OFFICIAL USE ONLY

ORNL-1456

CENTRAL RESEARCH LIBRARY
DOCUMENT COLLECTION
Cy. 1

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES
3 4456 0360649 9

BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT

FOR PERIOD ENDING NOVEMBER 10, 1952

Classification Cancelled
Or Changed To
By Authority Of AEC 9.13.71
By E. Goldhesy Date 11.10.71

OAK RIDGE NATIONAL LABORATORY
CENTRAL RESEARCH LIBRARY
CIRCULATION SECTION
4500N ROOM 175
LIBRARY LOAN COPY
DO NOT TRANSFER TO ANOTHER PERSON
If you wish someone else to see this report, send in name with report and the library will arrange a loan.
UCN-7969 (3 9-77)

OAK RIDGE NATIONAL LABORATORY
OPERATED BY
CARBIDE AND CARBON CHEMICALS COMPANY
A DIVISION OF UNION CARBIDE AND CARBON CORPORATION
OAK RIDGE, TENNESSEE

OFFICIAL USE ONLY

[redacted]

Index No. ORNL-1456

Subject Category: Experimental Biology

Contract No. W-7405-eng-26

BIOLOGY DIVISION QUARTERLY PROGRESS REPORT for Period Ending November 10, 1952

Alexander Hollaender, Director

E. J. Slaughter, Technical Editor

Quotations from or references to this report must not be made in open literature without written permission from the individual authors.

OAK RIDGE NATIONAL LABORATORY
Operated by
CARBIDE AND CARBON CHEMICALS COMPANY
A Division of Union Carbide and Carbon Corporation

Post Office Box P
Oak Ridge, Tennessee

[redacted]

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES
3 4456 0360649 9

[redacted]

Index No. ORNL-1456
Experimental Biology

Distribution, Series A:	No. of copies
Argonne National Laboratory	8
Atomic Energy Commission, Washington	3
Brookhaven National Laboratory	4
*Carbide and Carbon Chemicals Company	77
DuPont Company	2
General Electric Co., Richland	4
General Electric Co., Schenectady	2
Los Alamos	3
New York Operations Office	2
Patent Branch, Washington	1
Univ. of Calif. Radiation Lab.	5
Tech. Information Service, Oak Ridge	15
Mound Laboratory	1
UCLA Med. Res. Lab. (Warren)	1
Univ. of Chicago Air Force Lab.	1
University of Rochester	1
Total	130

*Carbide and Carbon Chemicals Company internal distribution as follows:

G. T. Felbeck	1
Central Research Library	2
Biology Library	2
Health Phys. and Metal. Library	2
Central Files	4
C. E. Center	1
C. E. Larson	1
W. B. Humes	1
L. B. Emlet	1
A. M. Weinberg	1
E. H. Taylor	1
E. J. Murphy	1
E. D. Shipley	1
J. A. Swartout	1

[redacted]

A. Hollaender	1	W. E. Cohn	1
J. S. Felton	1	A. W. Kimball	1
A. H. Snell	1	E. J. Slaughter	1
K. Z. Morgan	1	J. S. Kirby-Smith	1
F. L. Steahly	1	P. B. Pearson	1
M. T. Kelley	1	W. D. Claus	1
D. W. Cardwell	1	H. H. Plough	1
E. M. King	1	R. C. Briant	1
A. S. Householder	1	M. E. Ramsey	1
R. S. Livingston	1	C. S. Shoup	1
C. P. Keim	1	G. E. Stapleton	1
C. D. Susano	1	W. T. Burnett, Jr.	1
W. A. Arnold	1	L. P. Zill	1
S. F. Carson	1	Drew Schwartz	1
B. L. Strehler	1	Daniel Billen	1
S. C. Lind	1	K. C. Atwood	1
C. W. Sheppard	1	A. C. Upton	1
R. F. Kimball	1	John C. Bugher	1
W. L. Russell	1	E. F. Oakberg	1
M. E. Gaulden	1	W. K. Baker	1
C. J. Collins	1	G. H. Bowen	1
E. H. Anderson	1	J. R. Totter	1
A. H. Doermann	1	T. T. O'Dell	1
C. L. Comar	1	N. G. Anderson	1
Jacob Furth	1	J. H. Buck	1
K. M. Wilbur	1	Kenneth Kasschau	1
N. E. Tolbert	1	Reports Office, TID	4

Issuing Office
Technical Information Department, Y-12 Plant
Date Issued: FEB 2 1953

[redacted]

Reports previously issued in this series are as follows:

ORNL-13	Period Ending February 29, 1948
ORNL-61	Period Ending May 31, 1948
ORNL-150	Period Ending August 31, 1948
ORNL-220	Period Ending November 30, 1948
ORNL-318	Period Ending February 28, 1949
ORNL-244	Period Ending May 15, 1949
ORNL-457	Period Ending August 15, 1949
ORNL-537	Period Ending November 15, 1949
ORNL-644	Period Ending February 15, 1950
ORNL-727	Period Ending May 15, 1950
ORNL-807	Period Ending August 15, 1950
ORNL-889	Period Ending November 10, 1950
ORNL-969	Period Ending February 10, 1951
ORNL-1026	Period Ending May 10, 1951
ORNL-989	Period Ending August 10, 1951
ORNL-1167	Period Ending November 10, 1951
ORNL-1244	Period Ending February 10, 1952
ORNL-1297	Period Ending May 10, 1952
ORNL-1393	Period Ending August 10, 1952

[redacted]

[redacted]

TABLE OF CONTENTS

Page

REVIEW OF RESEARCH PROGRESS 1

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC 3

Publications 3

Scientific Society Lectures and Traveling Seminars 6

Visiting Lecturers 9

CYTOGENETICS 10

Cytogenetic Effects of Radiation 11

Investigations of the Effects of High-Intensity Gamma Rays on Paramecium 11

The Effect of Oxygen Tension upon the Division Delay and Nongenetic Death Produced by Hydrogen Peroxide 13

Development of Genetically Marked Stocks in Variety 1 of Paramecium aurelia 14

Radiation Studies on the Ciliate Protozoan Tillina magna 15

Infrared Effect on Neurospora 17

Homology Tests of Neurospora Lethal Mutations 18

Genetic Structure of Bacteriophage T4 20

Studies on the Effects of Combined Radiations on the Chromosomes of Tradescantia . . . 21

Effects of Radiation on Rate of Mitosis 24

Influence of Yolk on Grasshopper Egg Recovery of Grasshopper Neuroblasts from X-Ray-Induced Mitotic Inhibition 24

Influence of Yolk on Mitotic Activity 25

Effects of X Rays on Nucleic Acid Synthesis 26

The Effect of Krebs Cycle Intermediates on the Respiration of Intact and Homogenized Embryos 28

The Effect of Yolk from the Grasshopper Egg on the Respiration of the Isolated, Intact Embryo 29

The Separation of Free Neuroblasts and Other Embryonic Cells from Grasshopper Embryos 30

[redacted]

Page

Radiation Protection 32

Postirradiation Recovery of Escherichia coli 32

Investigations of Normal and Irradiated Rabbit Bone Marrow 35

MAMMALIAN GENETICS AND DEVELOPMENT 36

Genetic and Developmental Effects of Radiation in Mice 37

Radiation-Induced Mutations in the Mouse 37

A Preliminary Report on Radiation-Induced Presumed Somatic Mutation in the House Mouse 37

Further Observations on a New Semilethal Gene in the Mouse 42

PATHOLOGY AND PHYSIOLOGY 45

Pathology and Physiology 46

Studies of the Mechanism of Leukemogenesis by Radiation 46

Relationship between Thyroid Function and Growth of Pituitary Tumors Induced by I^{131} 48

Late Effects of Exposure to Thermal Neutrons in Mice 53

Modified Martins-Mallory Stain for Mouse Pituitary Gland 56

MICROBIOLOGY 58

Tracer Studies on Intermediary Metabolism 59

Studies on the Reversible Reaction, Succinate to Propionate Plus Carbon Dioxide . . . 59

α-Keto Acid Oxidation by Streptococcus faecalis 61

BIOCHEMISTRY 63

Studies on Nucleic Acids, Enzymes, and Energy-Transfer Systems 64

Nucleic Acid Structure 64

Enzyme Substrate Equilibria 65

Page

Conservation of Phosphorus in Exponentially Dividing Bacteria 65

Energy-Transfer Systems 66

Plant Physiology 68

Experiments with Heptuloses 68

The Use of Dowex-50 to Invert Sucrose Solutions 70

Acid-Soluble Phosphates of Crown Gall 70

Plant Biochemistry 73

BIOPHYSICS 74

General Physiology 75

Radiological Physics 75

Erythrocytes 75

State of Desoxyribonucleic Acid in Various Rat Tissues 76

Mass Isolation of Whole Cells from Rat Liver 76

Tradescantia Studies 77

REVIEW OF RESEARCH PROGRESS

Alexander Hollaender

Cytogenetics. — In studies of the combined effects of X rays and infrared radiation on Tradescantia, it was found previously that infrared alone produces no chromosome breaks. However, breakage is increased if the material is exposed to near-infrared radiation before or after X irradiation. Maximum effectivity is obtained if the Tradescantia is maintained somewhat below 20°C during infrared irradiation.

An interesting effect of the yolk on the recovery of the grasshopper neuroblast from X-ray-induced mitotic inhibition was found. It appears that protection against radiation damage can be effected by some constituents of the yolk. Analysis for nucleic acids has been refined to such a degree that one can follow the nucleic acid changes produced by relatively small amounts of radiation in the grasshopper neuroblast. The effect seems to be more pronounced on the ribonucleic acid than on desoxyribonucleic acid (DNA).

The test for postirradiation recovery of Escherichia coli has been modified sufficiently to permit recognition of as little as 0.01 μg of the factor in yeast extract which stimulates the recovery.

Analysis with radioactive formate of nucleic acid metabolism in surviving bone marrow cells showed that irradiation prevents the appearance of radioactivity in the DNA. The addition of either glucose or succinate relatively increases the incorporation of formate in the DNA.

Mammalian Genetics and Development. — The induction of the presumed somatic mutations in the house mouse has been studied carefully. The method consists of irradiating embryos and looking for the altered areas in the mature coat. The results obtained indicate that new spots appear at practically every location on the surface of the mouse. Spots are very irregular in outline and vary in numbers; their color can be classified by visual observation. Doses of 100 r were used more frequently. Spots appear at a rate of 1.7×10^{-7} per locus. Attempts have been made to modify the effects of new semilethal genes (skin defects) in the mouse.

Pathology and Physiology. — Further studies have been made on the modification of the development of leukemias by cortisone and other treatments. Interesting observations of the appearance of pituitary tumors after treatment with radioiodine have been mentioned previously. It has been possible to transplant these tumor to normal mice. The interrelation of

transplanted pituitary tumors, uptake of radioiodine, and the function of the thyroid has been investigated in great detail. Different methods of modifying the retention of radioiodine have been studied. These tumor studies have attracted the attention of many cancer workers to this laboratory.

The only significant difference of pathological effects of thermal neutrons and X rays appears to be the high cataract-inducing potency of the former. This was reported earlier and new evidence gives further confirmation.

Microbiology. — The interrelation of succinate activation requirements and their dependence on coenzyme A with a small adenosinetriphosphate requirement for propionic acid bacteria has been established. Attempts are being made to isolate the enzyme systems responsible for these reactions.

Biochemistry. — Further work on the identification of the nucleotides a and b has been conducted. Several interesting improvements in ion-exchange methods for sugar isolations have been developed. The enzyme substrate relationship has been investigated with a number of different compounds.

In a cooperative study with the bacteriophage group, it has been found that the bacteriophage transfers all the phosphorus in the bacteria into its own body constituents. None of the phosphorus escapes into the medium.

Methods have been devised for measuring very small absorption changes as well as fluorescence changes in illuminative plants. These advancements have been made possible by improvements in apparatus.

Plant Physiology and Plant Biochemistry. — Development and adaptation of ion-exchange methods for separation of different sugars continue as the principal subject of investigation in the Plant Physiology and Plant Biochemistry groups. Most of their efforts were concentrated on studies of heptuloses.

The accumulation of acid-soluble phosphates in crown gall tissues has been elucidated.

Biophysics. — Special equipment was developed for making the Y-12 cyclotron adaptable for biological work. Intensive investigations of the state of DNA in various rat tissues have been initiated. Modification of the state of the nucleic acid in these tissues, as produced by X rays, is being tested.

Differences in response to X rays have been found between onion-root tips kept in heavy water before irradiation and those kept in normal water.

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications. — Publications which have appeared during this quarter total 35 — 19 papers, 16 abstracts, and one book-length symposium. In addition to the listed papers, there are in press 60 papers, one book-length symposium, and 13 abstracts.

The proceedings of the Fifth Annual Biology Research Conference in Oak Ridge have been sent to press for publication under the title "Symposium on Some Aspects of Microbial Metabolism" as a supplement to the Journal of Cellular and Comparative Physiology. The contents of this book are as follows:

Introduction — Alexander Hollaender

C. B. van Niel. Introductory remarks on the comparative biochemistry of microorganisms.
W. W. Umbreit. Respiratory cycles. Eight Figures.
G. David Novelli. Studies concerning the structure of coenzyme A. Five Figures.
E. R. Stadtman. The enzymatic synthesis of acyl-coenzyme A compounds. Two Figures.
F. Lipmann. M. E. Jones, S. Black, and R. M. Flynn. Coenzyme A pyrophosphate, an intermediary in the ATP-CoA-acetate reaction.
I. C. Gunsalus. The chemistry and function of the pyruvate oxidation factor (lipoic acid). Two Figures.
B. L. Horecker. The metabolism of pentose phosphate. Sixteen Figures.
W. A. Wood and R. F. Schwert. Alternate pathways of hexose oxidation in Pseudomonas fluorescens. Ten Figures.
J. O. Lampen. Pentose and desoxypentose metabolism in bacteria. Eight Figures.
Ralph D. DeMoss. Routes of ethanol formation in bacteria. Four Figures.
Frederick W. Leaver and Harland G. Wood. Evidence from fermentation of labeled substrates which is inconsistent with present concepts of the propionic acid fermentation.
John R. Totter. The biological functions of pteridine derivatives. Four Figures.
Morris Friedkin. Purine and pyrimidine metabolism in microorganisms. Five Figures.

The quarterly publication list follows:

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Carlson, J. G.	Microdissection studies of the dividing neuroblast of the grasshopper, Chortophaga viridifasciata (Degeer)	Chromosoma, 5: 199-200, 1952
Chambers, M. A., L. P. Zill, and G. R. Noggle	The use of ion-exchange resins with glycosides	J. Am. Pharm. Assoc., 41: 461-464, 1952
Darden, E. B., Jr., C. W. Sheppard, and L. C. Emerson	A condenser type ion chamber for the measurement of gamma radiation in the presence of thermal neutrons	Rev. Sci. Instruments, 23: 568-569, 1952

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Denison, F. W., and E. F. Phares	A rapid method for paper chromatography of organic acids	Anal. Chem., 24: 1628-1629, 1952
Dent, J. N., and E. L. Hunt	An autoradiographic study of iodine distribution in larvae and metamorphosing specimens of Anura	J. Exptl. Zool., 121: 79-98, 1952
Furth, Jacob	Recent experimental studies and current concepts on the etiology and nature of leukemia	Proc. Inst. Med. Chicago, 19: 95-104, 1952
Furth, Jacob	Studies on endothelial fragility by ionizing radiation (Discussion following paper by Morton McCutcheon)	J. Cellular Comp. Physiol., Suppl. 2, 39: 123-133, 1952
Furth, J., E. L. Gadsden, and A. C. Upton	Hyperplasia and cystic dilatation of extrahepatic biliary tracts in mice bearing grafted pituitary growths	Cancer Res., 12: 739-743, 1952
Hollaender, A. (edited by)	Symposium on physiological effects of radiation at the cellular level	J. Cellular Comp. Physiol., 39, Suppl. 2, 1952
Hollaender, Alexander	Biology Division Quarterly Progress Report for Period Ending August 10, 1952	Project Report ORNL-1393
Noggle, G. R.	Review—Nitrogen in the life of plants. Dmitrii N. Prianishnikov, Kramer Business Service, Madison	Quart. Rev. Biol., 27: 320, 1952
Noggle, G. R. and L. P. Zill	The quantitative analysis of sugars in plant extracts by ion-exchange chromatography	Arch. Biochem. Biophys., 41: 21-28, 1952
Pomper, Seymour	A pH-sensitive, multiple mutant of Saccharomyces cerevisiae	J. Bact., 64: 353-361, 1952
Pomper, Seymour	The isolation of triploid Saccharomyces cerevisiae	Nature, 170: 892-893, 1952
Riley, H. P., N. H. Giles, Jr., and A. V. Beatty	The effect of oxygen on the induction of chromatid aberrations in Tradescantia microspores by X irradiation	Am. J. Botany, 39: 592-597, 1952
Sheppard, C. W., and Marian Stewart	The direct effects of radiation on erythrocytes	J. Cellular Comp. Physiol., 39, Suppl. 2: 189-215, 1952

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Strehler, B. L., and J. R. Totter	Firefly luminescence in the study of energy transfer mechanisms. I. Substrate and enzyme determination	Arch. Biochem. Biophys., 40: 28-41, 1952
Walker, W. G. and W. S. Wilde	The kinetics of radiopotassium in the circulation	Am. J. Physiol., 170: 401-413, 1952
Wheeler, H. E.	The use of radiocarbon for tagging fungi	Phytopathology, 42: 431-435, 1952

ABSTRACTS

Alexander, Mary L.	X-ray-induced mutation rates at specific loci on the third chromosome of Drosophila melanogaster	Genetics, 37: 563, 1952
Atwood, K. C.	Different actions of ultraviolet and X rays revealed by heterokaryon methods	Genetics, 37: 564, 1952
Baker, W. K.	Position effects of a gene normally located in heterochromatin	Genetics, 37: 564, 1952
Baker, W. K., and E. S. Von Halle	The effect of oxygen concentration on the induction of dominant lethals	Genetics, 37: 565, 1952
Conger, A. D., and L. M. Fairchild	Breakage of chromosomes by oxygen	Genetics, 37: 575, 1952
Doermann, A. H., and M. B. Hill	The arrangement of genetic loci in bacteriophage T4	Genetics, 37: 577, 1952
Furth, Jacob	Morphology of transplantable pituitary tumors induced by radioactive iodine and the associated secondary changes	Am. J. Path., 28: 548, 1952
Jonas, Herbert	Calcium translocation in calcium starved peas	Proc. Assoc. Southern Agr. Workers, p. 146, 1952
Kimball, R. F., and Nenita Gaither	The role of externally produced hydrogen peroxide in the action of X rays upon Paramecium aurelia	Genetics, 37: 594, 1952
Kirby-Smith, J. S., and D. S. Daniels	The relative effects of X rays, gamma rays, and beta rays on Tradescantia pollen	Genetics, 37: 596, 1952
Lovelace, Roberta	The effects of pre- and posttreatment with ultraviolet radiation on the frequency of gamma-ray-induced aberrations in Tradescantia	Genetics, 37: 602, 1952

AUTHOR(S)	ABSTRACTS	PUBLICATION
Noggle, G. R.	The biochemical effects of plant growth regulators	Proc. Assoc. Southern Agr. Workers, pp. 173-174, 1952
Riley, H. P.	Preliminary report on the effect of certain chemicals on radiation damage to chromosomes	Genetics, 37: 618, 1952
Russell, Liane B. and Mary H. Major	A preliminary report on radiation-induced presumed somatic mutations in the house mouse	Genetics, 37: 621, 1952
Schwartz, Drew	Evidence for sister-strand crossing over in maize	Genetics, 37: 623, 1952
Ward, C. L., and M. L. Alexander	A cytological analysis of X-ray-induced mutations in *Drosophila melanogaster*.	Genetics, 37: 634, 1952

Scientific Society Lectures and Traveling Seminars. — The Division has been represented by many speakers at scientific society meetings during the fall months. The papers are listed, along with the beginning lectures of the traveling seminar season.

Furth, J.	Detroit Inst. of Cancer Res. Detroit	The problem of autonomy and dependence of tumor cells
Billen, D., B. L. Strehler, and G. E. Stapleton	Tennessee-Kentucky Branch Soc. Am. Bact., Nashville	Postradiation loss of ATP from *Escherichia coli* B/r
Wilde, W. S., J. Ginsburg, and W. G. Walker	Am. Physiol. Soc. New Orleans	The distribution kinetics of intravenous potassium, K^{42}
Hollaender, A.	Detroit Inst. of Cancer Res. Detroit	Studies on the basic mechanisms of effects of radiation on individual cells
Sheppard, C. W.	Am. Physiol. Soc. New Orleans	Synthesis of dye dilution curves
Zill, L. P.	Agricultural Res. Conference Oak Ridge	Ion exchange as a tool for studying plant carbohydrates
Noggle, G. R.	Agricultural Res. Conference Oak Ridge	As above
Wilde, W. S.	Symp. on Potassium Metabolism Minneapolis	The distribution kinetics of intravenous potassium, K^{42}

Furth, J.	Am. Assoc. for Cancer Res. Southwestern Section, San Antonio	Conditioned and autonomous neoplasms of the pituitary
Chambers, M. A., L. P. Zill, and G. R. Noggle	Am. Pharmaceutical Assoc. Philadelphia	The use of ion-exchange resins with glycosides
Anderson, N. G.	Soc. Gen. Physiol. (AIBS) Ithaca	Observations on certain properties of the rat liver nuclear envelope
Jonas, H.	Am. Soc. Plant Physiol. (AIBS) Ithaca	Some effects of the concentration of potassium bicarbonate on the uptake of K^{42} by sunflowers as determined by a localizing scintillation count-rate meter.
Lovelace, Roberta	Genetics Soc. Am. (AIBS) Ithaca	The effects of pre- and post-treatment with ultraviolet radiation on the frequency of gamma-ray induced aberrations in Tradescantia chromosomes
Russell, L. B. and M. H. Major	"	A preliminary report on radiation-induced presumed somatic mutations in the house mouse
Conger, A. D. and L. M. Fairchild	"	Breakage of chromosomes by oxygen
Doermann, A. H. and M. B. Hill	"	The arrangement of genetic loci in bacteriophage T4
Kimball, R. F. and Nenita Gaither	"	The role of externally produced hydrogen peroxide in the action of X rays upon Paramecium aurelia
Kirby-Smith, J. S. and D. S. Daniels	"	The relative effects of X rays, γ rays, and β rays on Tradescantia pollen
Schwartz, Drew	"	Evidence for sister-strand crossing over in maize
Riley, H. P.	"	Preliminary report on the effect of certain chemicals on radiation damage to chromosomes

Ward, C. L. and M. L. Alexander	Genetics Soc. Am. (AIBS) Ithaca	A cytological analysis of X-ray-induced mutations in Drosophila melanogaster
Alexander, M. L.	"	Mutation rates at specific loci in the third chromosome of Drosophila melanogaster
Atwood, K. C.	"	Different actions of ultraviolet and X rays revealed by heterokaryon methods
Baker, W. K.	"	Position effects of a gene normally located in heterochromatin
Baker, W. K. and Eliz. Von Halle	"	The effect of oxygen concentration on the induction of dominant lethals
Noggle, G. R.	Bot. Soc. Am. (AIBS) Ithaca	Sugar utilization and transformation by a callus culture of Sequoia sempervirens
Ball, Ernest	"	Autoradiographic studies of the accumulation of S^{35}, P^{32}, and C^{14} by a callus culture of Sequoia sempervirens
_____	"	Histological effects of absorbed radioisotopes upon the callus of Sequoia sempervirens
Ball, Ernest and G. R. Noggle	"	Sugar utilization and transformation by a callus culture of Sequoia sempervirens
Billen, Daniel	Tulane University New Orleans	Studies of radiation-induced changes in the physiology of bacterial cells
_____	Louisiana State University Baton Rouge, Sigma Xi	As above
_____	Louisiana State University Baton Rouge, Biochem. Dept.	As above

Furth, J.	Baylor University Houston	Clinical pathology of irradiation injury
———	University of Texas Galveston	Carcinogenesis by ionizing radiations in man and animals

Visiting Lecturers. — Services on the Biology Division seminar platform have been rendered by the following investigators from other laboratories.

Dr. John F. Christman	Dept. of Agricultural Chem. and Biochem., Louisiana State University, Baton Rouge	A new interpretation concerning the mechanism of action of vitamins
Dr. Hans Grueneberg	University College London	Quasi-continuous variation in the mouse
Dr. F. S. Dainton	Chemistry Department, The University, Leeds, England	Hydrogen atoms in water
Dr. Howard H. Vogel, Jr.	Div. of Biol. and Med. Res. Argonne National Lab.	Radiobiology neutron chamber
Dr. Robert Briggs	Institute for Cancer Res. Philadelphia	Transplantation of embryonic nuclei into enucleated frogs' eggs, and the problem of nuclear differentiation during development.
Dr. E. C. Wassink	Laboratorium voor Plantenphysiologisch, Onderzoek der Landbouwhogeschool Wageningen, Holland	Discussion of problems in photosynthesis
Dr. Donald P. Costello	Dept. of Zoology, University of North Carolina, Chapel Hill	Discussion entitled: "The carrier effects as a means of protection against radiation damage from P^{32}"

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball (Leader)

A. D. Conger	Nenita Gaither
K. C. Atwood	Mary Kathryn King
A. H. Doermann	F. H. Mukai
Drew Schwartz	Betty B. Hill
C. P. Swanson*	Rachel C. Cheniae
R. T. Brumfield*	L. Roberta Lovelace*

Anna J. Bridgman*

Investigations of the Effects of High-Intensity Gamma Rays on Paramecium

(Kimball, Gaither, King)

Our previous investigations of the effects of high doses of ionizing radiation upon Paramecium have been carried out with the unfiltered beam of the Maxitron 250 X-ray machine. This beam includes a large component of very soft radiations. The high-intensity Co^{60} γ source of the Organic Chemistry Section made possible experiments with γ rays at intensities (16 kr/min) comparable to those used in part of the X-ray work. Methods were devised to expose two groups of paramecia simultaneously with continual bubbling of any desired gas during the exposure. A procedure was also worked out to keep the temperature within a degree or two of room temperature during the exposure. We are indebted to the Organic Chemistry Section for permission to use the source and advice concerning it.

The curves in Fig. 1 show results of two experiments in which one group of paramecia was bubbled with pure commercial nitrogen and one with pure commercial oxygen during the exposure. For comparison, previously obtained average points for X rays are included. It is seen that there is fairly good agreement between the results with the two radiations, suggesting that the doses as measured were quite similar in terms of biological effectiveness.

The relation between the high and low oxygen group is also in accord with previous results. As pointed out in ORNL-1393, the effect of oxygen tension during irradiation on division delay is extremely variable. In a number of experiments, more effect seems to be produced in nitrogen than in oxygen whereas in others there is little effect or even the reverse effect. The γ results show the same general picture with very little effect of the

* Research Participant

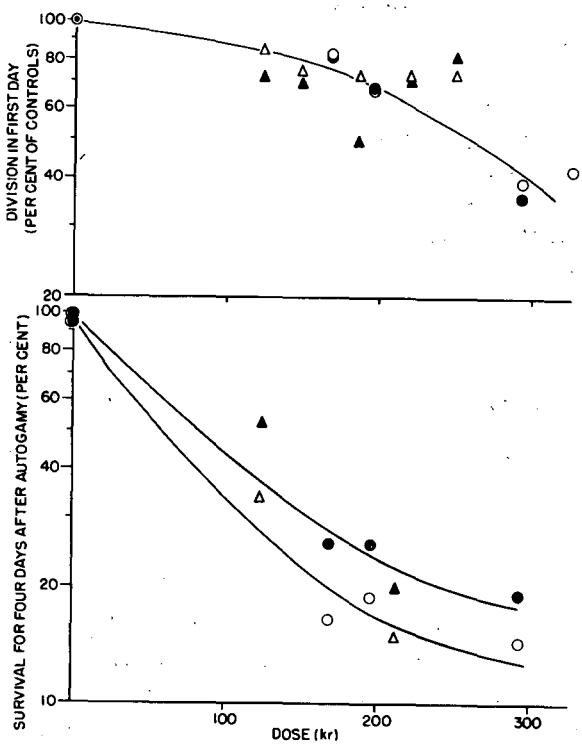

FIG. 1. Division delay and death after autogamy produced in Paramecium by gamma rays

O = gamma O_2 Δ = X rays air or O_2

● = gamma N_2 ▲ = X rays N_2 or 2% O_2

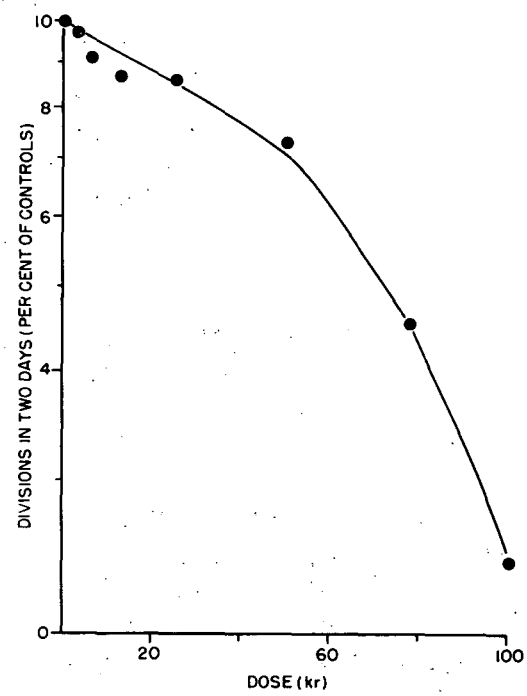

FIG. 2. Division delay in X-irradiated Tillina

oxygen tension. There is likewise some suggestion of a greater effectiveness of irradiation in nitrogen in that, at the highest dosage, all the animals in nitrogen died while most of those exposed in oxygen lived. The data for death after autogamy (a genetic effect) is similar to the X-ray data in showing a small but consistent protection by low oxygen tension.

The X-ray experiments were done with the animals in small hanging drops in chambers which were subject to gas leaks. Though several tests of the method seemed to show that it was satisfactory for maintaining the desired atmosphere, some question about it remained. The γ-ray experiments were carried out with animals in liquid which was vigorously bubbled with the desired gas throughout the exposure and for at least 15 minutes before it. Thus adequate saturation with the desired gas was almost certainly achieved and maintained. Since the results agree with those from X rays, the latter can be accepted with more confidence.

The fairly dense ionization produced by the soft X-ray component might possibly yield hydrogen peroxide without the intervention of oxygen. It was conceivable that this could account for a failure to find much effect of oxygen tension upon division delay and survival. The fact that the same small effect was found with Co^{60} γ rays makes this hypothesis unlikely.

The Effect of Oxygen Tension Upon the Division Delay and Nongenetic Death Produced by Hydrogen Peroxide

In a previous report (ORNL-1297) it was shown that hydrogen peroxide added to the medium produced division delay and death in a manner quite comparable to X rays and that the concentrations required were very little lower than the amount produced by X rays in the medium surrounding the paramecia. If, however, living bacteria were present in considerable numbers in the medium the effect of externally produced peroxide was probably small because of its destruction by the bacteria. In this latter case, however, it is conceivable that peroxide produced locally, immediately around the animal or within it, may be responsible in part for the effect of the radiation.

Later findings showed that when irradiation is carried out without bacteria much less effect is produced with low oxygen tensions, presumably because of the small amount of peroxide produced in the medium. However, when bacteria are present there is little if any oxygen effect, or there may be a reverse effect (ORNL-1393). One explanation that might be offered for these results is that hypoxia increases sensitivity to peroxide and that this tends to cancel its effect upon peroxide production.

We have recently run a series of experiments in which paramecia were exposed under various oxygen tensions to two different concentrations of hydrogen peroxide. The peroxide, first bubbled with the desired gas for 10 minutes, was then added to the paramecia by a pipette extending through a gas-tight joint into a box containing an atmosphere of the desired gas. The exposure was stopped by flooding the container with culture fluid from another pipette in the same box. The results in Table 1 show that there was a slightly greater effect of the peroxide in air than in nitrogen, thus ruling out the explanation of the oxygen results in terms of an increased sensitivity to peroxide in nitrogen. Apparently, the optimum oxygen tension for the action of peroxide is near that in air, for there was somewhat less effect when the paramecia were exposed in pure oxygen.

TABLE 1

INFLUENCE OF OXYGEN CONCENTRATION ON THE EFFECT OF HYDROGEN PEROXIDE ON PARAMECIUM

Expt. no.		Nitrogen (μg/cc peroxide) 0	8	10	Air (μg/cc peroxide) 0	8	10	Oxygen (μg/cc peroxide) 0	8	10
1	S*	100	97	47	100	23	0	-	-	-
	D**	4.6	3.1	1.4	4.5	2.4	-	-	-	-
2	S	100	50	23	100	30	20	-	-	-
	D	4.0	1.9	1.6	4.0	1.3	0.7	-	-	-
3	S	100	100	100	100	93	93	-	-	-
	D	3.5	3.4	3.2	4.0	2.8	2.6	-	-	-
4	S	97	87	10	100	33	13	100	73	10
	D	4.3	2.3	2.3	4.3	1.6	0.8	4.5	3.5	3.3
5	S	-	-	-	100	27	50	10	90	27
	D	-	-	-	4.0	2.4	1.5	4.1	2.3	2.0

* Percentage survival, usually from thirty treated paramecia

** Average number of division in first day among the survivors

Development of Genetically Marked Stocks in Variety 1 of *Paramecium aurelia*

Investigations of the genetic effects of radiation on *Paramecium* would be greatly facilitated if stocks with a number of marker genes were available. Recently, two stocks which differ from each other in five genes have been established. Two of the mutants in one of these stocks arose spontaneously in cultures in this laboratory while the other three were obtained from stocks at Indiana University through the courtesy of Dr. T. M. Sonneborn and Dr. G. H. Beale. One of the mutants which arose in this laboratory is especially useful since it can be used to mark individual paramecia. This mutant lacks the dark crystals which are usually scattered through the body,

and this lack can easily be seen with low powers of the dissecting micorscope. The other mutants can be determined only with difficulty, if at all, in single animals and require the raising of small cultures before classification. All five mutants show typical Mendelian inheritance and are probably not linked with one another.

Radiation Studies on the Ciliate Protozoan Tillina magna

(Bridgman, Kimball)

STUDIES ON TILLINA IRRADIATED IN CULTURE FLUID

Early visible effects. — Doses of 300 and 400 kr of X rays killed all animals. Most of those exposed to 200 kr died but a few rounded up, formed abnormal cysts, and even divided a few times (average 7.5) but all died in a few days within an abnormal cyst. Animals treated with lower doses (100, 75, 50 kr) showed the same tendency to round up but most returned to normal appearance and division rate. Animals exposed to 25 kr or less showed no visible effects.

Effects on division rate. — The number of divisions in 2 days was determined for 88 to 184 animals at each dose. The averages, expressed as per cents of the control, are plotted in Fig. 2. The division rate is reduced to about one-quarter at 100 kr, and there is a suggestion of a small effect even at the lowest dose used (2.5 kr). In general, Tillina seems to be somewhat more sensitive to this effect than Paramecium (ORNL-969).

There is also evidence that irradiation affects the mechanism which controls the number of animals produced at each division event. The usual division in Tillina results in four equal-sized daughter cells. In 67 cases out of 88 at 75 kr and 45 out of 58 at 100 kr, the first division resulted in two daughter cells while all controls gave rise to the usual four. At lower doses, unusual numbers (3, 5, 6, etc.) were often produced.

Delayed effects on mortality. — Many lines exposed to doses of 100 kr or less, which apparently returned to normal, after a few divisions began to divide abnormally, form cysts, and die. As shown in Fig. 3, deaths occurred in the controls at a low but constant rate. The irradiated lines had a much greater rate in the period from about 5 to 10 days, but then appeared to level off to approximately the control rate. Mortality during the first 10 days is thus a composite of radiation-induced and spontaneous events. The average number of divisions elapsing before death for all deaths in the first 10 days varies from 7 divisions at 100 kr to 10 divisions at 25 kr. It is quite possible that the average number of divisions for radiation-induced deaths is constant, and the greater number of divisions

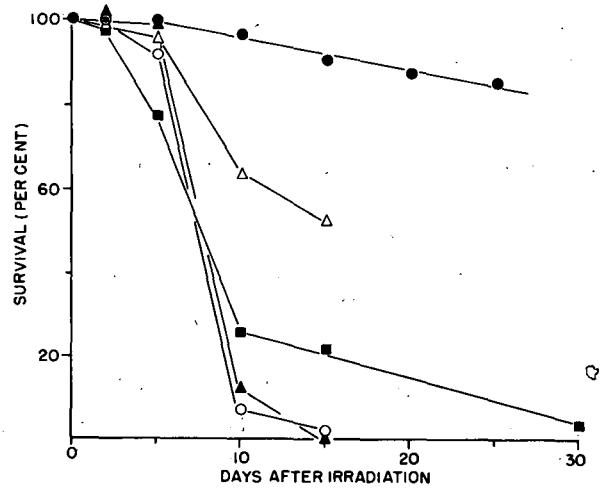

FIG. 3. Survival at various times after X irradiation of Tillina

● = control □ = 50 kr
Δ = 25 kr O = 75 kr
▲ = 100 kr

calculated for 25 kr is a result of spontaneous death making up a greater fraction of total death at low doses.

This phenomenon differs in several ways from the delayed death reported after ultraviolet in Paramecium (Kimball and Gaither, J. Cellular Comp. Physiol., 37:211-233, 1951), most importantly in that the surviving lines do not undergo a depression in fission rate at the time that late death is occurring. The average number of divisions to death is also considerably greater, since the paramecia died after only two to three divisions. Several interpretations might be offered. Mutations in the macronucleus with subsequent segregation, through unequal distribution of the mutated chromosomes, to daughter macronuclei offer one possibility. Other explanations involving either the genetic mechanism or some noninherited change might be visualized. In any case, an all-or-none phenomenon is involved. Either the line of descent dies, or it shows no late effect at all.

EFFECT OF OXYGEN TENSION

Hanging-drop preparations of the animals in culture fluid were exposed to X rays in atmospheres of nitrogen or of 20% oxygen. The results are summarized in Table 2. The marked effect of oxygen tension and especially the fact that there was less effect in nitrogen than in oxygen contrasts

TABLE 2

EFFECT OF OXYGEN ON DIVISION DELAY IN TILLINA

Dose (kr)	Experiment no.	20% Oxygen No. of animals	20% Oxygen Divisions in 2 days	Nitrogen No. of animals	Nitrogen Divisions in 2 days
0	B-18	15	4.9	15	4.7
	B-21	18	5.6	18	6.1
50	B-18	14	1.5	14	4.1
	B-21	21	1.2	30	3.2
100	B-18	25	0.9	30	1.1
	B-21	12	0.1	18	2.2

sharply with similar experiments on Paramecium (ORNL-1393). The results for Paramecium have been very variable but have indicated either very little effect of oxygen tension or a greater effect at low oxygen tensions. No explanation can be offered for this discrepancy except to remark that the Tillina results are in line with the more usual finding in other organisms.

IRRADIATED FLUID AND HYDROGEN PEROXIDE

Several experiments were made comparing the effects of irradiated fluid with the effect of fluid to which hydrogen peroxide was added in amounts equal to the measured concentration in the irradiated fluids. For this purpose 3% boiled culture fluid in distilled water was used. Animals in either of these fluids showed a significant decrease in 2-day division rate from the controls. There was a slight but consistent difference between irradiated fluid and fluid containing the same amount of hydrogen peroxide, the irradiated fluid having the greater effect. These results are plotted in Fig. 4. From this figure, it can be estimated that about two-thirds of the effect of the irradiated fluid can be accounted for by its peroxide content. These findings are in good agreement with similar findings for Paramecium (ORNL-1297).

Infrared Effect on Neurospora

(Atwood, Swanson, Mukai)

The potentiation of the chromosomal effects of X rays by infrared has been examined in the Neurospora heterokaryon system. Aqueous suspensions of conidia of the heterokaryon (29997)-(422-4894)A were placed in

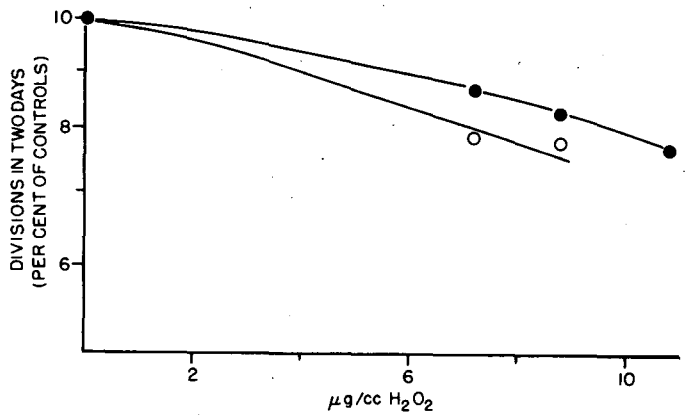

FIG. 4. Effect of H_2O_2 and irradiated fluid on Tillina

● = H_2O_2 O = Irradiated fluid

16-mm pyrex tubes and exposed for 1-1/2 hours to infrared from a 250-watt heat bulb with reflector, at 21 cm distance, through a Corning #2540 filter. The material was maintained at 15°C during treatment. It may be noted that marked potentiation is observed in Tradescantia when inflorescences are exposed under the same conditions, and that 1 cm of water transmits at least 60% of the effective light. The X-ray dose was 50,000 r delivered in 20 minutes at 250 kv and unfiltered except from 1 mm of Al inherent and 1 mm of glass. All samples were spread on the surface of minimal sorbose agar at room temperature (ca. 25°C), maintained at 25°C for 24 hours, then at 33° for 36 hours, after which the colonies were counted and isolated to punch tubes to be subsequently scored for recessive lethal mutations.

The results are shown in Table 3. Pretreatment or posttreatment with infrared had no effect on the frequency of X-ray-induced recessive lethals. Pretreatment significantly decreased the survival. A more detailed characterization of the effect on survival is in progress.

Homology Tests of Neurospora Lethal Mutations

(Atwood, Mukai)

It is desirable, for several reasons, to find the incidence and distribution of homology among the members of a series of separately induced recessive lethal mutations maintained by the heterokaryon method (ORNL-1167, p. 29). Such data will permit estimates of the number of different kinds of lethal mutations which are being induced, the relative incidence

TABLE 3

SURVIVAL AND MUTATION AFTER VARIOUS COMBINATIONS OF X-RAY AND INFRARED CONDITIONS DESCRIBED IN TEXT

Treatment	Dilution	Viable cells/cc	Surviving fraction	Total isolates	Recessive lethals	Per cent lethals
No treatment	10^{-4}	198.2 ± 17.2	1.0	120	2	1.7
Infrared only	10^{-4}	204.5 ± 12.7	1.0	120	3	2.5
X ray only	10^{-3}	54.1 ± 5.4	0.027	237	26	11.0
X ray with infrared pretreatment	10^{-3}	30.0 ± 4.6	0.015	241	25	10.4
X ray with infrared posttreatment	10^{-3}	54.8 ± 8.1	0.027	241	29	12.0

of each, and the size distribution of the deletions. The largest viable deficiencies will be identified and will provide instructive material for further experiments on the comparative radiosensitivity of normal and deficient nuclei.

One hundred X-ray-induced recessive lethals have been chosen for such an analysis, all from experiments yielding about 10% of mutants, so that the main test is for single-event phenomena. At present, 1440 of the possible 4950 two-by-two combinations have been made. These have shown 71.8% of the pairs to be nonhomologous, 14.8% to be homologous, and 13.3% of the test to be equivocal. In Table 4 the stocks are grouped according to the per cent of nonhomologous pairs among the total pairs formed by each stock. It is inferred that large deficiencies are present in the stocks which show small percentages of nonhomologous tests. The majority of the lethals are either small deficiencies or point mutations, however, since the stocks showing more than 60% nonhomologous reactions were homologous only with the presumed deletions, and not among themselves. This accounts for the larger occupancy of the 80-89% class compared with the 90-99% class in Table 2. Most of the stocks in the 100% class have not yet been tested against the major deletions, and are expected to shift to the 80-89% class as the experiment progresses. It is expected that the equivocal tests, when resolved, will fall mainly into the nonhomologous group.

TABLE 4

THE DISTRIBUTION OF LETHALS AMONG NONHOMOLOGOUS PAIRS OF LETHALS

Nonhomologous pairs (%)	Lethals (%)
0 - 9	4
10 - 19	6
20 - 29	4
30 - 39	1
40 - 49	3
50 - 59	4
60 - 69	10
70 - 79	8
80 - 89	32
90 - 99	4
100	24

Genetic Structure of Bacteriophage T4

(Doermann, Hill)

In collecting mutant types of bacteriophage T4 and determining their genetic position by recombination measurements (ORNL-1297:19-25), it was noted that the twenty-six tu (turbid halo) mutants tested fell into five clusters. Mutants were arbitrarily considered to be in one cluster where recombination values of 3% or less were found. The early data, even though inconclusive on this point, suggested that at least three nonallelic tu loci were represented in the tu_{42} cluster. In the intercluster data of the same report a slight positive correlation in the occurrence of adjacent recombinations was observed. This "negative interference" was explained on the assumption that the opportunities for genetic recombination are not equal among the various phage particles produced in one bacterial cell (cf. Visconti and Delbrück, Genetics, in press). This would lead to more double recombinants than predicted on the basis of the probabilities of the single recombinants. By using members of a cluster, the recombination values encountered will be small enough so that, on the hypothesis of a linear arrangement, significant numbers of double recombinants should not occur.

Thus the clusters offer an opportunity to test whether the loci are arranged in a linear order without complicating the predictions with double recombinants. With recombination values of the order of several per cent, one should be able to add recombination values for the intermediate loci to give the value for the terminal markers.

Investigation of this problem was begun by making two-factor crosses in mass cultures with members of the $\underline{tu}_{42}$ cluster which now contains twelve members. So far seven have been crossed with each other in all possible combinations. The data are summarized in Table 5. Perhaps the most surprizing aspect of the results is that recombinants were found in every cross, implying that none of the seven are allelic. In considering the quantitative aspects of the data, it should be pointed out that the wild type recombinant only was scored owing to the apparent unreliability of double mutant scoring. The recombination percentage given in the table is therefore two times the percentage of the wild type recombinant. Whenever double mutants were clearly distinguishable, they were isolated for obtaining stocks for future experiments using three-factor crosses for definitely ascertaining the order of the loci. Concerning the problem of linearity posed at the outset of this report, the data are sufficient only to say that they are compatible with a linear order. The problem of independence of recombinations in adjacent regions must await more complete data.

Tentatively, it seems probable that a, e, g, and c are located in that order with b and f nearly allelic with g. Results with b and f are held in abeyance, however, since both appear phenotypically to be double tu mutants and efforts to obtain segregants from them are in progress. The location of j could be either on the left of a or on the right of c as far as the present data are concerned. The order must ultimately be checked with three-factor crosses.

Studies on the Effects of Combined Radiations on the Chromosomes of *Tradescantia*

(Swanson)

The effectiveness of any given dose of infrared, when combined with X rays, will vary according to the temperature of the inflorescences of *Tradescantia* at the time when exposures to infrared are given. A range of temperatures from 2° to 30°C have been used in combination with 100 kvp X rays, with the results shown in Table 6.

An analysis has not yet been made for increases in the various types of aberrations, but it is readily apparent that the increases are not similar for all types. Thus, the isochromatid aberrations do not show as great an

TABLE 5

RECOMBINATION VALUES WITHIN THE $\underline{tu}_{42}$ CLUSTER OF BACTERIOPHAGE T4

Cross	Total plaques	Number of recombinants*	Percentage recombination
a x b	8427	20	0.48
a x c	8839	24	0.54
a x e	8381	15	0.36
a x f	8770	16	0.37
a x g	9670	19	0.39
a x j	8157	43	1.05
b x c	8434	13	0.31
b x e	8599	13	0.30
b x f	8066	2	0.05
b x g	8053	2	0.05
b x j	8844	49	1.11
c x e	8539	13	0.30
c x f	8794	6	0.14
c x g	8150	12	0.29
c x j	8443	48	1.14
e x f	8470	7	0.17
e x g	8881	9	0.21
e x j	9214	36	0.78
f x g	8278	1	0.02
f x j	9059	49	1.08
g x j	9240	49	1.06

* Only wild type recombinants scored

TABLE 6

INCREASE IN CHROMATID BREAKS AT INDICATED TEMPERATURES IN COMBINATION WITH X RAYS

Temperature (°C)	Percentage increase in breaks
2.0	50
7.0	77
12.5	127
15.0	79
15.0	86
19.0	28
21.0	10
23.0	0
26.0	5
30.0	0

increase as a result of the infrared pretreatment as do the single chromatid deletions and the exchange. It would therefore appear that this is not simply a dosage-increase phenomenon, but rather that certain aberrations are increased preferentially. Other experiments must be carried out, however, before we can be positive of the exact relationships between the various types of aberrations.

Initial experiments seem to indicate that the softer the ionizing radiation the more effective in increasing the aberration frequency is any given dose of infrared. Thus, a comparative study of cobalt γ radiation, 250 kvp X rays with 4 mm of Cu and 1 mm of Al, and 100 kvp X rays with 1 mm of Al — at a 50-r dosage — showed that the percentage increases were far greater with the soft X rays than with the γ radiation, and that the hard X rays were intermediate in this respect. The same relationships seem to hold when the infrared is given as a posttreatment, although our data are not as complete as when the infrared is given as a pretreatment.

EFFECTS OF RADIATION ON RATE OF MITOSIS

Mary E. Gaulden (Leader)
W. K. Baker
J. G. Carlson*
S. R. Tipton*
Georgia St. Amand
Elizabeth S. Von Halle
Katherine W. Loemker

Influence of Yolk on Grasshopper Egg Recovery of Grasshopper Neuroblasts from X-Ray-Induced Mitotic Inhibition

(Gaulden, Loemker)

Embryos of the grasshopper Chortophage were given 64 r of X radiation (32 r/min) in vivo, subsequently separated from their membranes, and dissected for observation of the neuroblasts. Half of the irradiated embryos were mounted, one to a cover slip, in hanging drops containing half the amount of yolk in one egg; the remainder were mounted in hanging drops of artificial culture medium only (modified Tyrode's solution). Embryos receiving no irradiation were mounted similarly, some with and some without yolk. Mitotic activity was determined as described previously in ORNL-727 and mitotic ratios obtained for treated and untreated embryos mounted with and without yolk (Fig. 5).

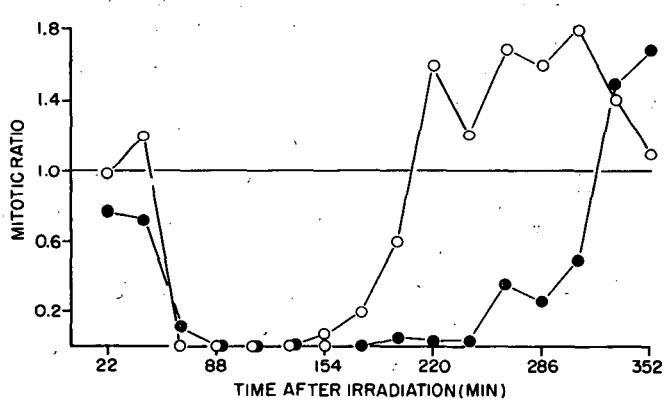

FIG. 5. O = Ratio of X-rayed cells/control cells mounted with yolk
● = Ratio of X-rayed cells/control cells mounted without yolk

*Consultant

An analysis of variance test of the data (Kimball, Mathematics Panel) showed that recovery of the cells from irradiation occurred more rapidly in those embryos mounted with yolk than in those mounted without yolk. It would appear, therefore, that the yolk contains some substance which the cells can utilize in effecting recovery from irradiation.

Influence of Yolk on Mitotic Activity

After noting the effects of yolk material on the recovery of cells from irradiation, it was decided to study further the influence of this substance and, if possible, to determine its biochemical nature.

Dissected embryos were mounted on cover slips in hanging drops in one of four ways; (1) with only culture medium, (2) with half the yolk from egg, (3) with a fourth the yolk, or (4) with an eighth the yolk. (Embryos could not be mounted in all the yolk from an egg because of osmotic pressure difficulties.) Mitotic activity was determined (see ORNL-727) using eight embryos for each of the four culture conditions. The average number of mid-mitotic cells per embryo for each of the four types of culture preparations were plotted against time after the preparation was made (Fig. 6).

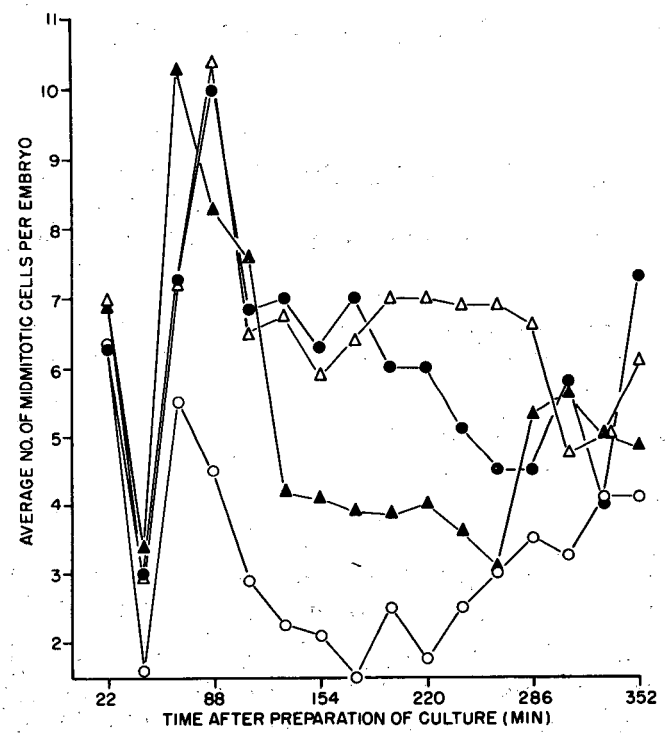

FIG. 6. O = no yolk
● = 1/2 yolk
▲ = 1/8 yolk
Δ = 1/4 yolk
in hanging drop

An analysis of variance test of the data (Kimball, Mathematics Panel) revealed that embryos in cultures containing only artificial culture medium showed a level of mitotic activity which was significantly lower than in the cultures containing yolk. There was no statistical difference in mitosis in embryos mounted in one-half and those mounted in one-fourth yolk from an egg. However, about 2 hours after preparation of the cultures mitotic activity in embryos mounted in one-eighth yolk dropped to a level significantly lower than in embryos mounted in one-half or one-fourth yolk. It is seen, therefore, that the level of mitotic activity in grasshopper embryos in vitro is enhanced by the presence of some substance in the yolk, and that the amount necessary to maintain a high mitotic activity for 6 hours lies somewhere above one-eighth of the amount found in one egg.

Experiments have been initiated in an effort to determine the nature of the substance or substances which evidently serve as nutrients to the neuroblasts. Yolk material was diluted by half with artificial culture medium and put through a fine Seitz filter. There was no difference in mitotic activity of embryos mounted in a drop of the filtrate as compared with that of embryos mounted in one-half the yolk from an egg—the substance appears to be water soluble. Preliminary work by Totter (Radiation Protection) has revealed that the filtrate is rich in phospholipids. Further preliminary experiments indicate that the substance does not pass through a dialysis membrane.

Effects of X Rays on Nucleic Acid Synthesis

(Totter, Radiation Protection; Gaulden, Loemker)

Preliminary experiments by Totter revealed that grasshopper embryos could metabolize formate in the synthesis of nucleic acids. Using formate labeled with C^{14}, a study has been initiated to determine whether synthesis of nucleic acids in grasshopper embryo is altered by X rays, and if so, whether there is a correlation between inhibition of synthesis and inhibition of mitosis.

Fourteen-day-old embryos were separated from their membranes and yolk, placed in polyethylene dishes containing culture medium (modified Tyrode's solution), and subjected to four doses of X rays: 64, 200, 500, and 12,500 r. These levels of radiation were chosen because (1) mitotic inhibition induced by 64 r is of relatively short duration (1 hour) so that the cells recover completely in a few hours; (2) 200 r stops mitosis for several hours; (3) 500 r and 12,500 r cause irreversible stoppage of growth, the latter probably causing more rapid and complete destruction of cell constituents than the former dose.

A period of about an hour was allowed to elapse after irradiation before the embryos, irradiated and control, were placed in the formate solution (0.1 cc equivalent to 0.94 μM potassium formate added to 2.00 cc of culture medium, solution containing 7.6 μc of C^{14}). All embryos were incubated at 38°C with the formate for 1.3 to 1.5 hours, following which they were fractionated according to a modified Schmidt-Thannhauser method.

The data obtained thus far are summarized in Table 7. It can be seen that the amount of ribonucleic acid in the cells is reduced by all four doses of X rays. The synthesis of desoxyribonucleic acid is affected by only the three higher dosages of radiation.

TABLE 7

UTILIZATION OF FORMATE IN THE FORMATION OF NUCLEIC ACIDS BY GRASSHOPPER EMBRYOS

				Ribonucleic acid			Desoxyribonucleic acid		
Treatment (X rays)	No. of embryos	Time between X irrad. and incubation (min)	Duration of incubation (min)	No. of units	Total counts	Cts/ sec/unit	No. of units	Total counts	Cts/ sec/unit
64 r	34	45	80	1.52	5.85	3.85	3.5	11.85	3.39
Control	34	45	80	2.58	11.0	4.26	3.34	10.70	3.20
200 r	34	60	90	1.46	3.25	2.22	1.64	3.85	2.34
Control	34	60	90	1.81	7.85	4.34	1.82	8.65	3.81
500 r	35	60	90	1.48	3.65	2.43	2.48	5.70	2.30
Control	35	60	90	1.16	4.70	4.05	2.66	9.25	3.47
12,500 r	56	45	90	3.00	1.65	0.55	3.97	2.10	0.52
Control	56	45	90	3.57	15.30	4.28	5.07	15.1	2.98

The Effect of Krebs Cycle Intermediates on the Respiration of Intact and Homogenized Embryos

(Tipton, St. Amand)

Studies on the respiratory metabolism of grasshopper embryos have been extended to determine the effective metabolic systems active in the embryo at a stage equivalent to the development at 14 days at 26 °C and possible substrates that might be utilized by these intact embryos. The embryos were dissected from the eggs, cleared of residual yolk and placed in 5-ml Warburg flasks with either isotonic salt solution (controls) or the desired substrate (sodium succinate, pyruvate, or acetate) in the sidearm. Forty embryos were used in each flask. The substrate concentration was such that, after tipping the sidearm contents into the main flask, the final substrate concentration was 0.05 M. All solutions were buffered at pH 6.8. Respiration was ordinarily determined for 1 hour before the sidearm contents were added and compared with that determined for an hour after tipping the sidearm. The experimental temperature was 38 °C and each flask was flushed with warm, moist oxygen for 3 minutes during the equilibration period.

The results obtained are shown in Table 8. The rate of oxygen consumption of the embryos generally decreased with time so that the respiration for the second hour was lower than that for the first hour. No differences were observed when the substrates were added. However, sodium

TABLE 8

EFFECT OF VARIOUS SUBSTANCES ON THE RESPIRATION OF EMBRYOS

Contents of flask sidearm*	No. of expts.	Respiration (Q_{O_2})**	
		Before substrate added	After substrate added
Salt solution	7	0.348	0.239
Pyruvate	5	0.351	0.254
Acetate	2	0.343	0.258
Succinate	6	0.346	0.238
Malonate	2	0.352	0.040

* Main flask in each case contained isotonic salt solution.

** Q_{O_2} expresses the respiration in mm^3 of oxygen per embryo per hour.

malonate depressed the respiration markedly. The malonate effect suggests that some of the Krebs cycle is active in the embryonic metabolism and that possibly the failure of succinate, pyruvate, and acetate to affect the respiration of the intact embryo was due either to a complete failure of these substances to penetrate the intercellular cement or the cell plasma membrane or to a very slow rate of penetration rather than to a deficiency in the appropriate enzyme systems. When succinate was added to isolated cell suspensions the respiration was not affected, indicating that the cell membrane is the restrictive barrier.

As a further test, succinate was added to homogenates of the embryo. These homogenates were prepared from 250 embryos in 1 ml of culture medium (salt mixture) or distilled water in glass homogenizers of the Elvehjem and Potter type (manometric techniques; Umbreit et al.). Homogenization with a motor-driven pestle at approximately 1200 rpm for 10 minutes resulted in a suspension with very few intact cells. In the presence of 0.05 M succinate, the respiration is increased 250-300% over that of the homogenates without added substrate. After homogenization by hand-operated pestle more than 50% of the cells are intact and many of the embryos are represented by small clumps of cells. This preparation showed an increase of only 67% when succinate was added. Obviously there is an active succinoxidase system in the grasshopper embryo. It appears that succinate either does not penetrate the intact cell or penetrates it very slowly. Our observation confirms, in some degree, the demonstration of a succinate effect on homogenates of Melanoplus embryos, but not on the intact embryo (Bodine et al., Proc. Soc. Exptl. Biol. Med., 78: 593-595, 1952). Our experiments indicate that malonate penetrates the intact cell, malonate inhibition being as great in the presence of succinate as in its absence.

The Effect of Yolk from the Grasshopper Egg on the Respiration of the Isolated, Intact Embryo

There is evidence that some material present in grasshopper egg yolk affects the mitotic rate of neuroblasts in the intact, isolated embryo (see Gaulden and Loemker, this report). We have compared the respiration of intact embryos in culture medium buffered at pH 6.8 with added yolk with that of embryos in culture medium without yolk. The embryos and yolk were separated from the embryonic membranes and transferred to the Warburg flasks (40 embryos to a flask) with a minimum of dilution (approximately 2 parts of yolk-embryo suspension to 1 part of salt solution). In such a small amount of fluid it was difficult to separate the embryo from the egg without injury. Some of the very low respiratory values for controls shown in Table 9 may be due to such injury. As indicated by the data in Table 9, the presence of yolk results in a higher rate of respiration of

TABLE 9

THE EFFECT OF GRASSHOPPER EGG YOLK ON THE RESPIRATION OF ISOLATED GRASSHOPPER EMBRYOS

Expt. no.	Respiration (Q_{O_2})*			
	Control – no yolk		Experimental – yolk present	
	First hour	Second hour	First hour	Second hour
9-8	0.257	0.190	0.386	0.273
9-11	0.256	0.202	0.295	0.295
9-17	0.284	0.126	0.364	0.286
9-18	0.290	0.298	0.290	0.260
10-1	0.205	0.112	0.349	0.203
10-2	0.251	0.192	0.394	0.285
10-10	0.228	0.205	0.236	0.209
Average	0.266	0.204	0.332	0.246

*Q_{O_2} expresses the respiration in mm^3 of oxygen per embryo per hour.

the embryos. However, the decrease in respiration with time is about the same in the presence of yolk as in its absence. Thus the yolk does not seem to "maintain" the respiration any better than the salt solution without yolk. The yolk suspension had no measurable respiration in the absence of embryos.

The Separation of Free Neuroblasts and Other Embryonic Cells from Grasshopper Embryos

A method for obtaining suspensions of relatively undamaged cells from Chortophaga embryos has been worked out. Neuroblasts as well as other types of cells from these suspensions appear to undergo normal mitosis and to maintain active respiration for several hours after separation.

Embryos were isolated in sterile, isotonic salt solution (a mixture of sodium, potassium, magnesium, and calcium chlorides, and sodium phosphate and carbonate). A sufficient number of eggs were opened to obtain 150

normal-appearing embryos. The embryos were then rinsed twice in fresh calcium-free medium and transferred to a 20-ml beaker containing 5 ml of a trypsin-hyaluronidase solution (7.5 mg of each per 10 ml of calcium-free medium). Trypsin is essential for solution of the intercellular cement while hyaluronidase treatment appears to result in less gel formation and less stickiness among the separated cells. The embryos were allowed to stand in this solution at 38°C for an hour. Agitation in this period results in the development of a high viscosity or gel in the suspension and to clumping of separated cells. These clumps under some circumstances are difficult to resuspend. At the end of the hour, 0.5 ml of bovine albumin (Armour) solution (5 mg albumin in 0.5 ml calcium-free medium) was added to the suspension. The clumps of cells representing the original embryos could now be separated into individual cells by drawing them several times into and expelling them from a small pipette with a rather large-bore tip. This must be done carefully and slowly since the cells are very sensitive to mechanical disturbances; blebs or blisters on the surface and a cessation of all mitotic activity can be readily induced by rough handling. When the suspension was quite milky and no longer showed large particles (clumps of cells) it was transferred to a 12-15-ml centrifuge tube. The beaker was rinsed with 5 ml of the medium containing calcium, and this washing fluid was added to the contents of the centrifuge tube. After centrifuging slowly (158 rpm) on a clinical centrifuge (distance from axis to bottom of centrifuge tube, 21 cm) for 15 minutes, the supernatant was discarded and the residue (approximately 0.2 ml) resuspended in 0.6 ml of the medium. This suspension has been used for the experimental observations; a second washing could be carried out if desired.

A suspension made as described contains an average of 2,000,000 cells which respire at the rate of 1.2 mm^3 oxygen per hour per million cells. The average number of cells per embryo is 13,334.

Hanging drops prepared under aseptic conditions will have dividing cells for hours. Special attention is being given to the neuroblasts. Free neuroblasts divide normally for as long as 7-1/2 hours after the embryos are removed from the eggs (the experiments have been terminated at this time). Only a small proportion of the cells have pycnotic nuclei. Further physiological and cytological studies are planned on the separated embryonic cells.

RADIATION PROTECTION

Alexander Hollaender (Leader)
G. E. Stapleton
E. H. Anderson
J. R. Totter
Ruth W. Whittle
Daniel Billen
C. W. Edington
Dorothy W. McKee

Postirradiation Recovery of Escherichia coli

(Stapleton, Billen, Edington)

The effect of postirradiation incubation temperature on viability of X- or γ-irradiated Escherichia coli has been described in previous reports (ORNL-1167, 1244, and 1297). The data presented indicates that the increase in surviving fraction of cells when incubated at a reduced temperature on complete media is a true recovery from the lethal effects of the radiation. The plot of surviving fraction of cells at a constant dose as a function of the postirradiation incubation temperature gives a curve which goes through a sharp maximum at 18°C, suggesting that the survival at any temperature may be a function of the rate of synthetic and destructive processes in the irradiated bacterial cell.

We have offered, as further proof of the synthetic nature of the process, the finding that at a particular temperature the number of survivors is enhanced only on a complete medium, those tested consisting of meat extracts, or yeast extract.

In recent experiments it has been observed that if cells are held at temperatures above 18°C on a synthetic medium which consists of inorganic salts and glucose, the viability of irradiated cells is decreased with time of holding. This synthetic medium will support maximal growth of nonirradiated cells. The loss of viability brought about by holding irradiated cells in the synthetic medium is most pronounced at 37°C, where the surviving fraction after 60 kr of X rays is less than 1% of that obtained on a complete medium. Addition of yeast extract in various concentrations increases the survival over that obtained in the synthetic medium, as shown in Fig. 7. It is clear from the graph that, at "high" concentrations of yeast extract (>100 μg/ml), a positive increase in survival is obtained by holding the cells at 25°C for 1 hour or more before plating. Addition of lower concentrations (<100 μg/ml), although yielding no increase in viable cells with time of holding at the same temperature, does prevent the loss of viability which occurs under the same conditions in the synthetic medium without any additions. The change in survival, about thirtyfold, with 3 hours of holding at 25°C after 60 kr of X rays, which can be seen to be a function of the yeast extract concentration, suggested the utility of this system for assay

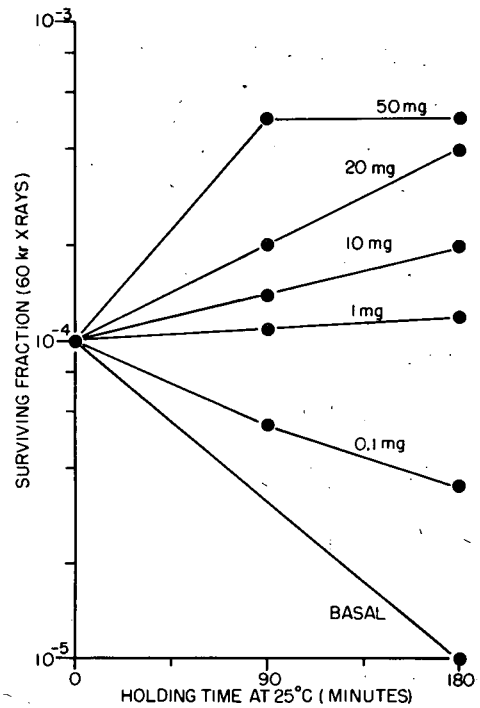

FIG. 7. Change in surviving fraction of Escherichia coli with incubation at 25°C. The numbers beside the curves refer to weight of yeast extract (Difco) added to 10 ml of basal medium before incubation.

of materials which may contain or substitute for the factor(s) present in yeast extract which stimulate recovery.

A variety of compounds and mixtures of compounds have been tested for activity in bringing about recovery in E. coli and, with few exceptions (see Table 10, rows d, e, and f), these mixtures have not closely approached the activity obtained by extracts of the natural materials. In the assay system described, the relative activity of a compound or groups of compounds can be described as the ratio of surviving fraction of cells incubated 3 hours at 25°C after 60 kr of X rays in the synthetic medium plus additions to that obtained in the same medium with no additions.

The testing of the purine and pyrimidine bases, nucleosides, and nucleotides, was prompted by the finding that fractions prepared from natural materials by several methods showed an ultraviolet absorption maximum near 260 mμ. Attempts at fractionation of yeast extract have resulted in no more than about a fivefold purification of the factor. Barium precipitation, plus further precipitation of barium salts by alcohol precipitation,

TABLE 10

COMPOUNDS AND MIXTURES ASSAYED FOR RECOVERY ACTIVITY

	Additions to basal medium	Survival ratio relative to that in basal medium
a	None	1.0
b	Amino acids*	2.5
c	Purines and pyrimidines**	2.0
d	Amino acids and purines and pyrimidines	6.0
e	Amino acids and adenosine (1 mg)	6.0
f	Amino acids and adenylate (1 mg)	8.0
g	Vitamin mixture†	2.5
h	Vitamin mixture and amino acids	4.0
i	Yeast extract (Difco) (2 mg)	30.0
j	Beef extract (Difco) (2 mg)	30.0
k	Peptone (Difco) (2 mg)	30.0

Basal medium used — KH_2PO_4 (0.1%), $MgSO_4 \cdot 7H_2O$ (0.07%), $(NH_4)_2HPO_4$ (0.4%), Na citrate (0.05%) plus glucose added aseptically after autoclaving

*Simulated casein hydrolyzate (17 amino acids included)

**1.0 mg each of adenine, guanine, uracil, thymine, and cytosine

†200 μg each of riboflavin, thiamin, calcium pantothenate, and nictotinic acid, 400 μg each of pyridoxal-HCL and PABA polyglutyl peptide, 500 μg of inosotol, 10 μg biotin, 100 μg folic acid, 0.2 μg of Vitamin B_{12}, and 20 units of lipoic acid.

yielded the aforementioned purification, all of the factor being recoverable in the barium-soluble alcohol-soluble fraction. The factor is not hydrolyzed by 2 N sulfuric acid at 120°C for 2 hours; it can be adsorbed on charcoal but is removed with difficulty. It can be extracted from aqueous solution with ethanol and in normal and secondary butanol, these extractions resulting in little if any more purification than has been accomplished by barium precipitation. Work is going ahead on extraction and purification of the factor in several natural materials which stimulate recovery of E. coli from the lethal effects of X and γ irradiation and further results will be reported at a later date.

Investigations of Normal and Irradiated Rabbit Bone Marrow

(Totter)

For investigation of nucleic acid metabolism in surviving bone marrow cells, radioactive formate was chosen as substrate. The C^{14} potassium formate (11 mc) was prepared by catalytic reduction of potassium bicarbonate. Preparation of this material with a high specific activity (8 mc/mM) was made possible through the collaboration of Mr. W. J. Skraba of the Organic Chemistry Group.

Numerous experiments were carried out in which the formate was incubated with bone marrow suspensions together with inactive substrates. Both normal and irradiated (at 0°C) marrow have been used. Fractionation of the material, after incubation, was carried out by the procedures of Ogur and Rosen.

The following tentative conclusions have been reached:

1. Irradiation prevents the appearance of radioactivity in the desoxyribonucleic acid (DNA). Approximately 3000 r *in vitro* at 0°C is sufficient to reduce the labeling by 50%.

2. Formate is incorporated chiefly into the DNA of bone marrow.

3. The addition of either glucose or succinate markedly increases the incorporation of formate into DNA.

4. Sarcosine and betaine interfere with formate-C^{14} incorporation but choline and glycine do not. These latter results are in accord with the belief that methyl groups of choline *per se* are not labile, whereas those of betaine are readily available for either transmethylation or formate formation. Glycine is not rapidly cleaved by marrow cells.

5. The possible protective effect of crude yeast extract is not readily tested by this method because of the presence of large amounts of betaine-like material in the extract.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENT EFFECTS OF RADIATION IN MICE

W. L. Russell (Leader)
E. F. Oakberg
Liane B. Russell
Josephine S. Gower
J. C. Kile, Jr.
Louis Wickham
Jane E. Crowell
Mary B. Cupp
Gloria J. Jasny
Elizabeth M. Kelly
Mary H. Major
Patricia A. Sarvella

Radiation-Induced Mutations in the Mouse

(W. L. Russell, Oakberg, Crowell, Cupp, Gower, Jasny, Kelly, Major, Sarvella)

It has been reported (ORNL-1393) that the data accumulated on mutation rates at specific loci for spermatogonia X-irradiated with 1000 r, when compared with the data obtained earlier from a 600-r exposure, and with the control rate, give some indication of a departure from linear increase in mutation rate with increase in dose. Additional data obtained since the last Quarterly Progress Report continue to support this indication, but it will be some time before definite conclusions can be drawn. The 300-r experiment, recently undertaken because of the importance of determining the curve for mutation rate and dose, especially if the indicated departure from linearity for irradiated spermatogonia proves to be correct, has already yielded a few mutations. However, it will presumably take a long time to obtain and test an adequate number of mutations for a reliable estimate of the mutation rate at this dose level.

A Preliminary Report on Radiation-Induced Presumed Somatic Mutation in the House Mouse*

(L. B. Russell, Major)

An attempt has been made to induce somatic mutations in a mammal by radiation. The work was originally started in the hope that it would provide a measure of somatic rates at specific loci which could be compared with the germinal rates at the same loci being measured in this Section. As will be shown, the results are probably not suitable for this but have provided other interesting information.

*This work was reported on Sept. 8, 1952, at the meeting of the Genetics Society of America at Cornell University, and will be published in Genetics.

Method. — The method consists of irradiating embryos (i.e., populations of pigment precursor cells), waiting for the mature coat to be formed, and then looking for the altered areas produced presumably from the multiplication of single mutated cells. The main experimental group consists of animals heterozygous for a number of coat color genes, obtained by mating C57BL females to the multiple recessive NB strain males. This group would show mutations to recessive or intermediate alleles at the specific loci, loss of the dominant either by deletion or chromosome loss, or mutation to dominants at the A locus or anywhere in the genome. Another group of embryos (obtained by straight C57BL matings) is homozygous dominant for the loci in question. It would therefore not show loss or recessive mutation of the wild type gene at the chosen loci. It would show mutation to new coat color dominants or to dominants at the a-locus. The main reason for including this group, however, is to provide a control for other, more or less nongenetic, effects of irradiation on pigment precursor cells — such as, e.g., complete loss of pigment.

In a preliminary experiment it was determined that the developmental stage at which the number of pigment precursor cells is sufficiently large to make the experiment practicable in terms of the number of individuals to be observed, yet small enough for the area later produced from the one mutant cell to be of perceptible size is day 10-1/4 postconception. This was rather unfortunate since it was already known from our main work on embryonic abnormalities that, in terms of neonatal death, this stage is the most susceptible to irradiation. In order to insure survival of animals irradiated as embryos to a stage where the coat could be observed, the experiment was begun with the relatively low dose of 150 r, but even this caused a high loss and it was necessary to switch to 100 r, which gives no greater loss than occurs in controls (Table 11) and at which the bulk of the work was done.

TABLE 11

THE INCIDENCE OF DEATH PRIOR TO OBSERVATION FOR COAT COLOR CHANGES, FOLLOWING IRRADIATION ON DAY 10-1/4 POSTCONCEPTION

Dose (r)	C57BL x NB		C57BL x C57BL	
	No. born	Percentage lost preobservation	No. born	Percentage lost preobservation
0	211	4.3	153	7.8
100	202	5.4	89	7.9
150	34	38.2	35	42.9

Animals are routinely observed at approximately 5 weeks of age and most of them also earlier, usually during the second week of life. If a spot is found, its outline is drawn, it is measured with calipers and hair samples are plucked for whole-mounts. The animals are then kept several more months to make sure the spot persists and does not turn out to have been due to moulting patterns. We have not yet tested whether any of the mutations involved germinal tissue.

Results. — (1) Mutant spots have been obtained at practically every location on the surface of the mouse and their distribution is probably random (Table 12). (2) In general, the spots have very irregular outlines and

TABLE 12

DISTRIBUTION OVER THE BODY SURFACE OF MUTANT COAT COLOR AREAS

Region	No. of areas
Dorsal-anterior, left	4
Dorsal-anterior, right	6
Dorsal-posterior, left	4
Dorsal-posterior, right	2
Ventral-anterior, left	2
Ventral-anterior, right	2
Ventral-posterior, left	4
Ventral-posterior, right	6

the same animal may have two or three apparently separate spots in the same general region. Within the mutant area there is a mixture of mutant and normal, black hair in most, if not all, cases. Out estimates have ranged from 10 to 100 for the percentage of hair mutant within the affected area. It is this factor, more than any other, which makes accurate determination of somatic mutation rates impossible. (3) The color of spots, as classified with the naked eye have been brown, tan, slate, gray, and near-white. Histological analysis of hair slides has not yet been completed. Mention should, however, be made of one case in which the hair could be classified as aa pp C- since, because of the linkage of p and C, it rules out whole chromosome loss or large deletion. (4) The frequency of animals with mutant areas is given in Table 13. The 150-r figures are open to a great deal of doubt because of the small sample sizes. Obviously, there can be no doubt about 100 r giving a great increase over the number

TABLE 13

PERCENTAGE OF ANIMALS SHOWING MUTANT COAT COLOR AREAS OR SMALL WHITE BELLY SPOTS FOLLOWING IRRADIATION ON DAY 10-1/4 POSTCONCEPTION

Animals	C57BL x NB aa Bb CP/$c^{ch}p$ DSe/d se			C57BL x C57BL aa BB CP/CP D Se/D Se		
	0 r	100 r	150 r	0 r	100 r	150 r
Total number	202	191	21	141	82	20
Percentage with mutant area						
Males	1.0	14.1	0	0	2.0	11.1
Females	0	7.5	8.3	0*	0	0
Total	0.5	10.5	4.8	0	1.2	5.0
Percentage with white belly spot						
Males	0	0	0	2.9	22.0	33.3
Females	0	0	0	9.9	34.4	63.6
Total	0	0	0	6.4	26.8	50.0

*One female had an enlarged nipple spot

of spontaneously occurring mutant spots. One spotted animal appeared in the straight C57BL irradiated with 100 r. The spot, occurring in a male, was of the same orange-tan color as the hair surrounding the nipples in normal females of this strain and it was located at or very close to the nipple position. The asterisk in the nonirradiated C57BL marks a female in which one nipple spot was diffusely enlarged. Therefore, because of the low frequency of spots in C57BL and their possible specialized nature, we may conclude that at least the great majority of the mutant areas in the heterozygous (C57 x NB)F_1 represent mutation or loss at the specific loci (B, C, P, D). (5) Estimates of the relative amount of mouse surface occupied by mutant hair are very much subject to error [see (2)] and must therefore be considered as only very approximate. On the assumption that all precursor cells present at the time of irradiation contribute equally to the adult coat, these approximate estimates are used to compute the number of pigment precursor cells present on day 10-1/4 postconception (Table 14). The larger areas (at the top of the table) are in the majority but there is, of course, the possibility of small ones having been overlooked. Only about six cell divisions separate the number of precursor cells necessary to account for the largest spot which occupied about one-seventieth of the surface and the smallest, which occupied less than one five-thousandth. This may well be due to differences in developmental age among embryos

TABLE 14

ESTIMATE OF THE NUMBER OF PIGMENT PRECURSOR CELLS PRESENT IN THE EMBRYO AT THE TIME OF OCCURRENCE OF THE PRESUMED SOMATIC MUTATION

(Figures in the body of the table indicate the number of spots of each size)

	C57BL x NB			C57BL	
No. of cells	0 r	100 r	150 r	100 r	150 r
0- 499	-	10	1	-	-
500- 999	-	3	-	-	-
1000-1499	-	2	-	1	1
1500-1999	1	-	-	-	-
2000-2499	-	-	-	-	-
2500-2999	-	-	-	-	-
3000-3499	-	2	-	-	-
3500-3999	-	2	-	-	-
4000-4499	-	-	-	-	-
4500-4999	-	-	-	-	-
5000-5499	-	-	-	-	-
5500-5999	-	1	-	-	-

of 10-1/4 days chronological age, particularly if multiplication of pigment precursor cells is rapid around that stage. The average number present at the time of irradiation is 1286. Using this figure, the somatic mutation rate can be calculated, although we hesitate to do so because of the inaccuracies of measurement involved. Using only the more extensive 100-r data and subtracting the spontaneous spot frequency and the frequency in straight C57BL (which is presumably not due to mutation at the specific loci), this rate is 1.7×10^{-7} per r per locus. This is very close to the much more accurately determined germinal rate. (6) The frequency of the mid-ventral white belly spot (or streak) normally occurring in about one-twentieth of our C57BL stock is enormously increased by irradiation on day 10-1/4 (Table 13), i.e., the stage found most favorable for the observation of somatic mutations. These white belly spots do not normally occur in the (C57 x NB)F_1 and are not induced by irradiation.

Further Observations on a New Semilethal Gene in the Mouse

(Oakberg, Crowell)

Preliminary observations on an apparently sex-linked semilethal gene in the mouse were given in a previous quarterly report (ORNL-1297). Data on total leucocyte and erythrocyte numbers (Table 15) have been expanded, and support the earlier observations. The rise in leucocyte number at 12 days, with a fall of normal levels at 18 days, suggests a pattern of response to a stimulus, with subsequent exhaustion. However, while total leucocyte counts return to normal around 18 days, the neutrophilia appears to be even more marked than at earlier ages. Erythrocyte counts are subnormal at all ages for which data are available, with the difference becoming pronounced by 14 days.

TABLE 15

MEAN LEUCOCYTE AND ERYTHROCYTE COUNTS OF SKIN-DEFECT MALES AND OF THEIR NORMAL BROTHERS

	Skin-defect males			Normal males		
Age (days)	No. mice	Leucocytes/ml	Erythrocytes/ml*	No. mice	Leucocytes/ml	Erythrocytes/ml*
9	6	8,877 ± 964	5.65 ± 0.118	4	6,465 ± 948	6.13 ± 0.271
12	4	32,370 ± 2,238	6.27 ± 0.205	4	7,365 ± 903	6.81 ± 0.147
14	4	19,805 ± 3,231	6.16 ± 0.226	4	6,102 ± 1,207	7.17 ± 0.344
18	5	7,308 ± 827	5.41 ± 0.415	4	4,160 ± 209	7.65 ± 0.461
24	3	6,687 ± 2,243	3.59 ± 1.097	3	5,920 ± 2,736	9.28 ± 0.256

*Millions per ml

The syndrome of "skin-defect" mice differs from any previously known condition due either to genetic factors, to nutritional deficiencies, or to infection by pathogenic microorganisms. One of the most attractive hypotheses is that the mutation has resulted in the loss of ability to synthesize certain hormones, or to utilize certain vitamins, proteins, or other essential materials. The problem of correction of hereditary metabolic disturbances in a mammal is difficult, especially if it is a reflection of deficiencies in cell metabolism. Injection techniques may be useful for replacing certain hormones, but are likely to be successful for nutritional deficiencies only if abnormally high requirements, failure of intestinal absorption, or lack of proper intestinal flora are basic factors.

In an attempt to prolong normal growth and survival time, skin-defect males and young male progeny from "carried" females have been given intraperitoneal injections of yeast extract, B-vitamins (Table 16), and whole

TABLE 16

AMOUNTS OF B-VITAMINS GIVEN IN DAILY INJECTIONS OF SKIN-DEFECT MICE

Vitamin	μg/day
B_{12}	10
Thiamin HCl	20
Folic acid	20
Pyridoxine HCl	20
PABA	20
Nicotinic acid	60
Calcium pantothenate	60
Choline chloride	120
i-Inositol	300
Biotin	8

fresh blood. The results are summarized in Figs. 8, 9, and 10, and it is apparent that neither a curative nor a preventive effect has been obtained with the techniques used. Weights were taken on alternate days, and were discontinued for controls when death of the skin-defect animals occurred. Where yeast extract (Fig. 8) and B-vitamins (Fig. 9) were used, injections were begun before clinical symptons appeared. Skin defects receiving the vitamin concentrate show the longest life span, but the data are not sufficient to support a hypothesis of increased viability. One group of animals receiving whole blood (Fig. 10) was started before symptoms appeared, a second group were given injections only after the condition was well advanced. Blood for injection was obtained by heart puncture from males of the closely related 129 inbred strain and injected immediately into the peritoneal cavity of recipient mice. Degree and rate of removal of corpuscular material is not known, but certainly this is an acute problem, and may have been somewhat of a liability even to normal mice.

Tissues of injected animals were not studied histologically, but the gross appearance and behavior were identical with skin-defect animals receiving no injections.

This particular mutation is an intriguing one both from genetic and pathological viewpoints. Additional experiments will be initiated in the hope that viability of skin-defect males can be enhanced sufficiently to obtain genetic tests, and in the hope that the physiological nature of the mutation may be elucidated.

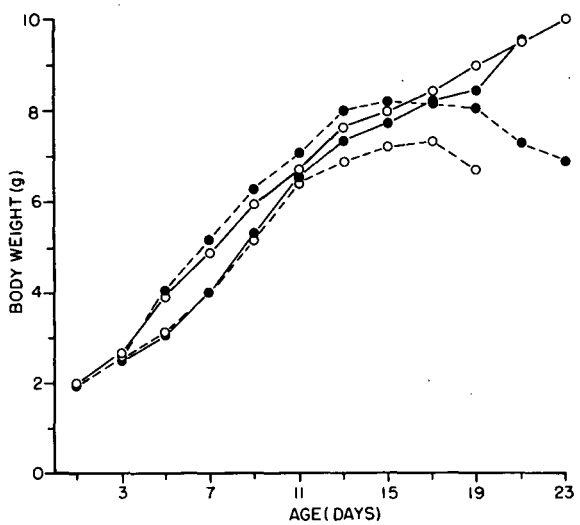

FIG. 8. Growth curves of normal and skin defect mice injected daily with a 2% solution of yeast extract.

●—● = Normals, no yeast extract
O—O = Normals, 2% yeast extract intraperitoneally 0.04 ml/g
●---● = Skin defects, no yeast extract
O---O = Skin defect, 2% yeast extract intraperitoneally 0.04 ml/g

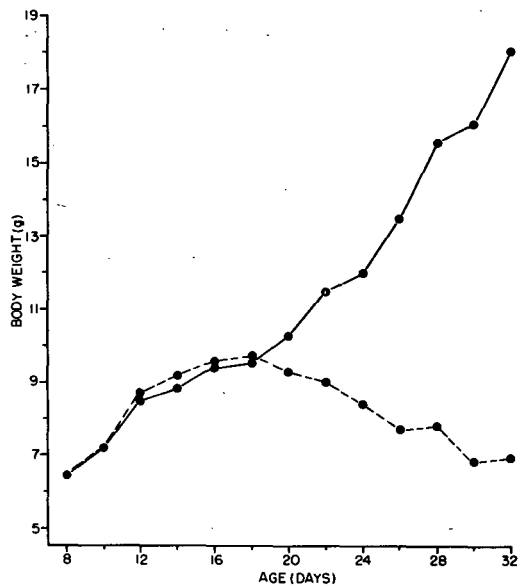

FIG. 9. Growth curves of normal and skin defect mice given daily intraperitoneal injections of the vitamin concentrate given in Table 2.

●—● = Normals
●---● = Skin defects

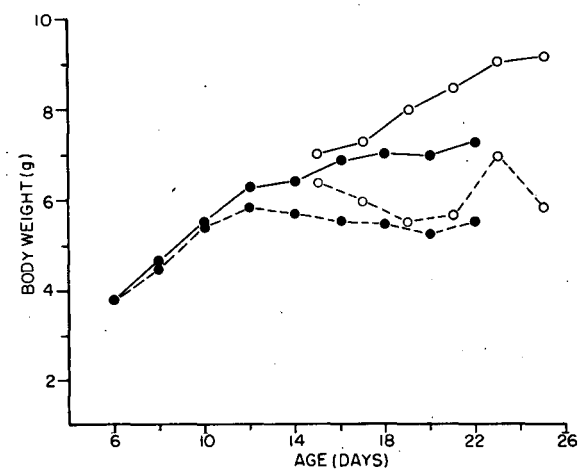

FIG. 10. Growth curves of normal and skin defect mice given daily intraperitoneal transfusion of fresh whole blood.

O—O = Normal, 0.2 ml blood injected intraperitoneally
O---O = Skin defects, 0.2 ml blood injected intraperitoneally
●—● = Normal, 0.15 ml blood injected intraperitoneally
●---● = Skin defects, 0.15 ml blood injected intraperitoneally

PATHOLOGY AND PHYSIOLOGY

PATHOLOGY AND PHYSIOLOGY

Jacob Furth - Section Chief
A. C. Upton
W. T. Burnett, Jr.
T. T. Odell, Jr.
Evelyn L. Gadsden
D. M. Woodbury
R. R. Bigelow*
K. W. Christenberry*
W. D. Gude
Frances Gamble
Frances F. Farbstein
Peggy Ledford

Studies of the Mechanism of Leukemogenesis by Radiation

(Upton, Ledford)

Experiments designed to study the effects of induced lymphoid atrophy upon the induction of leukemia by radiations, as well as on the incidence of spontaneous leukemia have been described in earlier reports and are still in progress. The following is an up-to-date account of existing data.

Table 17 shows that cortisone inhibits slightly the development of leukemia following irradiation and that the effect is the same whether cortisone is given before or after irradiation under the conditions of these experiments. It is also apparent that cortisone-induced lymphoid atrophy itself is not leukemogenic. In Table 17 all types of leukemias are considered together; currently histologic differentiation is made to separate lymphoid leukemias from other types. Cortisone is known to cause atrophy of the lymphoid tissues only if irradiation is known to produce both lymphoid and myeloid leukemia although only the former has been studied adequately thus far.

The effects of lymphoid atrophy upon the development of spontaneous leukemia in the high leukemia strain Ak mouse are being studied concurrently. Fig. 11 summarizes the effects of lymphoid atrophy induced by cortisone at 2-3 months of age on leukemia incidence. The findings indicate a significant reduction in the incidence of leukemia and a delay in its onset.

In order to compare the effects of transient lymphoid atrophy, such as that which follows a single cortisone treatment, with those of sustained lymphoid involution, a second series of experiments was undertaken in which three sets of cortisone injections were administered at intervals of approximately 6 weeks, beginning at 8-10 weeks of age. To compare the

*Consultant

TABLE 17

EFFECTS OF CORTISONE ON THE INCIDENCE OF LEUKEMIA IN X-IRRADIATED AND NONIRRADIATED MICE OF STRAIN Rf

Treatment*	No. of mice	No.** dead	Leukemia No.	Leukemia Percentage
Cortisone	132	52	27	20.4
Cortisone vehicle	146	69	23	15.8
X Radiation	124	109	76	61.3
Cortisone after irradiation	128	108	65	50.8
Cortisone before irradiation	128	101	64	50.0

*Females and males were distributed in approximately equal numbers within each group. A single dose of 350 r was given at 8-12 weeks of age. Cortisone was given by subcutaneous injections of 1.0 mg each on 3 successive days, beginning either 1 week before or 2-3 weeks after X irradiation. Cortisone vehicle (1.5% benzyl alcohol in physiological saline) was injected subcutaneously in the same amounts (0.4 cc) on 3 successive days at 8-12 weeks of age.

**Those alive are 21-24 months of age.

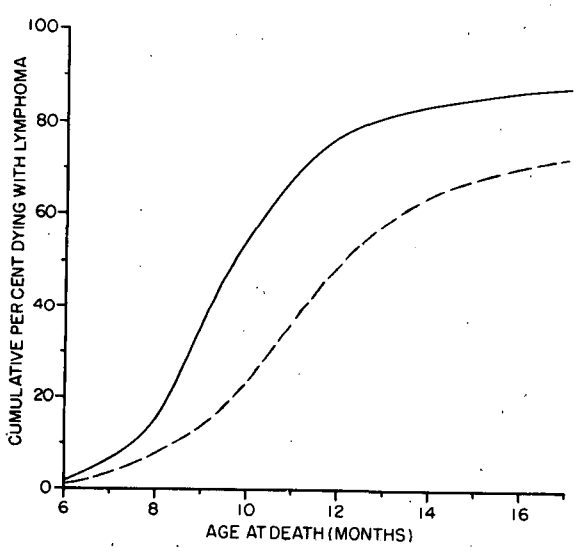

FIG. 11. The effect of cortisone on the incidence of spontaneous lymphoma in male mice of the Ak strain

—— = Vehicle treated

— — = Cortisone treated

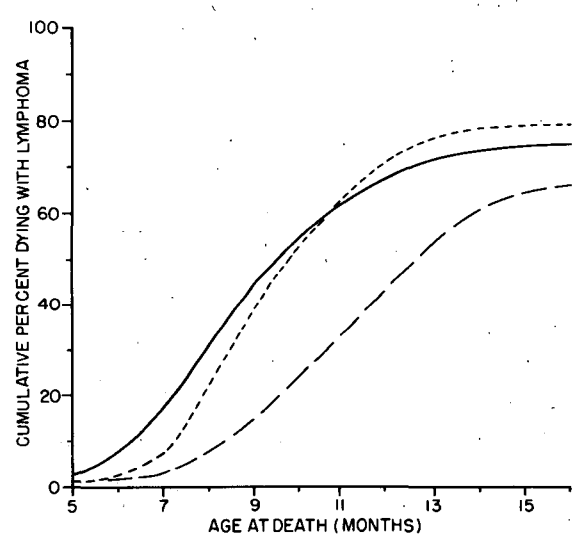

FIG. 12. The effect of repeated injections of cortisone on stress (acute starvation) on the incidence of spontaneous lymphoma in male and female mice of the Ak strain

—— = Vehicle treated

------ = Starvation treated

— — = Cortisone treated

effects of injected cortisone with those of stress, acute starvation was instituted for a period of 72 hours in litter mates, simultaneously with the cortisone injections. This causes a more sustained lymphoid atrophy than cortisone. The effects of these treatments on leukemia incidence are shown in Fig. 12. While it is still too early to draw final conclusions, it seems as if reduction in incidence of leukemia following repeated series of cortisone injections would parallel that seen after a single course of treatment. Although acute starvation stimulates cortisone in its action on lymphoid tissues, it appears less effective in inhibiting leukemogenesis.

It is significant that the development of leukemia should be inhibited by transient lymphoid involution. It is conceivable that prolonged, continuous lymphoid atrophy, as might be produced by stress or extended cortisone therapy, might completely inhibit the development of lymphoid leukemia.

Relationship Between Thyroid Function and Growth of Pituitary Tumors Induced by I^{131}

(Burnett, Gadsden, Woodbury)

Numerous experiments are under way to learn about the mechanism of induction of tumors by I^{131} in either the thyroid or the pituitary. A paper describing completed parts of this study has been submitted for publication. The following is the essential content of this paper:

Destruction of the thyroid gland by I^{131} in mice is followed by the development of TSH-secreting pituitary tumors. These tumors can at first be grafted only on mice similarly radiothyroidectomized, and not on normal animals; they are thus initially conditioned or dependent growths. The tumor cells gain autonomy in the course of passages as indicated by ability to grow on normal mice. In further subpassages in normal hosts they grow even better in normal than in radiothyroidectomized hosts. These findings are best explained by successive modification in cells of the pituitary from normal to autonomous type.

Dependent tumor cells (those requiring absence of the thyroid for their growth) resemble normal chromophobe pituitary cells but are probably related to basophiles. They exhibit no features of anaplasia even though they usually metastasize to regional lymph nodes. These characteristics have changed but little in the course of this work requiring 1-1/2 years. The autonomous growths are more anaplastic and invasive. A conspicuous morphological difference between dependent and autonomous growths relate to secondary changes in the tumors. Lack of secondary changes characterizes the dependent growth while extensive necrosis, hemorrhage, and fibrosis

are the rule with autonomous tumors. Anaplasia in the autonomous growths has increased markedly in the course of this study, and at its conclusion extensive lymphatic metastases in draining superficial and abdominal lymph nodes are now the rule. Nevertheless, ability to secrete TSH, as indicated by enlargement of the thyroid and its microscopic appearance, was invariably retained.

The mechanism of the pituitary tumor induction by radiothyroidectomy is not known with certainty. It has been postulated that depression of the thyroid gland with depression in TH secretion stimulates those cells of the hypophysis which control thyroidal function. The possibility that injury to the pituitary by radiations, incidental to radiothyroidectomy, is a contributing factor in the induction of pituitary tumors has, however, not been excluded thus far. This problem calls for studies of minimizing irradiation of the pituitary incidental to I^{131} treatment or avoiding irradiation altogether by performing surgical thyroidectomies.

The relation between pretreatment with I^{131} and pituitary tumor growth was examined by grafting the tumors on mice that had received 25, 75, and 225 μc of I^{131}, respectively. Four separate experiments were performed using our oldest dependent strain (3D) and two using more recently isolated strains. The procedures used in this work have been described in earlier Quarterly Reports and in open publications. The results of these experiments confirm those already mentioned in our last report. In the first and fourth experiments the pituitary tumors of strain 3D grew rapidly in the mice pretreated with 225 μc and very slowly in those given 75 μc; no tumor growth was observed in mice given 25 μc. In later experiments the 3D tumors appeared also in the 25-μc group but only after a long latency period. This was probably due to the acquisition of autonomy, as indicated by earlier experiments. The tendency to gain autonomy was marked with strains 3D and 19. Growth of strain 19 tumors was proportional to the I^{131} dose. Susceptibility of mice for tumor of strain 124 was markedly enhanced by pretreatment with 225 μc, slightly by 75 μc, and not at all by 25 μc.

The depression of thyroid function was not uniform in mice that were given the same dose of I^{131}. Traces of thyroid were present in several mice in the 225-μc groups and did not have an inhibitory effect on tumor growth. Great variations in thyroid depression in the 75-μc groups were indicated by I^{131} uptake studies, histological examinations, and by enhancement of tumor growth. The function of these thyroid remnants was minimal.

The ability of the thyroid hormone to inhibit the growth of pituitary tumors was further studied by microscopic examination of the thyroid glands following an assay of their functional capacity. The mice were sacrificed

1 day following intraperitoneal injection of near-tracer doses of I^{131} since the results of the experiment detailed in ORNL-1393 indicated that thyroid function can be evaluated by determining the 24-hour retention of I^{131}. Five experiments were performed using in each normal and I^{131}-pretreated mice with and without tumors. These were supplemented by experiments in which total-body retention of I^{131} was measured in the living animals by the γ-ray chamber and the retention in the thyroid with the scintillation counter. For comparison, similar tests were performed on mice that were given 25, 50, 100, and 200 μc 7 months earlier but were not grafted with tumors. There was some variability in values but the trend was the same in all experiments.

The mean total-body retention in 24 hours in normal mice was 19.2% of the tracer dose and of this 70.5% was in the thyroid gland. In contrast, the radiothyroidectomized mice retained 36.6% of the injected dose in the body but only a trace or none of the activity was in the thyroid region. The mean total-body retention in radiothyroidectomized mice bearing grafted tumors was 75.0% of the dose, with only a trace or none at the site of the thyroid. The enhancing effect of grafted pituitary tumors on the uptake of radioiodine was thus confirmed.

The total-body 24-hour retention of I^{131} was almost as much in normal mice bearing minute grafted dependent pituitary tumors as in normal mice bearing large autonomous tumors. Most of the radioiodine retained was in the thyroid gland (89.0%) and most of this was presumably hormonal (TH) iodine. The tumor nodules in mice of this group were rarely larger than a normal pituitary gland at autopsy performed 4-1/2-9 months after grafting. The rapidly growing autonomous tumor grafts weighed at autopsy 2-5 g, a thousandfold that of a normal pituitary gland. Secretion of TSH by minute tumor grafts over a long period of time produces a striking stimulation with great enlargement of the thyroid gland.

The effects of pretreatment with 75 μc of I^{131} on thyroid function of mice bearing tumor grafts were variable. There was, however, an inverse relationship between morphological appearance and function of the thyroid gland on the one hand and size of the tumor grafts on the other. This is clearly indicated by subdividing this group according to the quantity of I^{131} retention. In none of the four mice in which the 24-hour total body I^{131} retention was above 50% of the dose was the thyroid gland identified on gross examination, and in these mice the grafted tumors were large (~25 mm in average diameter).

The values obtained indicate that 75 μc injures the thyroid of different mice to a highly varying degree. The thyroids of all animals of this group had morphological changes indicative of stimulation by TSH and of varying grades of antecedent injury. One was markedly fibrosed. A feature of the

75-μc group was the coexistence of apparently resting and hyperstimulated follicles; similarly the autoradiographs revealed a highly variable uptake of I^{131} in different follicles.

The behavior of the mice in the 25-μc group bearing minute tumors was less variable than that of the 75-μc group. The two mice with the lowest I^{131} uptake by the thyroid had larger tumor grafts than those with high I^{131} uptake so that it is probable that 25 μc of radioiodine injured the thyroid gland of these two mice. If the remaining thirteen of the mice receiving 25 μc are compared with those of the 0-μc group bearing minute tumors, it may be noted that the I^{131} retention was about the same in both (60.8 and 61.9%, respectively) and most of the I^{131} retained was in the thyroid (86.1 and 89.0%). Thus the conclusion is warranted that in these thirteen mice 25 μc did not noticeably alter the thyroid.

Whether or not 25 μc had injured the thyroid could also be evaluated by comparing normal mice with those given 25 μc but not grafted with tumor. The I^{131} retention in the body and in the thyroid was only slightly greater in the latter. This can be explained by assuming that this dose depresses slightly the thyroid function of some mice.

The effects of radioiodine on the thyroid can be fully understood only by a consideration of a dynamic state covering the life span of the animal from the time of treatment until natural death. Depression of the thyroid can be assumed to be followed by stimulation of the pituitary with a secondary stimulation of the thyroid. The functional state of the injured thyroid can be expected to vary this time. A study is in progress to assess the long-term effects of graded doses of radioiodine on mice; function and morphology being correlated with tumor development.

To provide controls for the tumor-bearing mice pretreated with graded doses of I^{131}, similar mice without tumors, available 7 months after the administration of graded doses of I^{131}, have been included in the present study. The thyroids of the controls that were given 25 μc exhibited subtle changes which could be detected only by a close comparison with normal thyroids. There was a slight increase in size of the thyroid cells notably at the periphery of the gland, with some resolution of the colloid. The I^{131} uptake by thyroids of mice pretreated with 25 μc averaged 23.1% of the injected dose as contrasted with 14.5% in normals. Slight atrophy and fibrosis of the organ indicated injury by 25 μc of I^{131} whereas swelling of epithelial cells indicated stimulation by TSH. The epithelial cells contained many intracytoplasmic vacuoles and colloid droplets and exhibited some features of anaplasia. With tumors of long duration, adenoma formations were common.

Fifty and 75 μc of I^{131} produced conspicuous changes consisting of slight to moderate fibrosis, occasional atrophy, moderate to marked variability in the size and shape of the cells and acini, and in quantity and staining quality of the colloid. The lining cells of some acini were flat; others were greatly swollen and frequently the same follicle was lined with cells of greatly varying sizes. There were marked differences in size and chromatophilia of the nuclei. Some were small, others "giants," and some normochromic or hyperchromic with respect to affinity to hematoxylin. The cytoplasm of many cells was filled with colloid droplets varying greatly in size and number. Most cells were intensely vacuolated.

One hundred μc of I^{131} produced a marked reduction in the number of cells of the thyroid so that the organ could not be identified at a magnification of 5 x at autopsy. Microscopic examination of these mice disclosed a more intense change in the residual thyroid than in mice of the 50- and 75-μc groups. Anaplasia as concerns nuclear size, shape, and chromatophilia was particularly marked. Note that 50 to 100 μc markedly depressed thyroidal uptake of I^{131} but, in the presence of tumor, I^{131} uptake was fairly high (75-μc group). The presence of tumor markedly increased the total body retention of I^{131} (estimated 12-50% of the injected dose) and this enabled a greater uptake of I^{131} by the small residual thyroid tissue that had been stimulated by the TSH secreted by the pituitary tumors.

Two hundred and fifty to 400 μc of I^{131} destroyed the thyroid almost completely. Its site was identified by a marked stenosing "radiation arteritis" often with perivascular infiltrations by lymphoid cells and hemosiderin filled macrophages in an area of fibrosis adjacent to the parathyroid. Frequently, with an 200 μc dose, a few isolated acini remained, exhibiting the retrogressive changes already described, anaplasia being particularly marked. The presence of these rudimentary thyroid cells can be readily detected *in vivo* by the scintillation counter following administration of a tracer dose of I^{131}. Thus they function, but are unable to undergo compensatory hyperplasia when constantly stimulated by the tremendous quantities of TSH in the blood discharged by the grafted tumor. In mice receiving 300 μc or more, such rudimentary thyroid tissue was but rarely identified.

Thus the results of the morphological and iodine uptake studies are in agreement. The threshold dose of I^{131} for depressing the thyroid of mice appears to be in the neighborhood of 25 μc. This dose might cause a slight depression or even an apparent stimulation in some animals. Correspondingly, this is also the threshold dose for rendering some animals susceptible to tumor growth; in others the grafts are merely held *in situ* as they are in normal hosts.

The findings are being correlated with those of others and are concluded as follows:

The observations made indicate that proliferation of grafted TSH secreting pituitary tumor cells depends on the secretion of TH.

Near-complete or complete destruction of the thyroid follows administration of a single dose of 200 or more μc of I^{131}. This provides the maximum stimulus to pituitary growth. A lower dose, about 75 μc, produces a lasting depression of the thyroid, some follicles of which although normal in appearance, are incapable of undergoing fully compensating hyperplasia. In most mice 25 μc interferes little if at all with the thyroid function, with ability of this gland to be stimulated by TSH, and with its capacity to inhibit the growth of pituitary tumors. The inverse relationship between thyroid function, as indicated by its ability to take up I^{131}, and stimulation of the pituitary tumor grafts is almost quantitative.

Cells of thyroid glands which escaped destruction by large doses of I^{131} do not have the ability to undergo a fully compensating hyperplasia. This may be due in part to the stenosing "radiation arteritis" and diffuse fibrosis in the thyroid region caused by I^{131}.

Radiothyroidectomy causes an increased total body retention (or diminished elimination) of I^{131} during the first 24 hours following injection. Pituitary tumor grafts secreting TSH enhance further total body retention of I^{131}. The sites of this I^{131} retention are not precisely known. In radiothyroidectomized mice (with and without tumor) the elimination of the retained I^{131} is rapid. In normal mice bearing grafted tumors most of the retained radioiodine is in the thyroid and its retention is lasting.

These studies point to a simple isotopic assay of TSH and this is being worked out currently.

Late Effects of Exposure to Thermal Neutrons in Mice

(Upton, Farbstein)

These long-term studies, begun in 1949, have been repeatedly mentioned in previous Quarterly Reports. Rf mice of both sexes were irradiated at 8-10 weeks of age. One group was exposed in the thermal neutron column of the Oak Ridge reactor, and litter mates were given corresponding doses of X radiation or set aside as nonirradiated controls. The animals were followed until natural death or, in some instances, sacrificed at 24 months of age. Exposure in the thermal column of the pile for 80 minutes constituted an LD_{50}. In this facility the flux was approximately $0.9 \times 10^9 n/cm^2/sec$, with 6-7 r/minute of high energy γ radiation. The corresponding LD_{50} of 250 kvp X radiation was 80 minutes' exposure at 6.4 r/minute (512 r). Mice were exposed to both types of radiation for 80, 20, 5, and 2.5 minutes, respectively (Table 18).

TABLE 18

DOSAGES OF THERMAL NEUTRON-GAMMA AND X RADIATION IN THE GROUPS STUDIED

Duration of exposure (minutes)	Total thermal neutrons/cm^2	Total concomitant γrays (r)	Group designation	Matching dose, X rays (r)
80	4.3×10^{12}	520	LD_{50}	512
20	1.1×10^{12}	130	1/4 LD_{50}	128
5	2.8×10^{11}	32	1/16 LD_{50}	32
2.5	1.4×10^{11}	16	1/32 LD_{50}	16
0	-	-	-	-

The acute radiation-induced effects were essentially identical in both pile-exposed and X-irradiated groups, as reported earlier. Opacities of the lens occurred at all dose levels, and the relation of intensity and progression of opacities to the dose also has been described.

The pattern of longevity is indicated in Table 19. It is apparent that

TABLE 19

LONGEVITY OF MICE EXPOSED TO SLOW NEUTRONS AND TO X RAYS

	Males				Females			
	Slow neutrons		X rays		Slow neutrons		X rays	
Dose	Total no. of mice	Mean survival (months)*	Total no. of mice	Mean survival (months)*	Total no. of mice	Mean survival (months)*	Total no. of mice	Mean survival (months)*
LD_{50}	123	16	70	16	99	15	91	15
1/4 LD_{50}	130	17	116	18	131	17	111	17
1/16 LD_{50}	138	20	126	20	109	19	105	19
1/32 LD_{50}	109	20	80	21	77	19	49	20
Nonexposed	248	21	-	-	287	22	-	-

*Age in months at which 50% of mice dead

radiation resulted in a shortening of the life expectancy, the reduction of life span being proportional to the dose. Preliminary data suggest that this effect may be independent of radiation-induced neoplasia and leukemia.

The incidence of leukemias as diagnosed at autopsy is presented in Table 20. The leukemias are now being typed histologically. Those desig-

TABLE 20

INCIDENCE OF LEUKEMIA IN MICE EXPOSED TO SLOW NEUTRONS AND TO X RAYS

Group				Non-exposed	LD_{50}	1/4 LD_{50}	1/16 LD_{50}	1/32 LD_{50}
Female	Mediastinal	Neutron		9.7	26.2	17.6	15.6	22.0
		X ray			36.2	13.5	8.6	12.2
	Non-mediastinal	Neutron		20.2	27.2	35.9	28.4	25.9
		X ray			19.7	34.2	33.3	36.7
	Total leukemia	Neutron		29.2	53.4	53.5	44.0	47.9
		X ray			55.9	47.7	41.9	48.9
Male	Mediastinal	Neutron		5.2	20.3	6.9	3.6	12.8
		X ray			14.2	11.2	7.1	3.8
	Non-mediastinal	Neutron		33.5	26.0	36.9	36.2	23.8
		X ray			35.7	29.3	30.9	33.8
	Total leukemia	Neutron		38.7	46.3	42.8	39.8	36.6
		X ray			49.9	40.5	38.0	37.6

nated mediastinal are probably all lymphoid, and most if not all thymic in origin. The incidence of the latter was significantly increased after median lethal doses of either neutron-γ or X radiation, the peak occurring relatively early (6-12 months of age). Those referred to as nonmediastinal are predominantly lymphoid also, but of extrathymic distribution, and include myeloid, monocytic, and reticulum cell types. Further interpretation of these data awaits completion of histologic typing.

Neoplasms of various types have been observed. The most common are of pulmonary and ovarian origin. Their incidence, as diagnosed grossly, is given in Table 21. It would appear that the threshold dose for induction

TABLE 21

INCIDENCE OF PULMONARY AND OVARIAN NEOPLASMS IN MICE EXPOSED TO SLOW NEUTRONS AND TO X RAYS

	Percentage with lung tumor				Percentage of females with ovarian tumor	
	Males		Females			
Dose	Slow neutrons	X rays	Slow neutrons	X rays	Slow neutrons	X rays
LD_{50}	10.5	14.2	19.1	19.7	52.5	34.0
1/4 LD_{50}	13.8	20.7	20.6	22.5	51.1	53.2
1/16 LD_{50}	25.4	22.2	28.4	27.6	17.5	38.1
1/32 LD_{50}	22.9	28.8	20.7	26.5	11.7	8.2
Nonexposed	20.2		13.6		2.8	

of ovarian neoplasms is in the neighborhood of 16 r. The incidence of pulmonary adenoma was increased by irradiation in the female, as is consistent with earlier observations. The higher spontaneous incidence in the male is also in agreement with previous findings. The carcinogenic effect of even the lowest dose (1/32 LD_{50}) in the female deserves further investigation. The relatively small number of pulmonary tumors occurring after high doses of radiation may be related to the shorter survival of mice after heavier exposures, since these neoplasms arise late in life. The incidence of other tumors, such as hepatoma, may have been increased by irradiation; they were relatively few and will be analyzed later.

Final conclusions may not be drawn until histologic studies have been completed and the RBE for various parameters analyzed. Tentatively it may be inferred that the pathologic effects of thermal neutron radiation were not significantly different from those of X radiation. The relatively high cataract-inducing potency of thermal neutrons has been reported earlier.

Modified Martins-Mallory Stain for Mouse Pituitary Gland

(Gude)

Technical improvements essential to make headway in our program include histological and isotopic procedures. One of the limiting factors in the study of the mouse pituitary has been the lack of a staining method

which adequately stains all three cell types. In this laboratory a technique suitable for routine staining has been worked out, which combines Martins' technique and that of Mallory's aniline blue-orange G, and which differentiates acidophils, basophils, and chromophobes.

Martins' stain utilizes methylene blue as a stain for granules of the basophil cell. However, it is difficult to control the intensity of this staining reaction; thus, by substituting Mallory's aniline blue-orange G mixture for methylene blue, differentiation is easier to control and the basophil granules are stained intensely while Martins' acid fuchsin reproduces the acidophils more sharply than orange G. Staining of nuclei is reinforced with Harris' hematoxylin.

Satisfactory pituitary preparations can be obtained by leaving the gland attached to the bone at the time of fixation and decalcifying it subsequently with an electrolytic bone decalcifier, or with 10% formic acid. There is no loss of staining quality of the granules, and distortions due to dissection and handling of this minute organ are avoided; good visualization of the structures surrounding the pituitary (bone marrow, glandular tissue, and muscle) add to the value of this staining of the pituitary <u>in situ</u>.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson (Leader)
E. F. Phares M. V. Long
M. I. Dolin Suzanne Berger
J. M. Adams* E. A. Delwiche**

Studies on the Reversible Reaction, Succinate to Propionate Plus Carbon Dioxide

(Carson, Phares, Dolin, Delwiche, Long, Berger)

Previous experiments established the presence in the propionic acid bacteria of coenzyme A-adenosinetriphosphate-dependent activation system for succinic, propionic, acetic, and to a small degree, formic acids. However, substrate levels of CoA seemed to inhibit the over-all reaction of propionate formation from succinate. The nature of this inhibition has not been established and further work has been carried out using catalytic amounts of the coenzyme.

Necessity of ATP for formation of propionate and carbon dioxide from succinate. Using cell-free extracts treated with basic ion-exchange resin (Dowex-1), propionic acid and carbon dioxide are formed from succinic acid-1-C^{14} in the following ratios, based on C^{14} activity recovered (unity represents a 30% conversion of 5 μM of succinate).

	Propionate	CO
Dowex-treated enzyme + CoA	0.61	0.035
Dowex-treated enzyme + ATP	0.07	0.001
Dowex-treated enzyme + ATP and CoA	1.0	0.05

These data indicate a very strong dependency for CoA, with a smaller ATP requirement, which is well in line with the succinate activation requirements. The carbon dioxide ratios demonstrate the dependencies even more; however, the actual amount of activity found in the carbon dioxide is only 5% of that in the propionate, which suggests the esistence of a stable, 1-carbon product other than carbon dioxide, or of a very active product

*ORINS Predoctoral Fellow
**Research Participant

which is involved in competing reactions. The compounds containing the remainder of the radioactivity, which should be approximately equivalent to the radioactivity found in the propionate, have not yet been identified.

Succinate formation from propionate and CO_2. — Attempts to reverse the above reaction with only propionate and carbon dioxide present as substrates were unsuccessful, probably due in part to the extremely unfavorable equilibrium for the reaction. With large amounts of succinate present, however, conversion of propionic-2-C^{14} acid to succinic-2,3-C^{14} acid was accomplished to about the same extent as the reverse reaction. This large incorporation against an unfavorable equilibrium could have been due to a "pool" of the succinate; this phenomenon also resembled the oxalacetate exchange reaction, observed by Utter and Wood, in which the presence of oxalacetate was required in order to fix either pyruvate or carbon dioxide into oxalacetate. The existence of such a reaction in our present case was tested by using $C^{14}O_2$ along with the propionic acid-2-C^{14}. The succinate which was formed showed that the amount of propionate incorporated was eight times that of incorporated carbon dioxide. This is interpreted as evidence of a 1-carbon product from succinate with which propionate can combine in preference to carbon dioxide itself. When the Dowex-treated enzyme was used, a fivefold stimulation of carbon dioxide incorporation and a fifteenfold stimulation for propionate incorporation was obtained by the addition of CoA.

When formaldehyde-C^{14} and formic acid-C^{14} were used as 1-carbon substrates in place of carbon dioxide, essentially no incorporation into succinate was obtained. This would appear to further indicate that the 1-carbon "combining compound" is actually on the oxidation level of carbon dioxide.

Attempts were made to detect the accumulation of the active 1-carbon molecule along with the activated forms of propionic and succinic acids. Succinic acid was incubated with substrate amounts of CoA; the reaction was stopped by heating, hydroxylamine was added, and the formation of hydroxamic acids was tested by paper chromatography. No accumulation of any of the acids was detected by the procedure, although the activation of succinic, propionic, acetic, and formic acids can be shown by this technique when the hydroxylamine is added as a trap during incubation of these acids. It is possible that either heat or enzymes decomposed the product before addition of the hydroxylamine.

Enzyme fractionation. — Preliminary procedures for the isolation of the enzyme systems involved in these transformations were carried out. Cell-free juice was subjected to ammonium sulfate fractionation and the fractions tested for ability to activate succinic and propionic acids by the CoA-ATP reaction, as measured by the formation of hydroxamic acids. The fraction precipitating at 35-40% saturation with ammonium sulfate accounted for

10% of the activity of the original juice, with negligible amounts in the other fractions. This fraction, alone, and in combination with other fractions, showed only very feeble ability to produce propionic acid from succinate.

α-Keto Acid Oxidation by Streptococcus faecalis

(Dolin, Long)

The intermediate steps in keto acid oxidation are being further studied by comparing the metabolism of diacetyl and acetaldehyde with that of keto acids in the keto acid oxidation system of Streptococcus faecalis.

The oxidation of diacetyl and acetaldehyde carried out by vacuum-dried cell preparations conform to the following equations, as determined by performing chemical balances.

$$\text{diacetyl} + 2\ \text{ferricyanide} \longrightarrow 2\ \text{acetic} + 2\ \text{ferrocyanide}$$

$$\text{acetaldehyde} + 2\ \text{ferricyanide} \longrightarrow \text{acetic} + 2\ \text{ferrocyanide}$$

Anaerobically, in the absence of ferricyanide, these substrates undergo dismutation.

The anaerobic diacetyl reaction appears to be similar to the "diacetyl mutase" of pigeon breast muscle (Green et al., J. Biol. Chem., 167: 811 - 816, 1947), in that acetoin accumulates as the reduced product. In the presence of ferricyanide, only traces of acetoin are formed in the S. faecalis system. Although the reduced product in the acetaldehyde dismutation was not determined, it is probably ethyl alcohol.

Cell-free extracts have been obtained which oxidize diacetyl but not acetaldehyde in the presence of ferricyanide. This would indicate that the enzyme catalyzing the production of active aldehyde from $-\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}-$ does not activate free aldehydes. A further difference between dicarbonyl and aldehyde oxidation is shown in the requirement for lipoic acid in the former reaction but not the latter.

At present, the diacetyl reaction may be pictured as a hydrolytic or possibly thioclastic or phosphoroclastic split of the diketone, to give 1 molecule of acetate or acetyl and one of active aldehyde, the aldehyde then being oxidized to another molecule of acetate or acetyl, possibly via the keto acid pathway. This mechanism is supported by (1) the balance presented, which shows the production of 2 molecules of acetate per molecule of diacetyl oxidized with the transfer of one pair of electrons, and (2) the

observation that the S. faecalis enzyme fraction I, previously described (ORNL-1393) and which carries out no detectable stoichiometric conversion of keto acid in the absence of a second enzyme fraction (II), will oxidize ketobutyrate and diacetyl at similar rates, with ferricyanide as hydrogen acceptor. Hager and Gunsalus (personal communication) have described the oxidation of pyruvate by analogous fractions from Escherichia coli. The activation of diacetyl by S. faecalis fraction I is completely cocarboxylase dependent. The requirement for cocarboxylase in a reaction not involving decarboxylation, strengthens the hypothesis that this coenzyme is involved in the formation of active aldehyde.

Further evidence that enzyme fraction I is involved in reactions at the aldehyde level was obtained from isotope experiments which showed a small but definite incorporation of $C^{14}O_2$ into ketobutyrate in the presence of enzyme I, but virtually none in the presence of enzyme II. Unpublished experiments of S. Korkes have shown the incorporation of carbon dioxide into pyruvate with enzyme fraction A of E. coli (J. Biol. Chem., 193: 721-735, 1951).

Experiments are now in progress to determine the relationship of the aldehyde formed from diacetyl to that formed during keto acid oxidation.

BIOCHEMISTRY

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

W. E. Cohn (Leader)
D. G. Doherty
J. X. Khym
Elliot Volkin
Fred Vaslow
B. L. Strehler
M. Helen Jones
G. M. Cheniae

Nucleic Acid Structure

(Cohn, Khym, Volkin, Doherty)

Identification of nucleotides a and b. — The identification of the position of the phosphate group in our nucleotides a and b has proceeded slowly along the lines described in ORNL-1393. The enzymic hydrolysis of the nucleotides to their respective ribose phosphates is complicated by very low yields, so low as to be ascribable to impurities, and by subsequent degradation to ribose and phosphate by phosphatase. It has not been possible, up to now, to improve the enzyme preparation.

As an alternative, we have resorted to acid hydrolysis of the purine bases from the ribose phosphate. Treatment of the purine nucleotides with 1 N hydrochloric acid or acid Dowex-50 at 100°C leads to a preferential splitting at the glycoside linkage with the liberation of two (or sometimes three) sugar phosphates, ribose, and phosphate. The time and temperature characteristics of this degradation have been studied and it has been found that approximately 50% of the adenylic acid can be split to adenine and two ribose phosphates, without further degradation by acid Dowex-50, in 30 seconds at 100°C. Complicating this procedure is the rapid equilibration of the two nucleotides and of the two ribose phosphates under the same conditions. However, it is hoped that a disproportionation in the yields of the two ribose phosphates at short periods of heating will give unequivocal evidence as to which ribose phosphate comes from which nucleotide. The next step, identification of the ribose phosphates, should not be difficult.

A by-product of this study has been the appearance of a third sugar phosphate, which is slow to appear and relatively stable to loss of phosphate. We hope to identify it.

A third approach to the degradation, involving destruction of the base by bromine or otherwise, is yet to be tried.

The ribonuclease digest. — The completion of the analysis of the ribonuclease-produced fragments (see ORNL-1297; 74-75) has involved rather laborious ion-exchange recycling, enzymic degradation, and analysis of the fragments, all in quantitative fashion. This work is largely

completed and a paper is in preparation, although there is much more that we hope eventually to do with this digest. Among the new identifications made in this digest are: AAGC(peak 22 in the cited report), AAU(23), AAGU(25), AGGC(30). Also, we have established that GAC precedes AGC and that GAU precedes AGU.

Enzyme Substrate Equilibria

(Doherty, Vaslow, Cheniae)

In a continuing study of the nature of complex formation by chymotrypsin the following modified substrates were prepared: N-ethyl-L-tyrosine, N-formyl-L-tyrosine, L-tyrosinol, N-acetyl-L-tyrosinol, and β(p-hydroxyphenyl) propionic acid. These substrates were tagged in the usual manner with Br^{82} and the thermodynamic properties of their complex formation with chymotrypsin determined as previously described. L-Tyrosinol similar to other compounds with a free amino group α to the carbonyl exhibited a typical noncatalytic behavior with the enthalpy becoming positive and a very small change in entropy. The other four compounds exhibited catalytic properties to a varying degree. N-Formyl-L-tyrosine was bound more strongly than N-acetyl-L-tyrosine with a ΔH of -7500 cal and a large $-T\Delta S$ of +6000 cal. Thus, removal of the methyl group has permitted a tighter fit of the substrate to the protein surface and has therefore increased the strain on the catalytically sensitive bond, as indicated by the large $-T\Delta S$. N-Ethyl-L-tyrosine, with a more basic amino group, was weakly bound, ΔH = -3000 cal, and showed a small catalytic strain, $-T\Delta S$ = -2000 cal. N-Acetyl-L-tyrosinol was more strongly bound, ΔH = -4000 cal, but also had the same $-T\Delta S$ of -2000 cal. β(p-Hydroxyphenyl) propionic acid, with no acetamino group for binding in the α position, was bound strongly, ΔH = -4500 cal, but also had a $-T\Delta S$ of -2000 cal. To test the validity of these predictions of catalytic activity, hydrolyzable derivatives, i.e., esters or amides, of these compounds are being prepared and will be subjected to enzymatic action and the kinetics of hydrolysis determined.

Conservation of Phosphorus in Exponentially Dividing Bacteria

(Volkin; Doermann, Cytogenetics)

In an experiment originally designed for another purpose, a rather interesting observation on bacterial growth became apparent. The data and conclusions will be described briefly here.

Escherichia coli were made highly radioactive by growing through 4.27 generations at 37°C in 6 liters of nutrient broth containing 150 mc of P^{32}. They were then washed once with cold P^{32}-free broth, then transferred into 6 liters of P^{32}-free nutrient broth at 37°C and allowed to grow for 30 minutes. At this time three samples were removed: (1) approximately 3 liters into which was poured 160 ml of 100% TCA; (2) 2-3 ml for bacterial plate counting; and (3) 2-3 ml for counting radioactivity. To the remaining 3 liters of culture, an additional 3 liters of P^{32}-free nutrient broth at 37°C was added immediately. This process was repeated four more times at exactly 30-minute intervals. After all the 3-liter samples containing TCA were collected, they were separated into acid-soluble, lipid ribonucleic, desoxyribonucleic, and protein phosphorus fractions by a modified Schmidt-Thannhauser technique. The fractions were then assayed for radioactivity and phosphorus contents. By this procedure it was possible to follow the disappearance of P^{32} (turnover) from the phosphorus-containing cell constituents, in conjunction with the reproduction rate of the bacteria during the log phase. The 30-minute sampling time corresponded to an average of 1.36 bacterial divisions.

The results clearly demonstrated that, under these conditions of bacterial growth, the amount of isotope (and the specific activity) in each of the phosphorus-containing constituents, when multiplied by the necessary dilution factor for the appropriate time intervals, remained essentially unchanged throughout all 30-minute intervals. There was no significant loss of isotope to the medium. The conclusion seems apparent then, that in log-phase growing bacteria there is (a) no significant degradation of bacterial material, or (b) if breakdown of cell constituents does occur, the products are immediately reutilized for synthesis of higher molecular order material, without passage out of the cell. Thus during the log phase, the enzymatic functions are totally directed toward synthesis and growth.

Energy-Transfer Systems

(Strehler)

During the previous quarter, the work on photosynthetic and bioluminescent energy-transfer systems has been centered in two main lines of investigation. In photosynthesis, methods have been devised and are now being improved for measuring very small absorption changes in illuminated plants. Through the use of internal standards, it is hoped that the ultimate sensitivity of the method for studying spectral changes in narrow spectral regions of illuminated plants can be extended to 1 part in 10^4. A study has been made, in collaboration with W. A. Arnold, of fluorescence changes in illuminated plants. An electronic analog computer designed to obtain qualitative information on the effect of intermittancy and of parallel and

series reaction sequences in biological systems has been constructed in cooperation with J. Davidson of the Instrument Division. With this apparatus it has been possible to reproduce many of the induction and flashing-light phenomena in photosynthesis observed in our laboratory.

Additional information on firefly luciferin and one of its degradation products has been obtained. Electrophoretic mobility on a paper strip has been investigated as a function of pH as well as fluorescence changes as a function of the same variable. In addition, the mass spectroscopy of this compound and some of its pyrolysis products have been investigated through the cooperation of Dr. C. R. Baldock of the Stable Isotopes Division.

PLANT PHYSIOLOGY

G. R. Noggle (Leader)
E. G. Beck* M. Eleanor Schumacher

Experiments with Heptuloses

(Noggle, Schumacher)

In a previous report (ORNL-1393) the behavior of a number of heptuloses on paper chromatograms was given. Two additional heptuloses (D-idoheptulosan and L-guloheptulosan) were obtained from Dr. Nelson K. Richtmyer of the National Institutes of Health, Bethesda. The behavior of these heptuloses, along with the other heptuloses on paper chromatograms, is summarized in Table 22.

TABLE 22

Sugar	R_f (phenol)	Distance in mm from origin (ethyl acetate-acetic acid-water, 3:1:3)	Color with orcinol-TCA spray
D-Glucoheptulose	0.42	103	Blue
D-Mannoheptulose	0.40	108	Green-blue
L-Galaheptulose	0.48	99	Blue
Sedoheptulose	0.46	125	Blue
D-Idoheptulose	0.50	147	Blue
L-Guloheptulose	0.53	138	Blue
Sedoheptulosan	0.70	153	Blue
D-Idoheptulosan	0.64	194	Blue
L-Guloheptulosan	0.65	188	Blue

Sedoheptulosan may be converted to an equilibrium mixture of sedoheptulosan and sedoheptulose (80:20) by treating with hot 0.2 N hydrochloric

* Research Participant

acid. When this was done and the solution was freed of acid by treatment with Dowex-3 in the hydroxyl form, the neutral solution was paper chromatographed with water-saturated phenol (pH 5.5). The completed chromatogram was sprayed with orcinol-trichloroacetic acid and four blue spots were obtained with R_f values of 0.45, 0.64, 0.70, and 0.95. The 0.45 and 0.70 spots were sedoheptulose and sedoheptulosan but the other two materials remain unknown. When Dowex-50 in the hydrogen form was used in place of hot 0.2 N hydrochloric acid to convert sedoheptulosan into an equilibrium mixture of sedoheptulose and sedoheptulosan, only the two expected sugars were found on paper chromatograms.

Dowex-50 may also be used instead of 0.2 N hydrochloric acid to convert idoheptulosan and guloheptulosan into equilibrium mixtures of anhydrides and free sugars. The optimum conditions for these conversions have not been attained and additional work is in progress.

The heptuloses have been studied with regard to their behavior on borate anion-exchange columns. Ten-milligram quantities of each heptulose (sedoheptulosan, idoheptulosan, and guloheptulosan) were treated with Dowex-50 to obtain equilibrium mixtures of anhydrides and free sugars, and were run separately through a column of Dowex-1 (0.85 sq cm x 12 cm) in the borate form. The results are shown in Fig. 13. With the

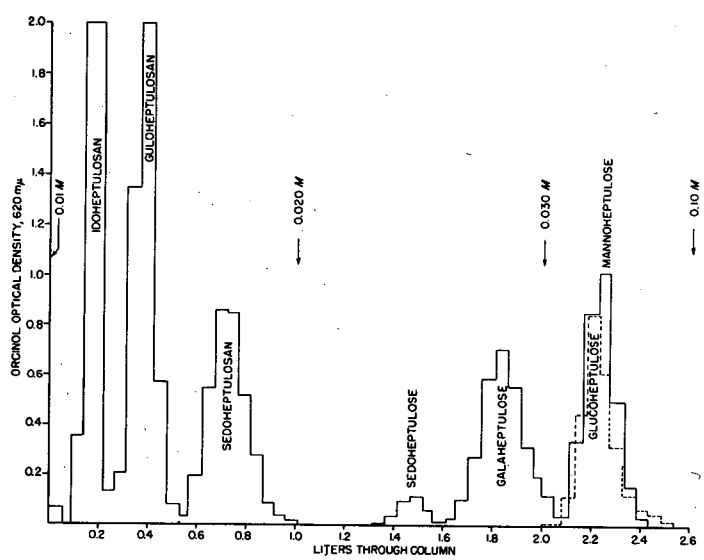

FIG. 13. Dowex-1 column, borate form, ca 300 mesh.

Bed dimensions, 0.85 sq cm x 11 cm. Flow rate 2 ml/minute.

Test materials, 10 mg each of idoheptulosan, guloheptolosan, galaheptulose, glucoheptulose, mannoheptulose, 8 mg sedoheptulosan, 2 mg sedoheptulose.

exception of glucoheptulose and mannoheptulose, the individual sugars may be separated by this procedure. These two sugars have been separated by a change in concentration of tetraborate solution used for elution. The free sugars, idoheptulose and guloheptulose, were not obtained; additional work is under way on this problem.

The Use of Dowex-50 to Invert Sucrose Solutions

The observation has been frequently made that sucrose can be inverted to glucose and fructose by passage through an ion-exchange column containing a cation-exchange resin in the hydrogen form. It was thought that it might be possible to develop a method of inverting sucrose that would do away with acid hydrolysis which entails the subsequent neutralization of the acid. Experiments have demonstrated that Dowex-50 in the hydrogen form may be used for this purpose. Sucrose (20-50 mg) was dissolved in 5 ml of water and 1 of moist Dowex-50 (in the hydrogen form) was added. The solution was placed in a boiling-water bath and stirred for 30 minutes after which it was filtered through a sintered-glass filter funnel. Complete inversion of the sucrose resulted, as compared with acid or invertose hydrolysis. Under the same conditions melibiose, maltose, cellobiose, and turanose were not affected. Raffinose was partially hydrolyzed to sucrose and galactose, while melezitose was partially hydrolyzed to glucose and turanose, although the turanose portion did not act typically on paper chromatograms. Additional work is in progress on this problem.

Acid-Soluble Phosphates of Crown Gall

(Beck)

It has been demonstrated a number of times that galls contain a higher percentage of phosphorus than normal tissues in the same region of the plant. In the study of the nature of the gall stimulus it was believed that an investigation of the phosphorus compounds of both gall and normal tissues might give some lead as to the metabolic variations due to the gall stimulus.

Crown galls were induced on 8-week-old tobacco plants (Nicotiana rustica) by inoculating internodes near the stem tips of the plants with the crown gall bacterium Agrobacterium tumefaciens. Control plants were wounded in the same region with a sterile needle. Wounded internodes of both control and inoculated plants were harvested every other day for the first week and once a week thereafter and a small fraction of each harvest analyzed for total phosphorus by the method of Fisk and Subbarow. The

remainder of the tissue was extracted with trichloroacetic acid. The acid extract was fractionated into five fractions by the method of Umbreit, Stauffer, and Burris, the compounds of each fraction identified whenever possible and, the total phosphorus of each fraction determined.

Fraction 1 was obtained by adding an equal volume of 95% ethanol to the acid extract. This consisted of a polysaccharide containing phosphorus. After the residue had been centrifuged off, the supernate was made to twice its volume with 95% ethanol, neutralized to pH 8, and 0.25 ml of 1.0 N barium acetate per gram of tissue extracted was added to the solution. This was allowed to stand for several hours and then centrifuged. The supernate contained fraction 2 which was composed of coenzyme I, as well as a number of unidentified phosphates. The residue was dissolved in 0.1 N hydrochloric acid and centrifuged. The residue contained fraction 3 and consisted of inorganic metaphosphates. Fraction 4 was obtained by increasing the pH of the supernate to four and centrifuging. The precipitated fraction consisted of inorganic phosphate, probably pyrophosphate. The supernate was then neutralized to pH 8 and fraction 5 centrifuged off. This fraction contained a comparatively large amount of orthophosphate with such organic compounds as hexose diphosphate, adenosinediphosphate, adenosinetriphosphate and phosphoglyceric acid. The supernate contained fraction 6, in which was found such compounds as hexose phosphate, ribose phosphate, triose phosphate, adenylic acid, and nucleotides.

There was a rise in the total phosphorus of both the crown gall and control tissues during the first few days after wounding. The increase reached its peak within a week in the control tissue and at the end of 2 weeks in the crown gall tissue. In both cases the same change was reflected in the phosphorus of the acid extract. The greatest differences between crown gall and control tissues appeared in fractions 1 and 5. The phosphate of fraction 1 (polysaccharide) of the gall extract increases to more than double the percentage of that which occurs in the control tissues. Fraction 5, barium precipitate insoluble at pH 8, made up over half of the acid extract after the first 2 weeks and increased up to 8% of the extract at the end of 5 weeks. In the case of the gall tissue this fraction never contained more than 10% of the phosphate of the acid fraction during the first 4 weeks. At the end of that time it began to increase and assume proportions similar to those of the control. The phosphorus content of the other fractions of the acid extract decreased rapidly with the age of the stem, in the case of the control, until they reached a level of 1 or 2% of the extract after 4 weeks. In the case of the crown gall tissue, the remaining fractions remained at about the same level attained during the first week, with some tapering off at the end of 4 weeks.

It is believed that the accumulation of the polysaccharide in the crown gall tissue was due to the inhibition of one phase of an enzyme cycle in the

process of respiration. Because fraction 5 of the control was much higher in organic phosphorus than the control in all the analyses made, it is believed that the smaller amount of phosphate, particularly organic phosphate, in the gall extracts is indicative of a high enzyme activity in connection with the compounds of this fraction.

PLANT BIOCHEMISTRY

N. E. Tolbert (Leader)
L. P. Zill Evelyn Purdy

Since the proposed program of investigation in plant biochemistry would require supplies of both radioactive and nonradioactive sedoheptulose, several samples were prepared by biosynthetic procedures.

Preparation of "cold" sedoheptulose. — Sedoheptulose was prepared by the method of LaForge and Hudson (J. Biol. Chem. 30: 61, 1917) from Sedum plant (probably *Sedum acre*). The yield was very low, only about 2 g of sedoheptulose being obtained from 5 kg of harvested plant material. Since the free sugar has never been crystallized, it was converted to the anhydride form which permits crystallization.

Preparation of "hot" sedoheptulose. — (With G. R. Noggle, of Plant Physiology). Potted Sedum plants (*Sedum spectabile*) were placed in a conventional photosynthesis chamber equipped with a circulating fan and device for production of $C^{14}O_2$. Labeled carbon dioxide was produced by the reaction of lactic acid with $BaC^{14}O_3$. The chamber was strongly illuminated from above by both incandescent and fluorescent lights. Plants were allowed to photosynthesize for 48 hours, during which 5 mc of labeled carbon dioxide was produced in the chamber. The plants were cut off at the base giving a harvested weight of ca. 155 g. Leaves and stems were disintegrated in a Waring Blendor, after which the procedure followed was identical with that for the "cold" sedoheptulose. The free sugar was again converted to the crystalline anhydride form. Analysis for uniformity of labeling and total incorporation of $C^{14}O_2$ is in progress.

BIOPHYSICS

GENERAL PHYSIOLOGY

C. W. Sheppard (Leader)
J. S. Kirby Smith E. B. Darden, Jr.
N. G. Anderson Patty Jean Matthias

Radiological Physics

(Darden)

A lead-enclosed facility for exposure of biological material to fast neutrons has been installed in the Y-12 cyclotron and a preliminary investigation of the neutron dose available has been completed. These results showed that fluxes up to 10^9 neutrons/cm^2/sec are available in the facility with a small but appreciable γ-ray component present. Results obtained by the activation of threshold detectors and exposure of nuclear track emulsion plates suggest that a detectable fraction of the total neutrons are thermal or epithermal and that a large percentage have an energy > 3 Mev. With the assistance of Dr. H. H. Rossi (Consultant, Columbia Univ.) measurements of the dose rate in rep units were obtained with his tissue-equivalent ionization chambers. The first biological experiments in the facility include irradiation of Tradescantia inflorescences by Kirby-Smith and by Swanson of the Cytogenetics Section and are now under way.

A scintillation spectrometer has been acquired for the purpose of determining the distribution of energies present in the radiation from various sources used here for exposure of biological material. Preliminary tests of the equipment have been completed and the energy spectrum of P^{32} β particles from phosphorus-bakelite plaques is now under investigation, using an anthracene scintillation crystal.

Erythrocytes

(Matthias, Sheppard)

Studies on the synthesis of acetylcholine by acetone-dried powders of human red cells have been made using our modification of the method by Greig and Holland (Arch. Biochem. Biophys., 39:77, 1952).

In all experiments the production of acetylcholine in preparations from acetone-dried powders of rat brain has been compared with the acetylcholine synthesis in preparations from the red cell powders.

To this time acetylcholine has not been detected in preparations from human red cells. In addition to this fact, inhibition of rat brain choline acetylase activity by red cell powders has been evident.

Portions of this work have been done in collaboration with Dr. Greig and additional collaboration is planned in order that the differences in our results may be reconciled.

State of Desoxyribonucleic Acid in Various Rat Tissues

(Anderson)

Preliminary studies have been made to determine whether marked variations occur in the degree of polymerization of desoxyribonucleic acid (DNA) in various tissues of the rat. Homogenates, prepared rapidly at low temperatures, were diluted to have approximately the same DNA concentration. These were made 1 M with respect to sodium chloride and studied in an Ostwald-Cannon-Fenske viscosimeter. DNA concentration was determined by using the diphenylamine reaction. The viscosity of a given preparation was expressed as relative to the viscosity of the same preparation after treatment with DNAase. Relative viscosities plotted against DNA concentration gave similar curves with liver, brain, kidney, and spleen. Thymus gave slightly lower viscosities but the differences may not be significant. These results tend to support the view that DNA in various somatic tissues of the rat is similar and that differentiation does not involve gross changes in the degree of polymerization of DNA.

Preliminary studies on the effects of X rays on tissue DNA viscosities using this method suggest that very marked differences exist in the degree to which DNA is depolymerized *in vivo* in various tissues by similar doses.

Mass Isolation of Whole Cells from Rat Liver

The effects of perfusion with solutions containing calcium binding substances on the percentage of whole cells remaining after standardized treatment in a loose-fitting homogenizer have been studied. Citrate, pyrophosphate, versene, and adenosinetriphosphate were almost equally effective in preventing cell breakage. A method, based on these findings, has been devised for preparing whole cells in 40-50% yield from rat liver.

Tradescantia Studies

(Kirby-Smith)

It was observed recently that onion root tips irradiated in deuterium oxide showed some differences in chromosomal aberration frequency when compared with similar material irradiated in water. These studies are being extended to include Tradescantia root tips and pollen. Methods for replacing unbound water in Tradescantia pollen grains by deuterium oxide prior to irradiation have been developed.

A study of the effects of neutrons on Tradescantia inflorescences in collaboration with Dr. Swanson (Cytogenetics) is underway.