000 r. At the time intervals indicated on the graph a sample was exposed to a standard $C^{14}O_2$ atmosphere for 10 min and determination was made of the total counts fixed. The wheat was kept in the light at all times.

Radiation Sensitivity in Formation of the Photosynthetic Process

F. B. Gailey N. E. Tolbert

As described,[4] the photosynthetic process already formed and functioning in a green plant is extremely insensitive to ionizing radiation. Those data substantiate the concept that radiation injury and possibly subsequent death of wheat must be caused by an effect or effects on some other process in leaf tissue than on photosynthesis and the enzymes catalyzing this process. At least one sensitive site of radiation damage has been established in the genetic composition of the nucleus. From the standpoint of comparative biochemistry, the next question would be whether development of the biochemical system is more sensitive to radiation than the preformed system.

In the normal process of greening, etiolated plants, when placed in light, form the complete and complicated photosynthetic mechanism.[5] This

[4] L. P. Zill, N. E. Tolbert, D. A. Mandon, and P. C. Kerr, *ibid.*, p 100.

[5] N. E. Tolbert and F. B. Gailey, *ibid.*, p 95–97.

TABLE 64. DISTRIBUTION OF PRODUCTS FROM $C^{14}O_2$ FIXATION AT VARIOUS TIMES AFTER GAMMA IRRADIATION WITH 100,000 r

Compound or Area	Control	Time After Irradiation (hr) → 0	1	5	24
		Percentage of Total Activity on Chromatogram			
Phosphate area	22	21	18	17	19
Sucrose	26	35	31	48	26
Glucose	1	2	3	1	4
Glycine and serine	32	27	32	21	34
Fructose	1	3	3	4	4
Alanine	1	1	1	2	2
Malic acid	11	6	7	5	7

formation, which takes many hours, includes the building of the chloroplast particles and at least some of the enzymes for photosynthesis. Details of this formation will require much more work, but for the present, relative tests of the radiation sensitivity of the photosynthetic mechanism may be made by measuring chlorophyll formation during this time.

The data to be presented show that, for etiolated plants irradiated at various times during greening, the subsequent formation of photosynthate in the leaves was inhibited only by large doses of radiation which were about equivalent to the doses needed to inhibit photosynthesis in a normal green plant. Furthermore, the inhibition of the formation of this process by massive doses of γ radiation (150,000 r) was only temporary, as has also been observed for photosynthesis in a green plant. Thus the formation of photosynthetic apparatus would appear to be no more radiation sensitive than photosynthesis itself. In both cases, the radiation effect may be caused by general killing of the plant rather than by any particular destruction of photosynthesis.

In these experiments, etiolated plants were placed in white light from a reflector flood bulb. Heat was removed with water filters between the plants and the light and, at intervals up to 48 hr afterward, total chlorophyll analyses were run on the plants as a measurement of their greening. In Fig. 42 is shown the rate of chlorophyll formation in the plants in various intensities of light. In 1000 ft-c of light, the plants greened at a nearly maximum rate; this intensity has therefore been used in the rest of the work.

Thatcher wheat plants were grown in the dark in 30-mm-dia sintered-glass Gooch crucibles filled with soil. The crucibles conveniently fitted into the holder used in the radiation exposure chamber, and the sintered glass provided drainage and aeration of the roots. The plants were irradiated in the dark in a Co^{60} exposure chamber delivering approximately 1365 r/min. For most of the experiment, the plants were prevented from receiving any light until after the γ irradiation by covering the crucible and plants with aluminum foil in the dark growing room before taking them to the cobalt source.

When the seven-day-old etiolated plants were exposed to various doses of γ radiation before exposure to any light, it was found that doses of thousands of roentgens were needed to inhibit chlorophyll formation. In Fig. 43 are drawn curves which show the effects of 50,000 and 150,000 r delivered immediately before placing the plants in the light. Large biological variation has been experienced in obtaining these data. Each point on the figure represents a separate experiment and batch of plants. At 50,000 r, there was approximately 50% reduction in the rate of chlorophyll formation, but by the end of two days the plants were about as green as their nonirradiated controls. At 150,000 r, there was complete inhibition of visible greening or significant chlorophyll formation for

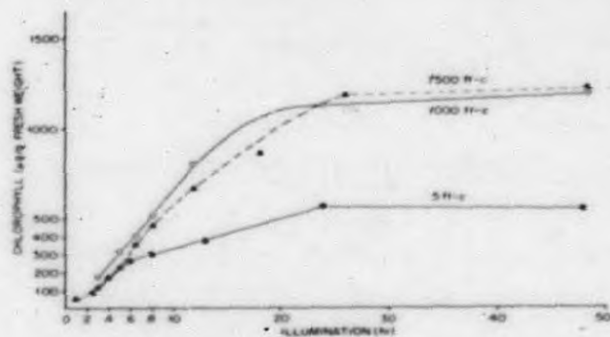


Fig. 42. Rate of Chlorophyll Formation in Etiolated Plants Placed in White Light of Increasing Intensity.

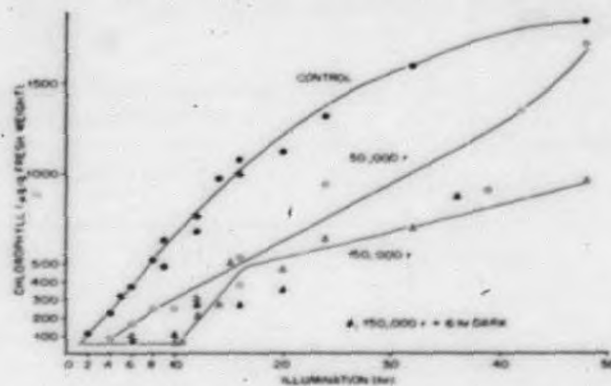


Fig. 43. Rate of Chlorophyll Formation in Etiolated Plants in 1000 ft-c of Light After Exposure to Gamma Radiation.

about 10 hr after plants were placed in the light; whereas, in the unirradiated controls after 2 hr in the light, some chlorophyll was present. After 150,000 r of γ radiation and 10 hr of light, the plants began greening, but for the first two days the total chlorophyll was much less than in the unirradiated controls.

The physiological form of these treated plants was interesting. The etiolated wheat leaves are rolled in a tight curl and, upon exposure to light, slowly unroll to become a flat blade after about 16 hr of light. Irradiation with 50,000 r delayed this unrolling for about one day and 150,000 r caused a delay of several days. This unrolling may be related to cell growth and elongation necessary to permit the change in shape of the leaf. Severe or complete inhibition of growth should be expected after 150,000 r. However, plants not used for chlorophyll analyses after 48 hr of continuous light have been kept in the greenhouse for observation. The leaf unrolled slowly into a flat blade, and other growth was evident by the development of new leaves. Growth was, however, very slow compared to nonirradiated controls and could arise by cell elongation from the leaf primordia.

Since 150,000 r produced a 10-hr period in which the plants did not green when placed in the light, experiments were run to determine whether light was necessary during this period to bring about chlorophyll formation. Figure 43 also shows data (▲) for plants kept in the dark for 6 hr after exposure to 150,000 r. These plants also required 10 hr in the light for an appreciable amount of chlorophyll to form. There was, therefore, a time lapse of 16 hr before greening began. In other experiments, plants were held for 2, 15, and 24 hr in the dark after 150,000 r of γ radiation, but always 10 hr of light was needed for greening to begin. In another experiment, plants, irradiated with 150,000 r followed by ½ hr of light and then darkness, also required about 10 hr of light for onset of greening.

In a nonirradiated wheat plant, rapid, sustained chlorophyll production does not begin until the plant has been in the light for about 1–2 hr. The reason for this time lapse is not well understood. Since the greening process is not radiation sensitive for plants irradiated just prior to exposure to white light, experiments were run to determine whether the plants were more sensitive during the first 6 hr in the light, when chlorophyll formation was actually getting under way. Two types of experiments were run – the γ radiation was given during dark periods alternating with nonirradiated light periods, or in one dark period after 2, 4, or 6 hr of light. Data for the latter experiments (Table 65) show that chlorophyll formation was not severely depressed by 50,000 r delivered during greening. The 150,000-r dose, if delivered 2 or 4 hr after the plant was placed in the light, inhibited greening for about 10 hr. The same dose, delivered after several hours of greening (after 6 hr of light), prevented further greening for many hours, but the greening formed before γ irradiation was not destroyed. Inhibition of chlorophyll formation was even less from the 150,000-r γ-ray dose delivered over 3–6 hr, alternating short γ-ray exposures with white light.

TABLE 65. EFFECT OF GAMMA RADIATION DELIVERED DURING GREENING OF ETIOLATED PLANTS

Illumination Before Irradiation[a] (hr)	Chlorophyll (μg/g of tissue)	
	50,000 r	150,000 r
2	207[b]	68[c]
4	208	93
6	291	353

[a]150,000 r at 1365 r/min from Co^{60}.

[b]Analyzed for chlorophyll 8 hr after beginning of illumination.

[c]Analyzed for chlorophyll 10 hr after beginning of illumination.

Parallel experiments on the rate and products from $C^{14}O_2$ fixation during greening of γ-irradiated etiolated wheat plants are being run. The $C^{14}O_2$ fixation follows chlorophyll formation, indicating that when the irradiated plant does become green, it is capable of photosynthesis.

Similar experiments have also been run with ultraviolet radiation from a germicidal lamp (2537 A maximum). Owing to epidermal shielding of the inner cells of the leaf and to the curled structure of the leaf, the ultraviolet experiments were not so satisfactory as those with the Co^{60} source. But approximately the same results were obtained, namely, large doses were necessary to inhibit temporarily the chlorophyll formation, as had been

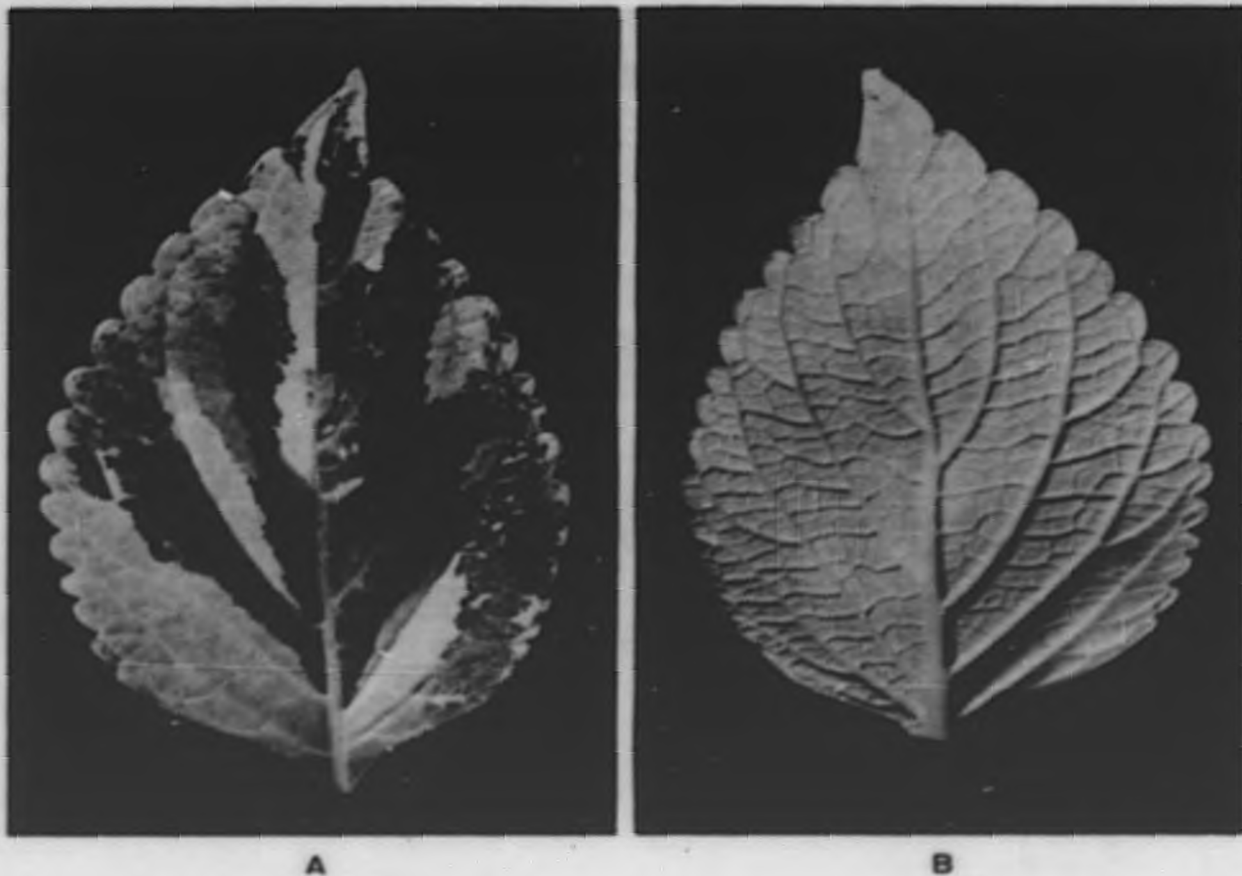
found for inhibition of the preformed photosynthetic process in a green leaf.

Photosynthesis in Anthocyanin-containing Leaf Tissue

C. W. Nystrom N. E. Tolbert

Filter Effect of Anthocyanins. – There are conflicting reports in the literature as to whether the rate of photosynthesis is affected by the anthocyanin pigments in the leaves of plants. In experiments reported here, the rate of $C^{14}O_2$ fixation and the intensity of chlorophyll fluorescence have been utilized to measure the rate of photosynthesis in both anthocyanin-containing and anthocyanin-free leaf tissues in different qualities of light. The results show that the anthocyanin does filter out much of the green light, making it unavailable to the plant for photosynthesis. However, anthocyanin does not absorb a significant amount of the red light, thus making it adviscble to carry out any photosynthesis rate studies in red light when anthocyanin-containing tissue is involved.

A variety of coleus was selected which had large, random sections which were either devoid of visible amounts of anthocyanin or were heavily pigmented with red. The anthocyanin was located entirely on the upper epidermis of the leaf (Fig. 44A) and there were no visible amounts of it on the lower epidermis (Fig. 44B) or in the palisade cells in the interior of the leaf. With such a leaf,



A B

Fig. 44.

it was possible to study the rate of photosynthesis in the anthocyanin-containing and anthocyanin-free sections during identical experimental treatment.

An aqueous HCl extract for the anthocyanin pigments of the leaf had a maximum absorption at 520 mμ, with very little absorption above 645 mμ. Although the absorption of the pigments in the leaf itself may not be identical to that of the HCl extract, it apparently is very similar, since a difference spectrum obtained by subtracting the spectrum of the anthocyanin-free section of the leaf from the anthocyanin-containing section had an absorption maximum at 525 mμ. Therefore, if there is a filter effect by the anthocyanin, in green light (465–545 mμ), the CO_2 fixation rate should be much lower in the anthocyanin-containing than in the anthocyanin-free section. In red light (645 mμ or greater), the rate should be nearly equal in both sections of the leaf.

Chlorophyll analyses and total amount of CO_2 fixed in 5- and 10-min intervals in both red and green light have been determined for the two sections of the same leaf. The anthocyanin-containing sections of the leaf also contained more chlorophyll (Table 66). This might be considered analogous to growing plants in low light intensity, whereby they produce more chlorophyll. The totals of C^{14} fixed in the soluble fractions are recorded in Table 66 on a fresh-weight basis, as well as on a chlorophyll basis. The results show that the rate of CO_2 fixation on a chlorophyll basis is markedly lower in the anthocyanin section of the leaf in green light; but there is little or no difference in the fixation rate between the two sections in red light.

Observation of the intensity of the chlorophyll fluorescence in both sections of the coleus leaf in green light gave further experimental evidence of the filter effect of the anthocyanins. Figure 45A, B shows the leaf photographed through a far-red filter which permits the passage of the red-chlorophyll fluorescence, but no appreciable amounts of the shorter wave lengths of light. The photograph of the top surface of the leaf exposed to green light (Fig. 45A) shows the highest chlorophyll fluorescence in the anthocyanin-free sections. In the lower surface of the leaf exposed to green light, the chlorophyll fluorescence is highest in the anthocyanin section, where the chlorophyll is in a somewhat higher concentration. These observations can best be explained by a filter effect of the anthocyanin.

Rate of Photosynthesis After Ultraviolet Irradiation of Anthocyanin-containing Leaves. – It has been suggested that anthocyanin pigments in plants may function by screening out deleterious ultraviolet rays from sunlight. This hypothesis has been tested by irradiating the leaves of the variety of coleus described in the preceding subsection with ultraviolet and then measuring the

TABLE 66. EFFECT OF LIGHT QUALITY ON THE RATIO OF $C^{14}O_2$ FIXED IN THE ANTHOCYANIN-CONTAINING TO THE ANTHOCYANIN-FREE SECTIONS OF THE COLEUS LEAF

Quality of Light	Time of PS* (min)	Section of Leaf	(counts/sec)/100 mg of Tissue	Chlorophyll (μg/g of tissue)	(counts/sec)/100 g of Chlorophyll	Ratio
Red	10	Anthocyanin	1775	412	441	1.0
		Antho-free	1520	345	430	
Red	5	Anthocyanin	1191	485	246	1.1
		Antho-free	1176	546	215	
Green	10	Anthocyanin	575	554	104	0.37
		Antho-free	1268	450	282	
Green	5	Anthocyanin	83	261	31	0.14
		Antho-free	243	112	217	

*Photosynthesis.

Fig. 45.

rate of photosynthesis in the anthocyanin-containing and anthocyanin-free sections of the leaf. No protective action by the anthocyanin pigments could be demonstrated, since the rate of photosynthesis appeared to be decreased more on a chlorophyll basis in the anthocyanin-containing section than in the anthocyanin-free section of the leaf.

Leaves were exposed at varying distances and for varying periods of time to a 15-w germicidal lamp, 2537 A maximum, or a Blacklite lamp, 3660 A maximum. After ultraviolet irradiation, the rate of $C^{14}O_2$ fixation was measured in red light. In unirradiated controls, this rate of photosynthesis in red light was directly proportional to the chlorophyll content in both sections; whereas, in green light, the rate in the anthocyanin section was much lower than in the anthocyanin-free section, owing to a filter action (described in the preceding subsection) by the anthocyanin pigments.

In one group of experiments, leaves were exposed to ultraviolet and then allowed to stand overnight; in another, the leaves were allowed to stand for 10 min in red light after ultraviolet exposure. In both groups, $C^{14}O_2$ fixation was measured for 10 min in red light. The results as shown in Table 67 indicate that short-range ultraviolet light around 2537 A has a marked effect on the

TABLE 67. EFFECT OF ULTRAVIOLET LIGHT, 2537 A MAXIMUM, ON $C^{14}O_2$ FIXATION IN THE ANTHOCYANIN AND ANTHOCYANIN-FREE SECTIONS OF THE COLEUS LEAF

Duration of UV Exposure (min)	Section of Leaf	(counts/sec)/100 mg of Plant Tissue	Chlorophyll (μg/g of tissue)	(counts/sec)/100 μg of Chlorophyll	Ratio
7*	Anthocyanin	1875	427	439	0.8
	Antho-free	2260	397	569	
15*	Anthocyanin	2201	610	394	0.6
	Antho-free	2306	336	686	
30*	Anthocyanin	366	563	66	0.5
	Antho-free	492	371	133	
7**	Anthocyanin	3886	558	661	0.9
	Antho-free	3845	539	713	
15**	Anthocyanin	2921	539	542	0.8
	Antho-free	2771	413	671	
30**	Anthocyanin	2026	502	403	0.9
	Antho-free	1989	448	444	

*Leaf allowed to fix $C^{14}O_2$ for 10 min in red light.

**Leaf allowed to stand for 16 hr after exposure to ultraviolet light.

rate of photosynthesis. Since long-range ultraviolet light (~3660 A) had little or no effect, the data are not presented.

In these experiments, a greater decrease in the rate of $C^{14}O_2$ was observed in the anthocyanin sections than in the chlorophyll sections of the leaf. Although this effect was not so marked after the leaf had stood overnight, there was still a definite trend in this direction. The extent of this inhibition is indicated by the ratio of the $C^{14}O_2$ fixation on a chlorophyll basis in the two sections of leaves after the ultraviolet treatment. These ratios varied extensively and only representative data are presented in Table 67.

A Form of Phosphorus Storage and Transport in Plants

N. E. Tolbert   P. C. Kerr

Recent studies on phosphorus metabolism have indicated the presence of numerous unknown compounds labeled with P^{32} which could be separated by chromatographic procedures. One of these has been selected for more extensive investigation because it contains more phosphorus than any other compound in the plant, except inorganic phosphate. In this report, it appeared to be a storage form of phosphorus.[6] Furthermore, this unknown was transported, along with inorganic phosphate, from the roots to leaves, where it was utilized in the metabolism of the leaf. This represents a unique function of the substance, since, of the many phosphorus-containing compounds of the root, only this one and inorganic phosphorus were transported to the leaf.[7]

Investigations toward identification on the chemistry of this unknown show it to be characterized by a great stability to acid or base hydrolysis and by the nonreactivity to numerous chemical tests expected of phosphorus compounds. This has resulted in slow progress in isolating it in sufficient quantities for chemical analysis.

[6]N. E. Tolbert and P. C. Kerr, *ibid.*, p 97–98.

[7]N. E. Tolbert, H. Wiebe, and P. C. Kerr, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 77–79.

In order to ascertain the optimum conditions for growing plants from which to begin the isolation procedures, a detailed study of the amount of the unknown storage compound was undertaken in plants growing in nutrient solution containing variable amounts of phosphorus. Of more importance, these nutrition experiments have shown the distribution of the amount and percentage of the phosphorus in the plant among this storage and transport compound, inorganic phosphate, and organic phosphate esters of metabolism, when plants are grown on phosphorus-deficient, normal, or phosphorus-rich media.

Sacramento barley plants were grown in nutrient cultures varying from 10^{-6} to 10^{-1} *M* total phosphate, but otherwise containing all the constituents of a normal Hoagland nutrient solution. A phosphate concentration of 10^{-3} *M* is considered a standard molarity since that is the concentration employed in Hoagland's nutrient solution.

Nutrient solutions were made up with the P^{32} tracer in sufficient amounts to produce autoradiographs of the chromatograms and to permit subsequent counting of radioactivity in each compound. The amounts of P^{32}, in microcuries per liter of nutrient solution, were 25 for 10^{-6} *M* phosphate, 125 for 10^{-5} *M* phosphate, 250 for 10^{-4} *M* phosphate, 500 for 10^{-3} *M* phosphate, 1000 for 10^{-2} *M* phosphate, and 2000 for 10^{-1} *M* phosphate. This specific activity was sufficient to cause visible radiation damage to the plants by the tenth day, especially in the range of 10^{-4} to 10^{-3} *M* phosphate.

Plants were harvested by grinding in liquid nitrogen, boiled in water, and aliquots were counted for total radioactive phosphorus and were chromatographed for separation of the compounds. In Table 68 these findings are reported for three representative analyses. Similar data for the leaves have been obtained; the experiments have also been repeated with Thatcher wheat, but these data are not recorded in this report.

The following tentative conclusions regarding phosphorus distribution in the roots of barley plants may be made.

1. The amount of P^{32} in the roots, representing the total phosphorus in the plant, increased with increasing phosphate concentration in the nutrient solution. This increase was about proportional to the phosphate concentration of the nutrient, up to 10^{-4} *M*, after which the phosphorus of the root continued to increase at a slower rate than the phosphate molarity of the nutrient solution increased.

2. The highest percentage of the phosphorus of the root in the unknown storage and transport compounds was at the lower phosphate concentrations in the nutrient solution. This indicates that the synthesis and storage of the unknowns had a great demand for the phosphorus of the root. This also probably indicates that other factors besides the inorganic phosphate of the nutrient solution, such as energy and nitrogen supply, were contributing to the synthesis of these compounds.

3. Highest yields of the unknowns were obtained at high phosphate concentrations of 10^{-3} and 10^{-2} *M* in the nutrient solution. The percentage of P^{32} in the unknowns decreased less rapidly than the amount of P^{32} in the roots increased. Thus excessive phosphate concentration in the roots favors still more synthesis of the unknowns.

4. The unknown storage compound accounted for as much as 10% of the phosphorus in the roots of these barley plants at the lowest phosphate concentration in the nutrient solution. The amount of phosphorus in the unknowns also increased with the age of the seedlings.

5. Unknown No. 2 has been described earlier[7] and as previously noted, the amount of it appears to coincide with the amount of Unknown No. 1.

6. Experiments were also run with no phosphate except the small amount of the $P^{32}O_4^{---}$ added to detect these compounds. Small amounts of P^{32} had been added to prevent too severe radiation damage, and as a result there was low radioactivity on the chromatograms used for analysis. The results indicated that at the very low phosphate concentration, less than 10^{-7} *M*, the amount of the phosphorus incorporated into these unknowns was less.

7. Experiments were also run at 0.1 *M* phosphate in the nutrient solution or at 100 times normal phosphate concentration. There were only trace amounts of the unknowns, but the amount of P^{32} in the inorganic phosphate was very high. Because of the very low activity in the unknowns, these chromatograms were not counted. The data suggest that the high osmotic pressure from the high phosphate concentration may have had some effect on the production of the unknowns.

TABLE 68. DISTRIBUTION OF PHOSPHORUS STORAGE AND TRANSPORT UNKNOWN (U_1) IN BARLEY LEAVES

Phosphate Molarity		P^{32} Activity* [(counts/sec)/root]	Percentage Distribution**		
			PO_4^{---}	U_1	U_2
5-day-old plants	10^{-6}	260	94.2	5.8	++
	10^{-5}	3,400	97.2	2.8	++
	10^{-4}	45,000	97.9	2.1	+++
	10^{-3}	109,000	99.2	0.8	++
	10^{-2}	293,000	99.5	0.5	+
9-day-old plants	10^{-6}	1,670	88.1	8.3	3.6
	10^{-5}	18,600	90.1	6.7	3.2
	10^{-4}	93,500	94.9	2.3	1.8
	10^{-3}	426,000	98.4	1.0	0.6
	10^{-2}	798,000	98.9	1.0	0.1
16-day-old plants	10^{-6}	4,300	88.4	11.6	+++
	10^{-5}	26,000	88.5	8.7	2.8
	10^{-4}	327,000	94.3	4.5	1.2
	10^{-3}	801,000	98.3	1.1	0.6
	10^{-2}			++	++

*Corrected to the date on which the 5-day-old samples were first counted and to the same specific activity as that of the first sample.

**Calculated on total radioactivity in PO_4^{---}, U_1, and U_2 which were the major P^{32}-containing compounds. P^{32} in other compounds was not counted since it represented only a small amount of activity.

Photosynthesis and Metabolism in *Sedum spectabile*

L. M. Rohrbaugh N. E. Tolbert

The phosphate ester of sedoheptulose has been recognized in the past few years as being an important intermediate in both the path of carbon in photosynthesis and in the aerobic glucose metabolism pathway for respiration. The present investigation, to determine why *Sedum spectabile* acquires so much free sugar, which normally does not accumulate in detectable amounts in other plants and animals, is approached by studying the effects of different environmental conditions on the carbohydrate metabolism.

Since the sedum leaf is thick and turgid and has a heavy epidermis, the penetration of $C^{14}O_2$ gas of the atmosphere into the leaf is slowed during short photosynthesis periods. Preliminary experiments indicate that removal of the lower epidermis increases the rate of $C^{14}O_2$ fixation, at least for short experiments and at partial pressure of CO_2 below 1%.

It was also necessary to know whether the distribution of the C^{14} among the products from photosynthesis was different after the removal of this epidermis. The lower epidermis was removed from one-half of each leaf used but left intact on the other half, which was used as a control. After the leaf had been allowed to fix $C^{14}O_2$ photosynthetically, it was split longitudinally along the midrib, and each half extracted separately. The photosynthetic products from each half of the leaf were then analyzed by paper chromatography for a number of photosynthetic conditions. In no case was a major difference observed in the products

of the carbon cycle in photosynthesis. However, one compound which could not be detected in the normal leaf was formed in appreciable amounts (but not more than 1 or 2% of total C^{14} fixed) from leaf tissue with the epidermis removed. Its R_f value in phenol-water was about 0.7 and in butanol–propionic acid–water was about 0.4.

Glutamic Acid Metabolism in Barley Leaves

A. W. Naylor N. E. Tolbert

The carbon chain of glutamic acid plays a central role in the nitrogen metabolism of plants and animals. At present, glutamic acid is thought to be formed by transamination from α-ketoglutaric acid – a member of the Krebs citric acid cycle. When fed to the plant or animal, it can be utilized in the synthesis of proteins; be deaminated and metabolized by way of the Krebs cycle; serve in the general metabolism of arginine and proline; be transformed into its amide, glutamine; and it can be decarboxylated.

Studies by others have made it clear that the pool size of glutamic acid is markedly affected by factors changing the general metabolism of the organism. It is known that, in plants, there is a change in pool size of a number of the amino acids following treatment with the hormone indole 3-acetic acid. In the present investigation, the many normal metabolic products associated with glutamic acid metabolism have been determined by feeding the plant uniformly labeled glutamic-C^{14} acid. These have been analyzed by paper chromatography for comparison in future studies of its metabolism by auxin-treated plants. Similar experiments, not reported here, have also been performed with glycine-C^{14}.

The major new discovery with labeled glutamic acid is that, under all environmental conditions tested, this amino acid was converted to γ-aminobutyric acid and that, under anaerobic conditions, this acid was the major product. There is no known biological function ascribed to γ-aminobutyric acid, though it is present in large amounts in such diverse tissues as the potato tuber and the brain. It is not a known constituent of protein.

Young Sacramento barley seedlings, grown in soil, were always employed. Immediately before use, the blade of the first leaf was cut off above the sheath and placed in an aqueous solution of glutamic-C^{14} acid with only 1–2 mm of the leaf submerged. The leaves were kept throughout experimental treatment in a glass chamber with a circulating atmosphere which could be varied in composition. When light was used, intensities of 500 ft-c or more were obtained at the leaf surface with a photoflood bulb. Heat was minimized by passing the light through a water layer maintained at constant temperature and by rapidly flowing air or nitrogen through the glass vessel. This prevented the temperature from rising above 28°C.

At the end of the feeding period, the leaves were killed by freezing in liquid nitrogen, grinding in a methanol-water mixture, and then boiling for 1 min in a hot water bath. Aliquots were subjected to analysis by paper chromatography. A typical autoradiograph of a chromatogram is given in Fig. 46. Identification of some of the compounds was made by cochromatography and specific spray tests, but at least five compounds have not been identified.

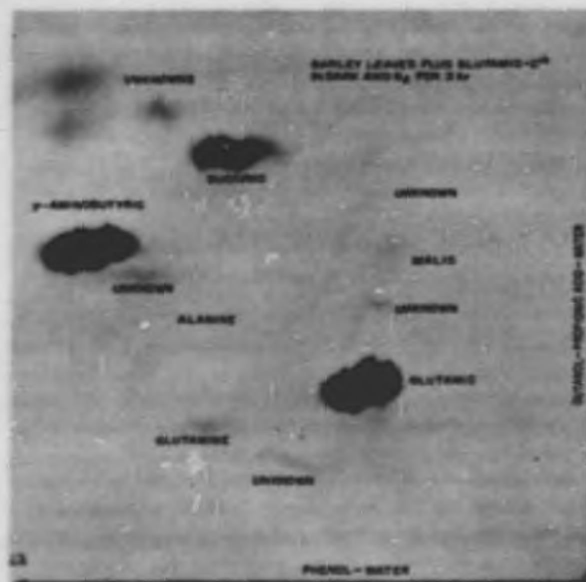


Fig. 46. Autoradiograph of Glutamic-C^{14} Acid Metabolism by Barley Leaves in an Atmosphere of Nitrogen.

Percentage distribution of the C^{14} in the leaf among the various products from the glutamic acid is recorded in Table 69 for four different experimental conditions of light or dark and an atmosphere of air or nitrogen. The time interval during which glutamic acid was available was varied from ½ hr to 3 hr. In Table 69 only 3-hr experiments in the dark and 1-hr experiments in the light are recorded. Data from 1-hr experiments in the dark or 3-hr experiments in the light showed no significant change in distribution of the metabolic products. Three hours in the dark was required to yield extensive metabolism of the glutamic acid, and 1 hr in the light best showed utilization of the glutamic acid with relatively little evidence of circulation of the C^{14} into the photosynthetic cycle.

The major product from glutamic acid under aerobic conditions was glutamine, the γ amide of glutamic acid. As expected, glutamine was not formed under anaerobic conditions, since its formation requires high-energy phosphate from oxidative phosphorylation. Under anaerobic conditions, however, glutamic acid was decarboxylated next to the amino group, yielding γ-aminobutyric acid as the major product. In light and nitrogen, a little oxygen was likely formed through photosynthesis, which would account for the results being part way between those for leaves in nitrogen and those in sufficient oxygen, as in air.

γ-Aminobutyric acid was identified by (1) the ninhydrin spray test on the chromatograms and its R_f values, (2) cochromatography of the radioactive compound with chemically synthesized γ-aminobutyric acid, (3) conversion of the unknown to its ureide, and (4) the failure of this compound to complex with $CuCO_3$ on paper chromatograms.

Glutamic acid decarboxylase is widely distributed in nature. When the glutamic-C^{14} acid was used as a substrate for this partially purified enzyme, γ-aminobutyric-C^{14} was formed in 100% yields, as shown by chromatography, followed by radioautography and the ninhydrin test. Thus the

TABLE 69. PERCENTAGE* OF C^{14} IN PRODUCTS FROM GLUTAMIC C^{14} ACID METABOLISM IN BARLEY LEAVES

Products	Dark and Air 3 hr	Light and Air 1 hr	Dark and Nitrogen 3 hr	Light and Nitrogen 1 hr
Glutamic acid	22.4	31.4	56.6	63.2
Glutamine	32.4	33.0	0.6	11.3
Malic acid	24.2	11.3	0.6	0.7
Succinic acid	2.4	3.3	5.4	1.5
Aspartic acid	6.5	2.3	Trace	0.1
Asparagine area	1.0	0.0	0.0	0.0
Unknown organic acid	4.3	1.3	Trace	1.1
Alanine	1.7	2.9	Trace	1.3
Threonine	0.6	0.3	Trace	0
Sucrose	0.4	5.4	0.5	0.5
γ-Aminobutyric acid	1.4	2.3	32.3	15.6
Unknown No. 1 (U_1)	2.1	1.1	0.9	0.3
Unknown No. 2 (U_2)	0.5	0.6	0.6	0.3
Unknown No. 3 (U_3)	0	1.3	Trace	1.7
Unknown No. 4, No. 5 (U_4, U_5)	0	0	2.5	1.2

*Difference between 100% and the sum of the values in each column is attributable to small amounts of other compounds not listed in this table.

product of glutamic acid decarboxylase action on glutamic acid has been identified as γ-aminobutyric acid. Furthermore, this enzyme functions actively in barley leaves under both aerobic and anaerobic conditions.

Other variations observed in glutamic acid metabolism are: (1) The labeling in succinic and malic acids indicates that glutamic acid is deaminated anaerobically *in vivo* to α-ketoglutaric acid and then to succinic acid, which cannot be further metabolized via the Krebs citric acid cycle in a nitrogen atmosphere. (2) Carbon-14 labeling in the organic and associated amino acids of the Krebs cycle does not appear rapidly in the photosynthetic products in the light, even in such metabolically active tissue as barley leaves. This indicates that the citric acid cycle is not functioning rapidly in the light. In terms of metabolic products, there appeared to be no major differences in glutamic acid metabolism in the light or dark.

Four apparently important compounds from glutamic acid metabolism remain unidentified. Unknown No. 1 has an R_f close to γ-aminobutyric acid. Unknown No. 2, on the chromatogram, had an R_f value near lactic acid, and unknown No. 3 was near fumaric acid. Unknowns No. 4 and No. 5 were formed in detectable amounts only in a nitrogen atmosphere. Their R_f values suggest that they may be cyclic compounds. Investigations are now in progress to determine whether they are produced from a further conversion of γ-aminobutyric acid.

Characterization of an Unidentified Compound Formed in the Metabolism of Biosynthesized Sedoheptulose-C^{14} in Plant Leaves

C. W. Nystrom N. E. Tolbert

In previous studies by Tolbert and Zill[8] on the metabolism of sedoheptulose-C^{14} in plant leaves, an unidentified compound containing C^{14} and formed in considerable quantities was observed on developed paper chromatograms of the extracts of leaves fed sedoheptulose-C^{14}. This compound, the one labeled "unknown" in the upper left-hand corner of the chromatogram shown in Fig. 47, had an R_f value of 0.80 in the phenol-water solvent system and 0.65 in the n-butanol–propionic acid–water solvent system. It was also formed when glucose-C^{14} was metabolized by either green or etiolated wheat and barley leaves. The compound has now been shown to be identical with phenyl glucoside and to be formed as a result of the detoxification of traces of phenol, which were acquired by the sugar solutions during their preparation from a plant source.

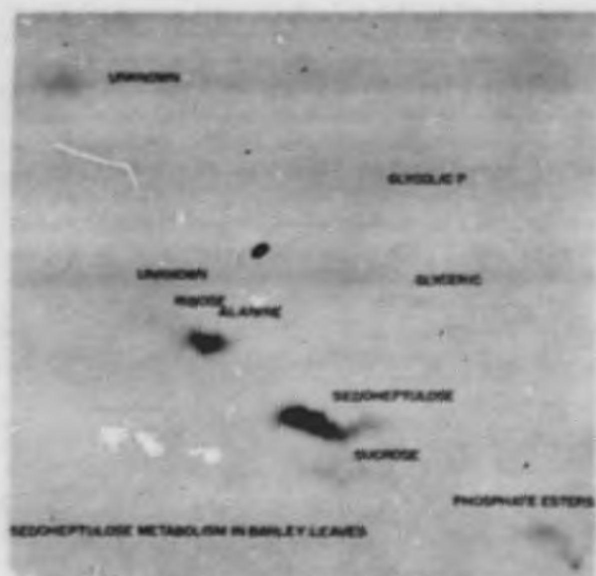


Fig. 47. Autoradiograph of a Chromatogram of Products from Sedoheptulose-C^{14} Metabolism in Barley Leaves.

Preliminary studies on the formation of the compound showed that, in either light or dark, the percentage of the C^{14} remaining in a leaf which metabolizes glucose-C^{14} or sedoheptulose-C^{14} increased in the compound with time. The compound was located on the developed chromatogram by its radioactivity and then eluted from the paper with distilled water. The eluate was treated with Polidase S and then rechromatographed. Glucose-C^{14} was the only C^{14}-containing compound found in the eluate. These results suggested that the compound might be either a detoxification product or, possibly, an active intermediate in glucoside metabolism.

[8]N. E. Tolbert and L. P. Zill, *Arch. Biochem. and Biophys.* 50, 392–398 (1954).

In order to test the possibility that the compound was a detoxification product of phenol used in the chromatographic preparation of sedoheptulose-C^{14} and glucose-C^{14}, a barley leaf was allowed to metabolize some glucose 6-C^{14} and glucose 1-C^{14} which would be free from such contamination. Only traces of activity could be detected in the area normally occupied by the compound on the developed chromatogram. Then 10 µl of phenol was added to the glucose-C^{14} solution, and the leaf was allowed to metabolize it. Chromatograms of the extracts of such a leaf contained several counts of C^{14} activity in the area occupied by the compound, indicating that the compound was probably a detoxification product of phenol. Some phenyl glucoside was synthesized and the compound cochromatographed in all the solvent systems tried with the synthetic phenyl glucoside.

The compound was eluted from a number of paper chromatograms and redeveloped in solvents free of phenol. Distilled water eluates of the area containing the compound on such chromatograms had an absorption spectrum identical with that of the synthetic phenyl glucoside. After boiling in dilute acid, the eluate had an absorption spectrum identical with phenol. Emulsin hydrolyzed the compound, yielding glucose-C^{14}. The compound, therefore, appears to be identical with β-phenyl glucoside.

Extraction of the biosynthesized glucose-C^{14} or sedoheptulose-C^{14} solutions with ether after acidification apparently removes all of the contaminating phenol, since none of the compound was formed when the sugar solutions treated in this manner were metabolized by barley leaves.

Growth Substance Activity of Serine

A. R. Kroll   A. W. Naylor

Carbon monoxide is metabolized by green barley leaves, yielding serine as the major product.[9] Since Zimmerman *et al.*[10] had reported that exposure of Turkish tobacco and tomato stems to CO was followed by adventitious root formation, it was thought this effect might be exerted through serine. With wild strains of *Neurospora*, this substance combines with indole in the formation of tryptophan.[11] Tryptophan is thought to be a precursor of indoleacetic acid, a well-known, naturally produced growth substance in plants.

A serine-lanolin mixture was smeared, in broad bands, around the stems of well-developed tomato plants. Lanolin alone was used on the control plants. In every instance, serine application was associated with the formation of large numbers of root primordia in the zone of treatment. In several cases, where the stems were horizontal, roots quickly emerged through the paste on the lower side of the stem. The lanolin controls exhibited no extra roots. Following some serine applications, leaves on the stems above the points of treatment showed epinasty. This did not occur when lanolin alone was used.

Subsequent to serine application, callus formation characteristically occurred. The first indication of an effect with serine is a darkening of the stem in the treated area. This is followed by a gradual, locally confined bleaching and enlargement in size. Numerous tiny mounds indicative of the presence of root primordia appear. This may be followed by emergence of individual roots or cracking of the cortex into strips, revealing hundreds of roots inside.

Additional evidence of growth substance activity has been shown with beans. The growing tips of two-week-old Black Valentine seedlings were severed about 3 cm above the unifoliate leaves. Normally, this operation is followed by rapid growth of the buds in the axils of the leaves, but such growth can be suppressed by the application to the top of the cut stem of substances showing auxin activity. Serine-lanolin paste applied to the cut stump inhibits growth of these buds but lanolin alone does not. This effect is shown in Fig. 48, where A represents a serine-treated group, B a tryptophan-tested group, and C a lanolin-treated group of bean seedlings.

Isolation of a Photosynthetic Oxidation-Reduction Coenzyme from Green Plants

A. R. Kroll

A compound which behaves physiologically as if it were a reduced coenzyme has been isolated from barley and corn leaves and from the green

[9] A. R. Kroll and N. E. Tolbert, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 102–103.

[10] P. W. Zimmerman, W. Crocker, and A. E. Hitchcock, *Contrib. Boyce Thompson Inst.* 5, 1–17 (1933).

[11] E. L. Tatum and D. M. Bonner, *J. Biol. Chem.* 151, 349 (1943).

Fig. 48. Bud Growth on Bean Stems Following Treatment of Severed Stem Tip with: A, Serine-Lanolin Paste; B, Tryptophan-Lanolin Paste; and C, Lanolin Paste. (Treated 8/7/55; photographed 8/12/55).

alga, *Chlorella*, by the column chromatographic method discussed previously.[12] Phosphorus-32-labeled tissue that is exposed to light of about 2000 ft-c up to the instant of immersion in boiling 30% ethanol contains a small amount of an unknown compound (5a).. If darkened a few seconds before exposure, it contains none of the material. If illuminated with flashbulbs (>>10,000 ft-c) at the instant of immersion, large amounts of label are found in the material. Thus its production is dependent on illumination of the tissue.

Label accumulates in 5a in tissue which is illuminated at normal levels (2000 ft-c) if the oxidative process known to be associated with CO_2 uptake or phosphate exchange is inhibited. The oxidative process, which terminates in cytochrome oxidase, may be inhibited by CO in the presence of red but not in yellow light. Since P^{32} accumulates in 5a in leaves illuminated with red light under CO but not in those illuminated with yellow light, the compound produced by illumination is oxidized via cytochrome oxidase in a coupled system which may be represented by Fig. 49.

It was also possible to pile up P^{32} in 5a by illuminating the leaves in a nitrogen atmosphere or under evacuation, both of which are conditions known to inhibit carbon dioxide uptake.

The compound is not the direct product of a light-

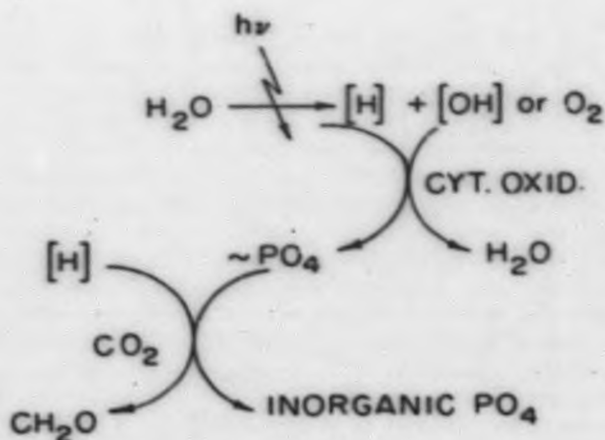


Fig. 49.

driven phosphorylation since its level changes only slowly upon cessation of oxidative activity. Its level is very high in those plants inhibited by CO under red light, conditions which give the lowest level of P^{32} attainable in adenosine triphosphate, which is also separable by the ion-exchange method used. No change in level of ATP can be noticed in the flash illumination experiments which pile up high levels of the substance. If, however, the tissue is exposed to an anaerobic environment for 1 hr or more, 5a does not pile up. This can be interpreted as evidence for lability of the phosphate bond involved, requiring its continual resynthesis in the plant. This lability is borne out by the rapid decomposition of the material to inorganic phosphate after isolation.

This compound may be labeled with S^{35} as well as P^{32}. Paper chromatography of the two differently labeled materials give spots at the same place on the chromatogram. The isolated material gives a positive test for SH groups, the decolorization of an iodine-azide solution being used as a measure of –SH. Calvin has proposed that lipoic acid is the compound first reduced upon photolysis of water. As yet it has not been possible to confirm the presence of lipoate in this compound with the pyruvic oxidase test of Gunsalus *et al.*[13] This may be caused by low concentrations of the material, or by its being some other compound than lipoic acid.

[12] A. R. Kroll and L. P. Zill, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 83.

[13] I. C. Gunsalus, M. I. Dolin, and L. Struglia, *J. Biol. Chem.* 194, 849–857 (1952).

GENERAL PHYSIOLOGY

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Horizontal Tubeless Centrifuge Head

N. G. Anderson

The technique of gradient differential centrifugation[4] by use of sector-shaped tubes has been extended with the design of a centrifuge head (Fig. 50) in which a doughnut-shaped mass of fluid having a gradient of decreasing densities from the edge to the center is spun. The gradient is introduced through an annular opening in the center and flows to the outer edge through two stainless steel tubes while the head is in motion. The brei is layered on top of the gradient through the central opening shown. After centrifugation is completed, the gradient is removed by displacing it with a denser liquid. As the gradient is raised, it drains out through small tubes in the bottom of the head into the collecting head shown in the lower part of Fig. 50. All operations are conducted with the head spinning. The model now in use utilizes a 5-liter gradient and operates at 1000 rpm. It is designed chiefly for the large scale separation of nuclei and mitochrondia. Higher speed models are contemplated.

Organization of the Soluble Phase of the Rat Liver Cell

N. G. Anderson M. L. Anderson

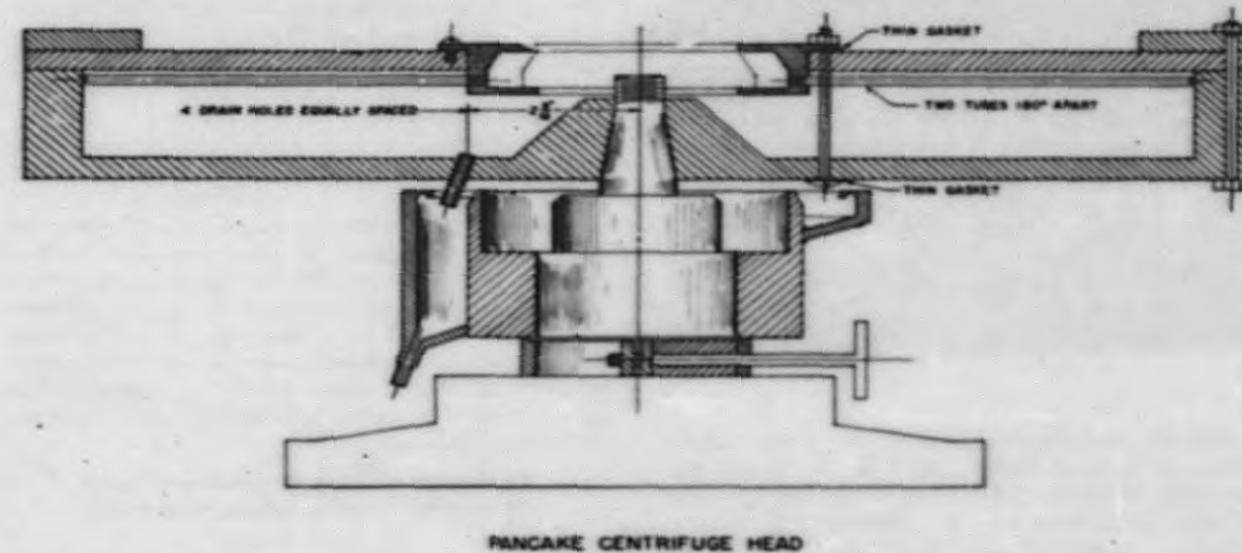
As a preliminary step in a long-term project (the Cell Molecular Census Project) aimed at the isolation of the major classes of proteins from various cells and tissues, a study of the soluble proteins of rat liver has been undertaken. These have been isolated from livers rapidly perfused with Locke's solution. Breis are prepared in

[1] Research participant.

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

[3] ORINS predoctoral fellow.

[4] N. G. Anderson, *Science* 121, 775–776 (1955).



PANCAKE CENTRIFUGE HEAD

Fig. 50.

0.25 *M* sucrose by a loose fitting coaxial "homogenizer" in which the plunger is moved vertically. Centrifugation is carried out in two steps – at 18,000 × *g* for 20 min and at 104,000 × *g* for 60 min. Electrophoresis in veronal buffer (pH 8.6) and phosphate (8.6) gave reproducible patterns in the ascending limb (Fig. 51), but anomalous disturbances and precipitations appeared in the descending limb. Evidence indicates that the precipitation is caused by the migration of certain complex-forming proteins away from the proteins which they normally solubilize. Changes observed in the cytoplasm of cells placed in an electric field might be explained on this basis. Several other properties of living cytoplasm have their counterpart in this preparation. Thus freezing and thawing produce either precipitation or gelation. However, the addition of glycerol, which is now widely used to preserve cell viability during freezing and thawing, prevents the changes otherwise observed in the soluble phase. Incubation at 37°C for 15 min results in the formation of a voluminous precipitate, a change considered to be analogous to the gelation which occurs during cytolysis. A detailed study of the mechanisms of these reactions is in progress.

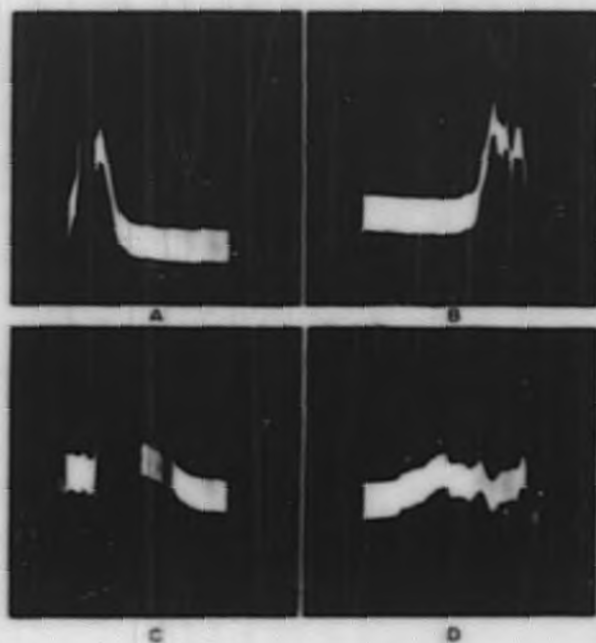


Fig. 51. Electrophoresis of Rat Liver Soluble Phase in Veronal Buffer, pH 8.6. A, Descending boundary showing precipitation as blank space; B, ascending boundary; C, descending boundary showing precipitation area and precipitated protein as shaded area; D, ascending boundary showing multiplicity of peaks.

The isolation of classes of soluble proteins and of individual molecular species by three methods has been started; namely, isoelectric precipitation, zonal electrophoresis on starch, and electroconvection. The first method was adopted after a study of the effect of pH on the stability of the fresh undialyzed preparation showed it to be stable between pH 1.2 and 4.2, and pH 6 and 10. At pH 5, 25% of the soluble nitrogen was precipitated in 90 min at 0°C. Several reprecipitations, with necessary changes in pH, were used to purify the precipitate. Electrophoretic examination at pH 8.6 showed that one very fast component predominated.

Electroconvective separations carried out at pH 7.8 in such a manner that only proteins isoelectric at this pH were separated are being characterized. Carbon electrodes, which were used in orienting experiments, were unsatisfactory since a consistent drop in pH with time was observed even though the buffers in the two electrode compartments were constantly mixed. Installation of platinum electrodes corrected this difficulty completely.

Theoretically, a protein fraction isoelectric at the pH used may be obtained in better than 90% purity in one run by electroconvection. Further purification is difficult since insufficient impurities are present to electroconvect. This problem has been solved, insofar as pure protein antigen production is concerned, by the addition of pure plasma albumin from the animal in which the antibodies are to be produced. By this method, purities of 99.9% with respect to contamination by other rat liver proteins is theoretically possible in three or four runs.

Production of Density Gradients in the Horizontal Tubeless Centrifuge Head

J. R. C. Brown

Continuous density gradients of known composition and rate of change with radius have been

produced in the horizontal tubeless centrifuge head. For production of the gradient, a Lucite box having a volume equal to the total volume of solution desired in the gradient is divided into two compartments by a thin partition. The curvature of the partition is mathematically calculated to produce the desired gradient, provided that the levels of the solutions (light and heavy) in the two compartments are kept equal at all times during outflow. Since the heavy solution is much denser and more viscous than the light solution, a hand-operated leveling device on the outflow is necessary to maintain equal levels in the two compartments. A magnetic mixer installed in the line assures thorough mixing of the two solutions prior to their introduction into the centrifuge head.

Calculation of the curvature of the partition must take into consideration the fact that the volume of the head increases as the square of the radius. Thus, for a straight-line gradient, the curve should be that of the parabola $V = c\pi r^2$, where V is the volume, c the constant depth of the head, and r the radius. Since the head is not filled to the center, only that sector of the parabola between r_{min} (distance from center to surface of gradient) and r_{max} (distance from center to outer limit of gradient) is used. Figure 52 shows the theoretical gradient and the actual recovery of material from a sample run with a gradient formed by mixing 13% (wt/vol) sucrose and 60% (wt/vol) sucrose as the light and heavy solution respectively. The concentration of the recovered material was determined by refractometer on samples taken at 500-ml intervals. The obvious mixing of material at the start of the gradient may be corrected by installation of radially arranged baffle plates which have since been installed but not yet tested.

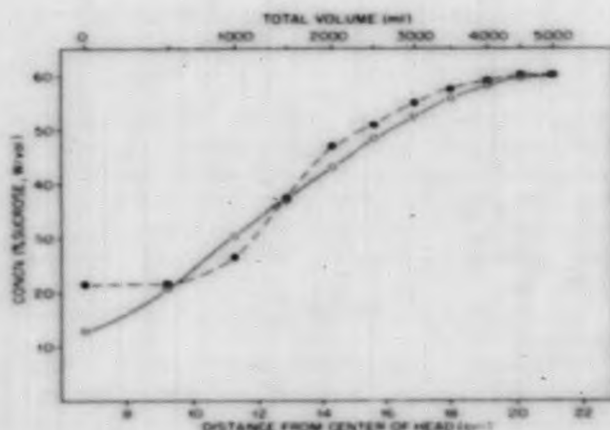


Fig. 52. Theoretical Gradient and Actual Recovery of Material from a Sample Run. O—, Theoretical curve; ●---, recovery curve on run July 14, 1955.

Purity of Nuclear Fractions of Tissue Homogenates

J. R. C. Brown

Nuclear fractions of thymus homogenates isolated in 0.25 *M* sucrose–0.0018 *M* $CaCl_2$ have been shown to contain large numbers of intact small thymocytes, identifiable on basis of their osmotic activity as contrasted with the nonosmotic behavior of isolated nuclei.[5] These thymocytes, which are probably identical with small lymphocytes, have a very thin layer of cytoplasm and are indistinguishable from isolated nuclei by microscopic examination of the homogenate. Further investigations have demonstrated that exposure of nuclear fractions and homogenates containing osmotically intact thymocytes to enzymic digestion by chymotrypsin or deoxyribonuclease leaves these cells unaffected. Since resistance to enzymic digestion is considered one of the criteria of living cells, this indicates that these cells are not only osmotically intact, but in all probability still viable.

Nuclear fractions of thymus homogenates prepared in the citric acid media, or in markedly hypotonic media, do not contain osmotically active or enzyme-resistant cells. Low pH, ion binding, and hypotonicity appear, therefore, to favor rupture of the cell membrane.

Repetition of these experiments on spleen homogenates has indicated a similar contamination by whole cells in the nuclear fraction of this tissue.

[5] J. R. C. Brown, *Science* 121, 511–512 (1955).

An Analysis of the *in vivo* Effect of Nucleoprotein from Ehrlich Ascites Cells [6]

E. C. Horn M. E. House

The intraperitoneal injection of nucleoprotein (NP) extracted from Ehrlich ascites tumor cells reduces the mean survival time of mice when they are subsequently challenged with living ascites tumor cells.[7,8] This study was made to determine the cause of accelerated death in such pretreated mice.

The method of preparing the NP has already been outlined.[7]

Difference in Mean Survival with Time After a Single Pretreatment with NP. – Thirty mice were injected intraperitoneally with 0.4 ml of an NP preparation (N/P = 5.86; 10.3 mg dry weight). Thirty control animals were set aside at the same time and treated identically except for lack of pretreatment with NP. At weekly intervals beginning on the fourteenth day and continuing for five weeks thereafter, five experimental and five control mice were challenged with an ascites cell suspension. Their deaths were recorded twice daily and the mean survival time for each group calculated. The results are shown graphically in Fig. 53. Since the difference between the mean survival time of the controls (S_c) and that of the experiments (S_e) fluctuates with the number of ascites cells used for challenge, a quotient obtained by dividing the difference in mean survival time of the two groups by the mean survival of the controls $(S_c - S_e)/S_c$ allowed for comparison between different challenges. This quotient is plotted against time in all the graphs which involve differences in mean survival time. The data in Fig. 53 for the simultaneous injection of NP and ascites cells (day zero) and that for the seven-day interval were taken from another experiment which was comparable in treatment but in which the same series of animals was not used.

The data show some irregularity but a maximum decrease in mean survival time (high quotient) is evident three weeks after the injection of NP; thereafter this difference decreases somewhat more gradually than the initial rise to the maximum. Similar results were reported earlier in much less complete form and have been corroborated by additional experiments.

Modification by a Second Injection of NP. – Twenty mice, prepared identically to those used for the time data, were given a second injection (0.1 ml) of NP (N/P = 5.41; 1.8 mg dry weight) on the thirty-fifth day of the experiment. Ten animals received the material intravenously; ten, intraperitoneally. Five of each group were challenged with ascites cells 7 and 14 days after the second injection (or 42 and 49 days after the initial injection). From Fig. 53 it is readily apparent that the mean survival time for the mice receiving a second injection of NP has been drastically shortened not only when compared to controls but also to those mice receiving but one injection.

Modification by an Injection of Killed Ascites Cells or Ascites Fluid. – In these experiments cell-free ascites fluid or washed ascites cells killed with weak formalin solution were used for a second injection into NP-prepared animals. The fluid was obtained by centrifuging clear, pooled ascites suspensions and passing the supernatant fluid through a sterilized, sintered glass

Fig. 53. Relative Differences in Survival Between Control Mice (S_c) and NP-injected Mice (S_e) When Challenged with Ascites Suspensions at Different Time Intervals After the NP Injection. The dotted arrow indicates a second NP injection at 35 days. Broken lines connect points indicating relative survival for mice receiving the second injection either intraperitoneally (IP) or intravenously (IV).

[6] This work was supported in part by research grant (RG-4203), National Institutes of Health, Public Health Service.

[7] E. C. Horn and M. E. House, *Biol. Semiann. Prog. Rep.* Feb. 15, 1955, ORNL-1863, p 109–111.

[8] E. C. Horn, *Biochim. et Biophys. Acta* 16, 440–442 (1955).

filter. The formalin-killed cells were obtained by suspending saline washed cells in 0.5% formalin-saline for 24 hr at 4°C. These cells were then washed thoroughly in 0.14 *M* NaCl by successive suspension and centrifugation. Final suspensions for injection were made in saline.

Twenty-one days after the initial NP injection, four mice were injected intravenously with 0.5 ml of a 50% suspension of killed cells; five uninjected controls received the same treatment. Twenty-one days later (day 42 of the experiment), both groups were challenged with the same ascites suspension used for the injection of mice already described for day 42 in the time study. The mice which had received killed cells alone showed the greatest decrease in mean survival time yet observed, whereas the mice prepared first with NP and then with killed cells showed no difference in mean survival time from the control ascites-injected mice of day 42 (Fig. 54).

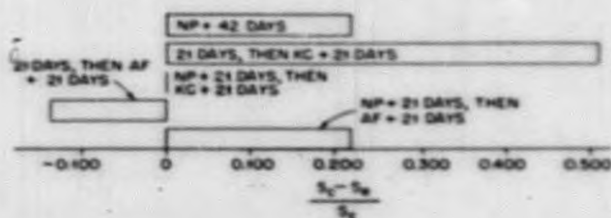


Fig. 54. Relative Differences in Survival When Challenged with Ascites Suspensions 42 Days After Treatment with NP and with Killed Cells (KC) or Ascitic Fluid (AF).

Five NP-injected mice were given, intravenously, a second injection of 0.4 ml of undiluted cell-free ascites fluid (approximately 8 mg of protein) 21 days after the initial NP injection; four unprepared controls received identical treatment. Twenty-one days later (day 42 of the experiment), both groups were challenged with the same ascites cell suspension used for all other 42-day challenges. The animals receiving the fluid alone actually survived slightly longer than the ascites control whereas those that received NP and later the fluid injections showed a difference in mean survival from the controls identical to that of the group which had been prepared with NP alone 42 days earlier (Fig. 54).

Modification by Cortisone. – A fourth group of 20 mice which had been prepared with NP on day zero, identical in every respect to all previously mentioned NP-injected mice, were given an additional 0.1-ml injection, intravenously, of NP on the fifty-sixth day of the experiment. Ten of these mice were administered 1 mg each of cortisone intramuscularly on days 60, 61, and 63. On the sixty-third day, all 20 of these animals were challenged, together with uninjected controls. As expected, the "boosted" NP-injected animals succumbed earlier than the controls, but the NP-prepared animals which received the cortisone treatment outlived the controls (Fig. 55). Although suggestive, these results must be conditioned by the indication, in a separate experiment, that cortisone given in the manner described does extend slightly the mean survival time of untreated mice challenged with Ehrlich ascites tumor (Fig. 55), contrary to the findings of others.[9]

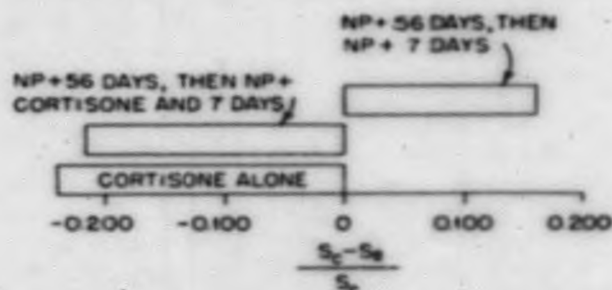


Fig. 55. Effect of Cortisone on the Difference in Mean Survival Time in Treated and Untreated Mice Challenged with Ascites Tumor Cells.

Difference in Mean Survival Time as a Function of the Amount of NP Injected. – Three groups of mice were injected intraperitoneally with an NP preparation as follows: 16 mice received 0.1 ml, 16 received 0.5 ml, and 12 received 2.0 ml. Each week thereafter for four weeks, untreated control animals of the same age and four each of the first two injected groups were challenged with ascites tumor cells. The data from the differences in mean survival time are plotted in Fig. 56. Those receiving 0.1 and 0.5 ml showed the usual

[9]R. G. Gottschalk and A. Grollman, *Cancer Research* 12, 651–653 (1952).

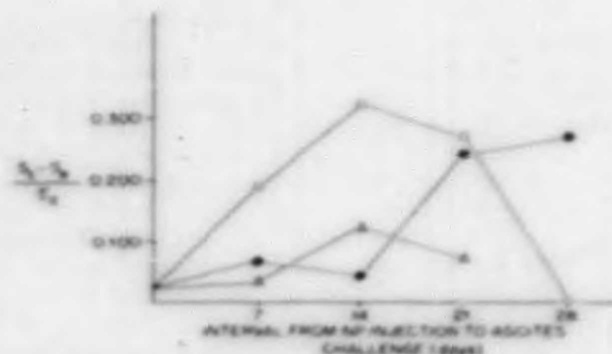


Fig. 56. Relative Differences in Survival When Challenged with Ascites Suspensions at Different Time Intervals After Different Initial NP Injections. ●, 0.1 ml; O, 0.5 ml; Δ, 2.0 ml.

response, within the limits of the experiment, although the mice with the lower dosage of NP responded later. The mice receiving the highest dose (2.0 ml) did not show the characteristic response during the first three weeks.

Specificity of the Reaction. – A number of substances have been tested to determine the specificity of the response to the NP, including protamine, histone extracted from chicken erythrocyte nuclei, histone extracted from ascites cell nuclei, and NP extracted from rat lymphoma tissue.

Protamine and chicken erythrocyte histone produced no significant depression of the mean survival time. Since ascites cell histone, on the other hand, produced irregular reductions in the mean survival time, an account of its preparation is given here.

Isolated nuclei, prepared as for NP extraction, were treated with 0.2 *N* HCl overnight at 4°C. The suspension was centrifuged at 8000 × *g* for 30 min, and the clear supernate was neutralized by the gradual addition of dilute NaOH. Any precipitate which appeared during this process was removed by further high-speed centrifugation for 30 min. At or near pH 6, a white precipitate invariably appeared – very likely, an acid protein component. Any clear supernate with pH 7 which was obtained was dialyzed against several changes of distilled water for 24 hr. The histone solution was then centrifuged again at 8000 × *g* whether or not it appeared clear, and the supernate was quick frozen and lyophilized. The pure white, water-soluble product was administered as an aqueous solution.

Twenty mice were each given 4.64 mg of ascites cell histone as a 1-ml intraperitoneal injection; 20 additional mice of the same age served as untreated controls. Groups of five treated and five untreated mice were challenged each week for four weeks, and their survival recorded (Fig. 57). Although obvious differences in mean survival time are exhibited, the pattern is erratic.

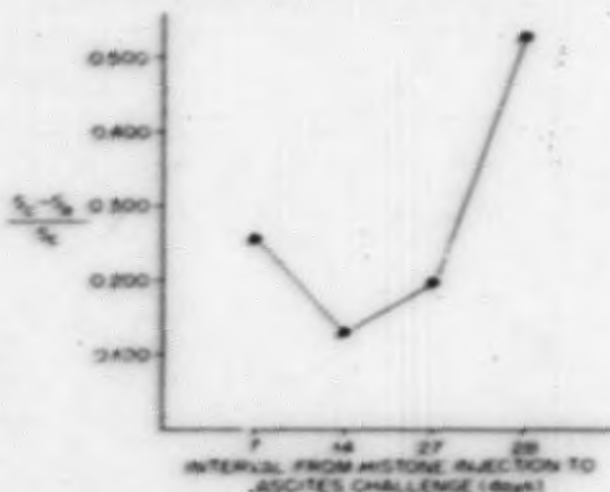


Fig. 57. Relative Differences in Survival When Challenged with Ascites Suspensions at Different Time Intervals Following an Initial Injection of NP Treated with Deoxyribonuclease.

No depression in the mean survival time was discovered in a small series of experiments involving ten mice injected with an NP preparation derived from a rat lymphoma.

In an attempt to elucidate the criticality of undissociated NP for the effect, 20 mice were prepared with an intraperitoneal injection of NP treated with deoxyribonuclease (the injected material had lost all typical viscosity). These animals were challenged in groups of five each 14, 21, 28, and 42 days after the initial injection. The differences in mean survival time are shown in Fig. 58, a result resembling, in its irregularity, that obtained with histone.

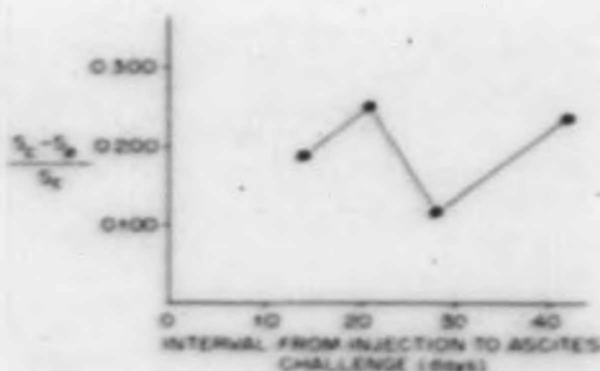


Fig. 58. Relative Differences in Survival When Challenged with Ascites Suspensions at Different Time Intervals Following an Initial Injection of Histone Extracted from Ascites Cell Nuclei.

Effects on Tumor Cells. – A few animals which had received an NP injection 35 days prior to an ascites challenge were sacrificed along with untreated controls at days 2, 3, 4, 5, and 7 after the tumor inoculation. The spleen, liver, fat bodies, and a kidney were fixed, stained, sectioned, and examined for tumor invasion. The results were essentially those reported by Klein and Revesz,[10] namely, initial invasion of the tissues was not observed until the fourth or fifth days after tumor inoculation. No difference was seen between treated and untreated animals.

Cell counts and hematocrits were also made of the ascites cell suspensions from control and treated animals. Again no difference in tumor-cell counts per unit volume of ascites fluid was found between controls and treated animals, although the hematocrits were invariably higher in the ascites from treated mice, suggesting either larger tumor cells or more contaminating cells of other types. This finding is being investigated more fully.

Tests for NP-induced Sensitivity. – Different tests were conducted in an effort to show more conclusively whether the NP injections produced in the mice a sensitivity demonstrable through anaphylactic responses. Twenty-one, 28, and 35 days after an initial intraperitoneal injection of NP when, according to the mean survival data, a titer against NP should be high, a number of animals were given a second injection of NP at different dose levels (0.1, 0.4, and 0.5 ml) and by a variety of routes (intravenous, intraperitoneal, subcutaneous and intravenous, and intravenous and intraperitoneal). In no case could any anaphylactic manifestation be demonstrated either by examining the ear vessels[11] or by noting more obvious symptoms. The Arthus reaction, so clearly demonstrated with bovine plasma albumin,[12] was attempted, with no success.

It should be pointed out that, during the experiments, the expected anaphylaxis was not noted in many of the animals which had received as a second injection varying amounts of killed cells or ascitic fluid. Furthermore, there was no death in animals which had received many times the amount of NP used routinely to produce the reduction in survival time, nor in any animals injected with NP alone.

In conclusion, the variation in mean survival time with the amount of NP injected and with the time after the injection proceeds in such a manner as to suggest the production of a sensitivity to the nucleoprotein in the treated mice. The earlier death of treated mice is thought to be caused by a reaction between the sensitized mouse and the reintroduced antigen in the form of the ascites cell challenge. The data showing the response to two widely spaced injections of NP prior to the ascites cell challenge strongly suggest the typical "secondary response" which is elicited when an antigen is reintroduced into an animal already immunized to it.

Antigenicity of Ascites Tumor-Cell Components

E. C. Horn M. E. House

Because of the conclusions reached in the preceding paper demonstration of the antigenicity of nucleoprotein preparations extracted as already described[8] was considered imperative. At the same time preparations of other ascites cell components, whole ascites cells, and a general mouse liver homogenate were obtained as antigens for comparative serological purposes.

[10] G. Klein and L. Revesz, *J. Natl. Cancer Inst.* **14**, 229–277 (1953).

[11] F. D. McMaster and H. Kruse, *J. Exptl. Med.* **89**, 583–596 (1949).

[12] A. A. Benedict and R. L. Tips, *Proc. Soc. Exptl. Biol. Med.* **87**, 618–622 (1954).

Thus far, rabbit antisera to washed, whole ascites cells (AC), ascites cell mitochondria (AM), ascites cell nuclei (AN), and ascites cell nucleoprotein (ANP) have been obtained.

The testing of these antisera presented complications. Precipitin reactions were worthless because of NP insolubility in physiological saline. Attempts to extract the water-soluble pseudoglobulin fraction from ANP sera and to make tests with NP in distilled water gave highly dubious results. Agglutination of cell suspensions proved quite feasible for AC (Fig. 59). Uniform, easily reproducible suspensions of nuclei and mitochondria were extremely difficult to prepare, and agglutination results were untrustworthy. Complement fixation appeared to be the best test reaction on which to base a comparison of the various antisera in their reactions with all the different antigens employed.

Table 70 presents the complement fixation titers of the various antisera reacted with the antigens thus far tested. Ranges are given because the titers, when repeated often vary by one dilution. The dilutions used throughout are twofold, beginning with 1:10. The titers are 50% end points; two units of complement were used throughout.

The results indicate widespread interaction between an antiserum to ascites cells, or to ascites cell components, and any of the antigens. In all instances, these ascites antisera reacted equally well with some heterologous antigen and with their homologous antigen. These results could be explained simply if each antigen preparation contained the other antigens. Methods of antigen preparation do not exclude this possibility in at least one respect, i.e., there could exist a surface antigen common to the cell surface, the nuclear surface, and the mitochondrial surface. If such a common antigen exists with specificity typical of the ascites cell (in this instance), this in itself would be of profound interest and importance. Contamination of the antigens on the particle level is highly dubious. The presence of even small amounts of mitochondria in a nuclei of

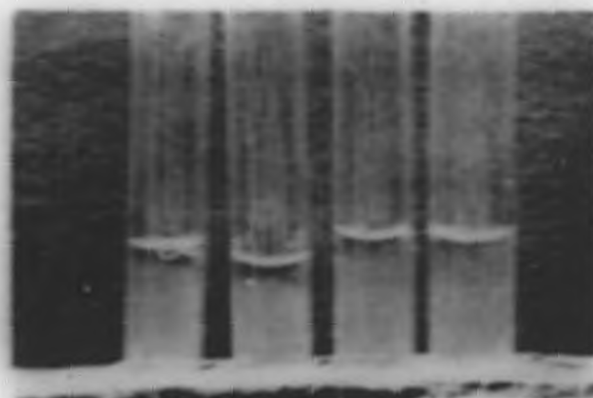


Fig. 59. Agglutination of an Ascites Cell Suspension as a Test Method. From left to right the tubes contain, in addition to equal volumes of a known ascites cell suspensions, equal volumes of Tyrode's solution, NRS, ANP serum, and AC serum (1:160). The tubes are standard 10 × 75 mm.

TABLE 70. COMPLEMENT FIXATION TITERS FOR THE VARIOUS ANTISERA AND ANTIGENS FROM ASCITES CELLS AND THEIR COMPONENTS

Antisera	Antigens			
	Cells	Nuclei	Mitochondria	Nucleoprotein
AC	80–160	(40)[a]	160	10–20
AN	40–160	160	320	80
AM	80–160	(160–320)	160–[illegible]	20
ANP	40	(20–40)	80	40–80
AL[b]	0[c]	(10)		0

[a] All titers in parentheses indicate a single antiserum; the rest are of pooled antisera.
[b] Antiserum to mouse liver homogenate. This had a titer of 160 to mouse liver homogenate.
[c] This titer was obtained with agglutination rather than complement fixation.

nucleoprotein preparation, for example, has not been confirmed by phase-contrast microscopy, nor is it likely that mitochondria would survive as centrifugally precipitable particles in the 1% citric acid–distilled water washes used for nuclear isolation. However, examination of the complement fixing ability of the AN and ANP sera with mitochondria as the antigen reveals high titers in both instances.

At present, no attempt will be made to draw conclusions from these data. It is hoped that the program now in progress, the systematic study of absorption of these antisera with the variety of antigens available, will reveal more specifically the meaning of these interactions. Evidence for fundamental differences in these antisera, not yet revealed by serological tests thus far used, is provided in the succeeding section.

Cytotoxicity of Different Antisera to Ascites Tumor Cells

E. C. Horn M. E. House

To supplement the serological data on rabbit antisera to different fractions of the Ehrlich ascites tumor cell, *in vivo* and *in vitro* exposures of ascites cells were made and their effect on mouse survival was tested by intraperitoneal injection of known numbers.

***In vivo* Exposures.** – Five groups of ten mice each received the following intraperitoneal injections: 0.1 ml of undilute antisera to AN, AM, and ANP; dilute (1:10) to AC; and undilute NRS (normal rabbit serum). Ninety minutes after initial injection, each mouse was challenged with an injection of 6.25×10^{6} ascites cells in saline. Serum injections were repeated on days 2, 4, and 6 following the tumor challenge, and the survivors noted for each day until all mice were dead.

No significant increase in mean survival time was noted in experimental groups over the NRS group (Fig. 60). It is possible, of course, that a larger amount of antiserum might have produced a significant difference in the mean survival time of any experimental group.

***In vitro* Exposures.** – Equal aliquots of a saline suspension of ascites cells were combined with equal parts of various antisera (AC, AN, AM, ANP), NRS, and saline. The mixtures were incubated for 1 hr at room temperature. All sera were undilute except AC, whose dilution was 1:20 before addition to the cell suspension. During incubation, cells agglutinated rapidly in the AC

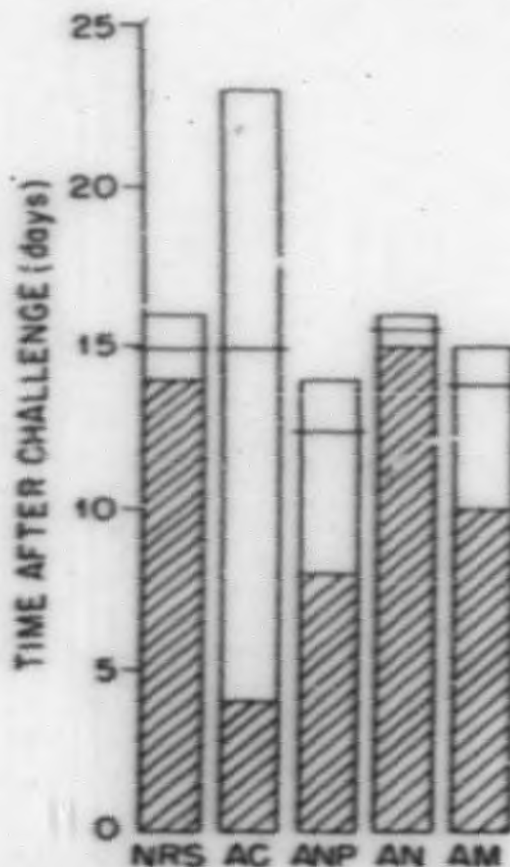


Fig. 60. Pattern of Survival for Mice Injected with Ascites Tumor Cells and Successive Aliquots of Different Rabbit Sera. Hatched bars indicate period of survival for all animals of a group; open bars, period from first to last death in a group. The horizontal line marks the mean survival time of the group. See text for serum abbreviations.

serum; very slowly in AN, AM, and ANP; very slightly in NRS; and not at all in the saline suspension.

Each group of incubated cells was then thoroughly resuspended and injected intraperitoneally into ten mice for each group, each mouse receiving 6.25×10^{6} cells. Survival of the mice was followed and noted by two daily observations until all were dead. The results (Fig. 61) indicate that the AN and AM sera cause a lengthened mean survival time.

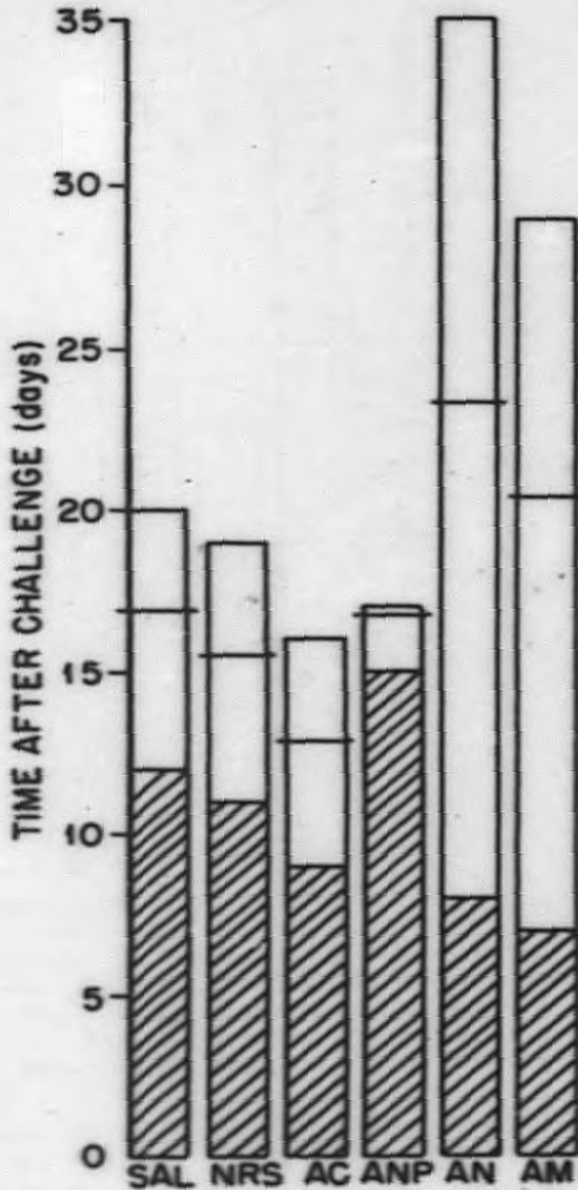


Fig. 61. Pattern of Survival for Mice Injected with Ascites Tumor Cells Which Have Been Incubated in Different Media. See Fig. 60 for explanation.

The experiment was repeated in essentially the same manner except that the AC serum was used undiluted and three additional antisera were tested – antimouse liver homogenate (AL), anti-ascitic fluid (AAF), and antibovine plasma albumin (ABPA). AC, AN, and AM sera were tested at different low dilutions. There was little or no indication that the effect was obtained after one twofold dilution. Results from this second *in vitro* test are shown in Fig. 62. Survival times are different from the previous test because the ascites cell suspension dilution was different. Although not all experimental animals had died

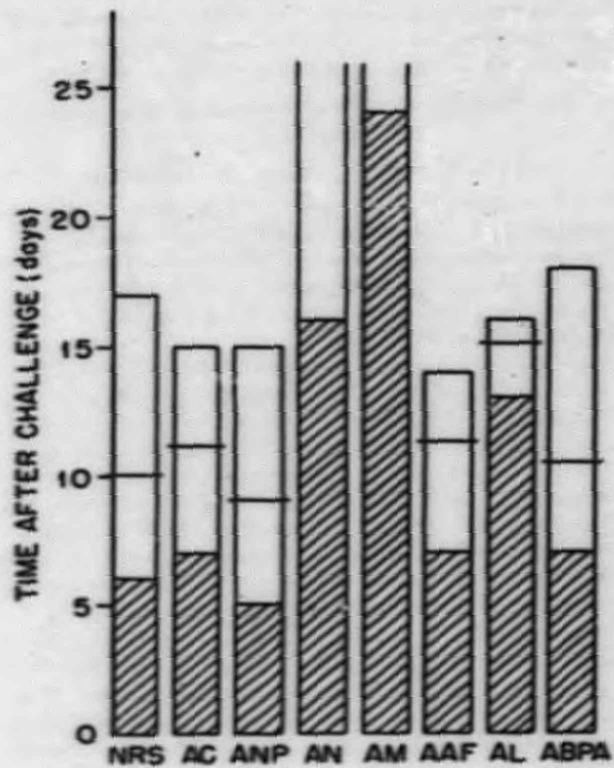


Fig. 62. Pattern of Survival for Mice Injected with Ascites Tumor Cells Which Have Been Incubated in Different Rabbit Sera. Some mice of groups AN and AM still alive.

when the data were collated, the results are sufficiently complete to bear comparison with the earlier experiment. The effect of AN and AM sera is even more striking than before in the lengthening of the mean survival time of the mice within these two groups. Of the other antisera tested, only that prepared against mouse liver homogenate deviated enough from the control to warrant special attention. Not only was the mean survival time of this group lengthened somewhat beyond that of the control, but the pattern of death in this group was remarkable in its brevity; all died in the short span of three days compared, for example, to the ten days during which control mice died.

Since these antisera are the same as those used to accumulate the data on titer of the previous section, it is of especial interest to compare their effectiveness in increasing the mean survival time of mice with titer. Examination of the titers of the AC, AN, and AM sera against whole cells (Table 70) shows them to be essentially the same by the complement fixation test. The results of the *in vitro* test reported here, however, indicate that differences exist which are not yet revealed by complement fixation. There is the possibility that absorption of the antisera with different antigens will supply some clue to these differences.

MOLECULAR BIOPHYSICS AND RADIOLOGICAL PHYSICS

J. S. Kirby-Smith — J. N. Dent[1]
M. Slater — D. L. Craig

Molecular Biophysics

Effect of Radiation on *Tradescantia* (J. S. Kirby-Smith and D. L. Craig). – Investigations of the relative biological effectiveness (RBE) of various ionizing radiations on the induction of chromosome aberrations in the *Tradescantia* pollen grain mitosis have been concluded with a study in which were used 2.5-Mev monochromatic neutrons from the Biology Division Cockcroft-Walton facility. An RBE of 12.5 for these fast neutrons relative to Co^{60} γ rays has been observed, slightly larger than the value of 10 previously observed in work with neutrons of mean energy 1 Mev from the ORNL 86-in. cyclotron. The results of these experiments and previous figures for other radiations[2] are given in Table 71.

TABLE 71. RELATIVE BIOLOGICAL EFFECTIVENESS OF VARIOUS RADIATIONS ON CHROMOSOME ABERRATIONS IN *TRADESCANTIA* POLLEN

Radiation	RBE
Co^{60} γ rays (1.17 and 1.33 Mev)	1
P^{32} β rays (mean energy 500 kv)	1
200-kv mean-energy X rays (0.06 A)	1.5
60-kv mean-energy X rays (0.20 A)	2
Fast neutrons (1-Mev mean energy)	10.0
Fast neutrons (D-D; 2.5 Mev)	12.5

An investigation of the time versus intensity relations for chromosome aberration in *Tradescantia* pollen exposed to X and γ rays has been continued. These experiments temporarily complete the studies of the response of this material to radiation and are designed to give some idea of the average time during which breaks remain open in pollen. Dose curves obtained at intensities over a range of 5 to 380 r/min have shown no significant intensity effects, indicating that the primary breaks produced have an average lifetime far in excess of the figure of 4 min found for *Tradescantia* microspores.

Amphibian Radiobiology (D. L. Craig and J. S. Kirby-Smith). – Study of the elementary cytology and radiation sensitivity of regenerating tail-tip tissue of salamanders of the genus *Gyrinophilus* includes investigation of the effects of X rays on mitotic inhibition and the production of chromosomal aberrations in this material.

The change in the number of dividing cells during a 47-hr period after administration of 100 r of 250-kvp X rays was followed in the regenerating tail tips of these animals (body shielded). The mitotic index dropped, after irradiation, to a minimum of near zero and lasted about 8–22 hr following the treatment. X rays produce in the chromosomes of this tissue the same types of aberrations as are found in irradiated plant chromosomes. Chromatid and isochromatid aberrations and symmetric and asymmetric exchanges were observed at 47 hr following irradiation with 100 and 200 r of X rays.

Metamorphosis in *Gyrinophilus palleucus* (J. N. Dent, J. S. Kirby-Smith, and D. L. Craig). – It has been previously reported[3] that the cave-dwelling salamander *Gyrinophilus palleucus*, which has been found only in the larval state, will metamorphose when treated with either thyroxin or thyrotrophic hormone; however, various lines of evidence indicate that it is ordinarily neotenic. One large larva was observed to metamorphose spontaneously after being maintained at 75 ± 2°F for 11 months. This indicates that, although this animal does *not* metamorphose at cave temperature (56°F), it may metamorphose at higher temperatures. This possibility is being investigated.

Cytological Studies on the Pituitary of Thyroidectomized Newts (J. N. Dent). – Pituitary changes following thyroidectomy have been followed in mice by Halmi and Gude,[4] who employed cytochemical staining techniques (aldehyde fuchsin-light green and periodic acid Schiff reagent).

[1] Research participant.

[2] J. S. Kirby-Smith and D. S. Daniels, *Genetics* 38, 375–388 (1953).

[3] J. N. Dent, J. S. Kirby-Smith, and D. L. Craig, *Anat. Record* 121, 429 (1955).

[4] N. S. Halmi and W. D. Gude, *Am. J. Pathol.* 30, 403 (1954).

Careful studies on such changes have not been reported for lower vertebrates.

Two types of basophiles have been found to stain, in the newt hypophysis, with PAS and with aldehyde fuchsin. They have tentatively been identified as thyrotrophs and gonadotrophs. A study is being made of these cells in animals which have been thyroidectomized or castrated. Halmi and Gude reported that pituitary tumors develop from greatly enlarged, lightly staining "thyroidectomy cells" in the radiothyroidectomized mouse. Similar cells have appeared some 15 days following thyroidectomy in the newt and are numerous after 30 days.

Steinitz and Stone[5] observed changes in the ultimobranchial body of the thyroidectomized newt and suggested that it may take on a thyroid function in the absence of the thyroid. If this is the case, the ultimobranchial body would be expected to take up no radioiodine after thyroidectomy. Newts given radioiodine six months following thyroidectomy, however, showed no iodine uptake in their ultimobranchial bodies.

Radiological Physics

Cockcroft-Walton Fast Neutron Facility (M. L. Randolph, E. B. Darden, Jr.,[6] and T. A. King[7]). – The progress of the accelerator program can conveniently be considered under the headings: dosimetry, fast neutron yield, and neutron exposures.

Three independent methods have been employed to determine the dose rate from $D(d,n)He^3$ nuclear reaction: a homogenous ethylene-polyethylene ion chamber, a proportional counter to measure the proton flux from the companion $(Dd,p)T$ reaction, and a long counter calibrated versus various standard Po-Be fast neutron sources. Since the dose rate may be calculated from measurements with the ion chamber in two ways – from the volume, Bragg-Gray principle and absolute resistance measurement or from comparison of currents induced in the neutron field and in a known γ field near a standard radium source – there are four at least semiindependent simultaneous values of dose rates which may be calculated and compared. In making these intercomparisons, allowances have been made for such factors as building scatter (based on measurements of flux from a standard source in a scatter free area and in the accelerator room), target scatter, γ attenuation for the ion chamber, recoil proton attenuation owing to the aquadag layer in the ion chamber, and proton attenuation of the foil covering the window of the proton counter (based on measurements with different foil thicknesses). The averages of intercomparisons of any two methods of obtaining dose rates are in agreement to within less than 5%. The agreement of the dose-rate values obtained by the total energy dissipation method (ion chamber) with the values obtained by the flux methods (proton counter and long counter) as well as measurements with a lead-lined ion chamber indicate that the γ-ray dose is less than 5% of the total dose. Methods to make a complete study of the spatial distribution of dose around the target are now available. Final measurements are to be made with optimum target conditions of minimum scatter, γ-ray production and energy degradation, and of maximum neutron yield such as will be employed for biological exposures.

Following the realization of satisfactory dosimetry, the chief emphasis has shifted to obtaining maximum fast neutron yield. Since (1) the dosimetry has been developed for the *d-d* nuclear reaction, (2) the problems of obtaining maximum yield by the *d-d* and *d-t* reactions are very similar, and (3) there is some contamination hazard in handling tritium, the *d-d* reaction has been employed almost exclusively. The high-voltage power supply has been completely rebuilt by the manufacturer and now operates steadily at 250 kv with varying loads up to almost 2 ma. The ion source, after slight modification, has yielded ¾-ma beam current focused through a ½-in.-dia aperture near the target. With a power dissipation of about 200 w and probably several thousand watts per square centimeter at the target, the problem becomes that of producing a deuterated target which will retain deuterium at high temperature or else very efficiently cooling the target, preferably with an assembly of small mass and nonhydrogenous coolant. A number of deuterated zirconium targets on various backings have been produced and tested with a variety of cooling systems. As a compromise between the effectiveness of massive water cooling and the low scatter and energy degradation of air cooling, a scheme of injecting into the air

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[5] H. Steinitz and L. S. Stone, *Anat. Record* 120, 435 (1954).

[6] Now in General Physiology Section.

[7] Instrumentation and Controls Division.

stream small quantities of water to be vaporized at the target has been devised, and has given encouraging preliminary results. Approximate steady state yields and dose rates obtained with the *d-d* reaction at 250 kv on a particular target under various cooling situations are given in Table 72.

A single tritium target with water cooling has been used briefly with a maximum total emission of roughly 2.4×10^9 neutrons/sec or 150 rep/hr at 5 cm from the target with a 160 kv beam of less than 100 μa.

In general, as a result of the agreement of independent dosimetric methods and *d-d* yields obtained, the accelerator now appears as a nearly established facility for the exposure of biological materials to *d-d* neutrons, and two instructive orientation experiments have been carried out. Although emphasis has been given to the *d-d* reaction, it is felt that the transition of dosimetry and target methods to the much more prolific *d-t* reaction will be much less difficult than the establishment of the facility as a *d-d* source.

Neutron Dosimetry (M. Slater). – The neutron source for the chronic facility will have an initial emission rate of 2.5×10^8 neutrons/sec and thus will provide an excellent opportunity for an intercomparison of neutron dosimetry methods. In preparation for this, the existing neutron dosimetry equipment has been overhauled, added to, and carefully investigated for reliability and stability. The primary comparisons will be between various ion chambers[8] and the neutron proportional counter.[9] Methods have been devised and tested to give each of these measurements an accuracy of the order of 1%.

Increased precision in the measurement of the dose rate from a point source has been obtained by a new method for inverse square determinations. The measuring device (e.g., ion chamber or proportional counter) is constrained to move along a straight line, and dose-rate measurements are made at accurately measured distances from an arbitrary zero on this line. The problem of making accurate measurements of the distance from the center of the source to the center of the chamber is thus replaced by the simpler one of measuring distances along a line. A simpler mathematical analysis then gives the dose rate from the source and also the location of the effective center of the measuring device. An example of the effectiveness of this technique is an ion chamber measurement of the dose rate from a 20-mg radium source, with a standard error of the mean of 0.4%, differing from the National Bureau of Standards value for the source of 0.9%.

In order to use this technique with the ion chamber, a lightweight, flexible cable is necessary between the ion chamber and vibrating reed electrometer. Various cables, connectors, and fabrication procedures were investigated, which would lead to a final cable having a resistance greater than 10^{14} ohms, a noise voltage of a few hundred microvolts, and which recovers quite rapidly (a few minutes) when flexed.

[8] E. B. Darden, Jr., and C. W. Sheppard, topical report, ORNL-1002 (1951).

[9] G. S. Hurst, *Brit. J. Radiol.* 27, 353 (1954).

TABLE 72. APPROXIMATE STEADY-STATE YIELDS AND DOSE RATES BY THE *d-d* REACTION AT 250 kv

Cooling Agent	Beam Current (μa)	Total Emission		Dose Rate (rep/hr) at 5 cm from Target	
		Neutrons/sec	Neutrons/μc	90 deg to Beam	0 deg
Massive water	200	1.08×10^8	5.4×10^5		
	670	3.8	5.7	12	26
Water injection + air	400	2.05	5.1	6.5	14
	700	0.8	1.1		
Air	300	0.7	2.3		

A proportional counter dosimetry system was built up and investigated with a 10 curie Po-Be source. The results are extremely sensitive to the location of the zero of the pulse height discriminator which measures the height of the pulses out of the linear amplifier – a 0.1-v error in the zero producing a 1% error in the results. A method was devised, with a pulse generator and count rate meter, to measure the zero accurately, and to monitor the amplifier gain and counter high voltage to achieve 1% stability in this method of dosimetry.

A series of calculations on the ORACLE, performed with the assistance of J. Z. Hearon and J. Vander Sluis of the Mathematics Panel, on the energy absorbed by various materials irradiated by γ rays and fast neutrons at various energies, gave results which are useful in the interpretation of the readings of various types of ion chambers exposed to mixed beams of radiation.

Fast Neutron Biological Exposures with an Internal Target Cyclotron (C. W. Sheppard,[10] E. B. Darden, Jr., and M. Slater). – In November 1951, biological material was exposed in the ORNL 86-in. cyclotron, but serious work was not begun until August 1952 when a preliminary program of physical investigation was instituted to determine to what extent the machine could serve as a source of fast neutrons for radiobiological studies. These studies indicated that quite high doses of neutrons could be obtained (40,000 rep were given on one occasion). With a small lead box with 2-in.-thick walls placed 30 deg below the axis of the proton beam as it strikes the target, there were about 2–12% γ rays in the field and a negligible amount of thermal neutrons. Since the neutrons are produced by proton bombardment of beryllium, most of them are in the energy range below 3 Mev. The ORNL machine does not accelerate deuterons. Dosimetry was based on the readings of two Victoreens, one with a bakelite thimble and one with a thimble made of conducting lucite-graphite plastic developed by Raper and Zirkle during World War II.[11] Ratios of rep to n-unit to convert the Victoreen readings to dose values were determined by a limited number of dose determinations with tissue-equivalent chambers provided by H. H. Rossi.

The over-all physical situation was not ideal for precise work. The neutrons were highly scattered and degraded, which precluded the use of techniques for outlining the neutron spectrum in detail. Other difficulties have been described.[12] Nevertheless, because of the imminent need for data required in Operation Upshot-Knothole a limited program of biological investigation was instituted and service was provided to a number of investigators for semiquantitative exposure of their material. Ion chambers were also calibrated for use in the operation. Biological findings were published in a series of preliminary reports.[13–16]

In the summer of 1953 the Pathology and Physiology Section became interested in the facility and began to make exposures with the assistance of the Health Physics Division. Their biological and physical findings made on the axis of the proton beam in a large lead box with 1-in.-thick walls are now in press.[17,18] Comparison of the earlier dosimetry with the Health Physics results soon indicated that an error of ~30% had been made in the earlier figures.

In February 1954, an intensive program of physical observations was begun to investigate the discrepancy. At the same time, further biological work was in progress and, occasionally, biological measurements from the early period were repeated by the more satisfactory dosimetry. Some of the studies were made in the experimental pathology box and others in the old box. The principal conclusions concerning the dosimetry were:

1. Tissue-equivalent ion chambers cannot be relied upon in their present form unless freshly filled with gas before use. This conclusion may not apply, possibly, to chambers of more recent design and constructed of different types of plastic than the type used in our instrument.

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[10] General Physiology Section.

[11] J. R. Raper and R. E. Zirkle, U.S. Patent No. 2,596,080.

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

[13] J. S. Kirby-Smith and C. P. Swanson, *Science* 119, 42–45 (1954).

[14] W. K. Baker and E. S. Von Halle, *ibid.*, 46–49.

[15] G. H. Mickey, *Am. Naturalist* 88, 241–255 (1954).

[16] W. L. Russell, L. B. Russell, and A. W. Kimball, *ibid.*, 269–286.

[17] G. S. Hurst, W. A. Mills, F. P. Conte, and A. C. Upton, *Radiation Research*, in press.

[18] A. C. Upton, F. P. Conte, G. S. Hurst, and W. A. Mills, *ibid.*, in press.

2. Victoreens and tissue-equivalent chambers used with a Victoreen electrometer have an appreciable ion-collection defect for neutrons which sets in with the 25 r Victoreen usually at exposure rates corresponding to about one-third full scale deflection per minute. The defect also occurs when deflections greater than one-third full scale occur because of the falling off of the collecting voltage at large deflections.

3. The "educated guess factor" of 2.5 often applied to Victoreen readings may suffice for very rough work with the high-energy neutrons produced by bombarding beryllium with deuterons. With lower-energy neutrons, the value for a bakelite thimble is about 3.0–3.2.

4. Monitoring a cyclotron by measuring the background "fog" of neutrons at some point well removed from the target is limited in precision when, as in the internal target machine, degradation by the target structures is appreciable. Movement of the proton beam on the target can alter the degradation pattern and cause the radiation field close to the target to change relative to the background fog. There will be changes in the dose and also, to some extent, the spectrum.

The ~30% increase in all the earlier dose figures does not affect the ratio of the Upshot-Knothole results to those obtained in the cyclotron since the Upshot-Knothole dosimeters were calibrated in the cyclotron. The results of Atwood and Mukai[19] were done later and do not contain the error. In Mickey's cyclotron results,[15] an additional error in dosimetry was encountered. His exposures involved a larger than normal correction for ion collection defect and his final doses are about two times greater than before. This is the worst error which was made, however.

It is felt, that in the more recent work, the final dosimetry was improved to the point that approximately ±15% confidence limits could be placed on the results. This was achieved by repetitions of the biological determinations and by making large numbers of dose determinations. The fluctuations do not seem to have been very severe since, usually, quite smooth dose-effect curves were obtained. Nevertheless, since occasional drifts and jumps were obtained, it is not felt that results based on any single exposure can be trusted. With the completion of the beam deflection program with this machine, a better situation should be achieved in future work. The development (M. Slater) of an integrating type of tissue-equivalent dosimeter in the latter part of the work will also improve the situation to some extent.

In addition to the Experimental Pathology studies already reported[17,18] the following biological investigations in the cyclotron were made:

1. dominant lethal mutations in mice;
2. spermatogonial degeneration in mice;
3. developmental disturbances in the mouse embryo;
4. radiation protection in mice (preliminary study);
5. lethality studies in *Escherichia coli*;
6. chromosome and chromatid aberration production in *Tradescantia*;
7. *Drosophila* dominant and recessive lethal mutations at specific loci;
8. translocations in *Drosophila* and detachment of attached X chromosomes;
9. chromosome breakage and rejoining in *Vicia faba*;
10. mutations in micronuclei of *Paramecium*.

A 140 page collation of the physical data and factors pertinent thereto has been prepared and distributed among the various biologists involved in the cyclotron work. This completes the physics service provided to this project.

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

END