OFFICIAL USE ONLY

ORNL-1297

Cy. 1

TRAINING SCHOOL LIBRARY
CENTRAL RESEARCH LIBRARY
DOCUMENT COLLECTION

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES
3 4456 0360974 4

BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT

for Period Ending May 10, 1952

DECLASSIFIED

CLASSIFICATION CHANGED TO:
BY AUTHORITY OF: E. J. Murphy 1-4-60
BY: M. Tallent 3-28-60

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-1297

Subject Category: Experimental Biology

Contract No. W-7405-eng-26

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

Alexander Hollaender, Director

Edited by E. J. Slaughter

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 0360974 4

Index No. ORNL-1297
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 (Y-12 Area)	81
DuPont Company	2
General Electric Co., Richland	4
General Electric Co., Schenectady	2
Los Alamos	3
Naval Radiol. Defense Lab.	2
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	136

*Carbide and Carbon Chemicals Company internal distribution as follows:

G. T. Felbeck	1
Chemistry Library	2
Physics Library	1
Biology Library	2
Health Phys. and Metal. Library	2
Training School Library [redacted]	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

F. C. VonderLage	1
A. Hollaender	1
J. S. Felton	1
A. H. Snell	1
K. Z. Morgan	1
F. L. Steahly	1
M. T. Kelley	1
D. W. Cardwell	1
E. M. King	1
A. S. Householder	1
R. S. Livingston	1
C. P. Keim	1
C. D. Susano	1
W. A. Arnold	1
S. F. Carson	1
B. L. Strehler	1
S. C. Lind	1
C. W. Sheppard	1
R. F. Kimball	1
W. L. Russell	1
G. R. Noggle	1
Seymour Pomper	1
M. E. Gaulden	1
C. J. Collins	1
E. H. Anderson	1
A. H. Doermann	1
C. L. Comar	1
Jacob Furth	1
W. E. Cohn	1
A. W. Kimball	1
J. Moshman	1
E. J. Slaughter	1
J. S. Kirby-Smith	1
Shields Warren	1
P. B. Pearson	1
W. D. Claus	1
H. H. Plough	1
R. C. Briant	1
M. E. Ramsey	1
C. S. Shoup	1
G. E. Stapleton	1
W. T. Burnett, Jr.	1
L. P. Zill	1
Drew Schwartz	1
Daniel Billen	1
A. D. Conger	1
K. C. Atwood	1
A. C. Upton	1
John C. Bugher	1
E. F. Oakberg	1
R. H. Storey	1
W. K. Baker	1
Reports Office, TID	4
J. H. Buck	1
Kenneth Kasschau	1

Issuing Office
Technical Information Department, Y-12 Area
Date Issued: JUL 24 1952

OF[redacted]ONLY

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

OFFICIAL USE ONLY

TABLE OF CONTENTS

Page

REVIEW OF RESEARCH PROGRESS 1

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC 4

Publications 4

Scientific Society Lectures and Traveling Seminars 7

Visiting Lecturers 12

CYTOGENETICS 13

Cytogenetic Effects of Radiation 14

The Role of Externally Produced Hydrogen Peroxide in the Action of X Rays upon *Paramecium* 14

Effect of X Rays upon Micronuclear Number in *Paramecium* 16

Breakage of Chromosomes by Oxygen 17

Genetic Structure of Bacteriophage T4 19

Studies on the Distribution of Genetically Marked Nuclei in *Neurospora* Conidia . . 25

Radiation Studies on Yeast 28

Effects of Radiation on Rate of Mitosis 30

Fragmentation of Chromosomes in Grasshopper Neuroblasts by β and X Rays 30

Study of Mitosis in the Presence of Homogenates of Irradiated Embryos 31

Effect of Dinitrophenol on Respiration of the Grasshopper Embryo 32

Effect of X Rays on Respiration of Individual Embryos 33

Mutation Rates at Specific Loci in the Third Chromosome of *Drosophila melanogaster* 34

Cytological Analysis of X-Ray-Induced Mutations in *Drosophila melanogaster* . . . 37

Radiation Protection 40

Recovery of Bacterial Cells from X Rays 40

Radiation Protection Studies—Sulfhydryl Compounds 41

MAMMALIAN GENETICS AND DEVELOPMENT 42

Genetic and Developmental Effects of Radiation in Mice 43

Histological Study of a Semilethal in the Mouse 43

Page

PATHOLOGY AND PHYSIOLOGY 47

Late Effects in Mice of Exposure to Thermal Neutrons 48

Studies on the Pathogenesis of Radiation-Induced Cataract 51

Permeability Problems of Ionizing Radiations 52

Role of Platelets in Postradiation Hemorrhagic Syndrome; Platelet Transfusions in Dogs with Exteriorized Lymph-Venous Anastomosis 53

Studies of the Mechanism of Leukemogenesis by Radiation 56

Characteristics and Secretions of Pituitary Tumors Originating in Radiothyroidectomized Mice 59

Comparison of the Long-Term Distribution of C^{14} from Glycine-2-C^{14} and $NaHC^{14}O_3$ in Mice 59

MICROBIOLOGY 64

Tracer Studies on Intermediary Metabolism 65

Biosynthesis of Kojic Acid 65

BIOCHEMISTRY 71

Studies on Nucleic Acids, Enzymes, and Energy-Transfer Systems 72

Studies on Nucleic Acids and Enzymes 72

Nucleotide Studies 76

Enzyme Substrate Equilibria 76

A Direct Ion-Exchange Method for the Quantitative Analysis of Some Biologically Important Phosphate Esters 77

Biological Energy-Transfer Systems 78

Studies on the Formate Pigeon Bone Marrow System 81

Plant Physiology 82

Separation of Labeled Sugars by Ion-Exchange 82

Ion-Exchange Separation of Sugars 83

The Behavior of Glycosides on Anion-Exchange Colums 83

Some Effects of the Concentration of Potassium Bicarbonate and 2,4-D on the Uptake of K^{42} by Sunflowers as Determined by a Localizing Scintillation Count-Rate Meter 84

BIOPHYSICS 86

General Physiology 87

Effects of X Radiation on the Bioelectric Response in Dionaea muscipula 87

Page

Erythrocytes 87

Ionizing Radiation on Rat Liver Homogenates 88

Comparison of the Effects of X Rays, γ Rays, and β Rays on Tradescantia 88

Effects of Deuterium Oxide on Aberration Frequency 89

Radiological Physics 89

Gamma Radiation Work 89

Beta-Ray Depth Dose Studies 89

Energy Distribution of P^{32} Beta Rays 90

Photosynthesis 90

[redacted]

REVIEW OF RESEARCH PROGRESS

Alexander Hollaender

Cytogenetics. — During the present quarter, more exact measurements of both the amount of hydrogen peroxide formed by a given dose of radiation and the amount of effect upon Paramecium have been made; the conclusion has been reached that hydrogen peroxide can account for part of, but not all the effect of irradiated D medium. X rays have no marked effect on number of micronuclei in Paramecium, except at very high doses. This is in contrast to grasshopper neuroblasts where there is a very definite mitotic effect of oxygen at low-energy levels. Careful analysis of the genetic structure of bacteriophage T4 has led to an interesting theory of its genetic make-up. The data are compatible with the hypothesis that genetic loci are distributed in a linear order and that the recombination values are approximately independent of one another. No detectable effects were found on mitotic rate or cell structure of the grasshopper neuroblast by the presence of homogenates of irradiated or unirradiated embryos. Close observations of the respiration of grasshopper embryos brought out that the percentage of decrease of oxygen consumption following irradiation is not affected by dinitrophenol. No effect of X radiation at low level was found on respiration of the individual embryos. To meet the need for more reliable data for the comparison of rates of X-ray-induced mutations in the mouse and in Drosophila, a redetermination was made of the mutation rate at specific loci in Drosophila melanogaster. The results obtained in this experiment agree with the tentative conclusion, reached from experiments that afforded less exact comparison, that the X-ray-induced mutation rates in Drosophila is considerably below that reported for the mouse. In a parallel experiment cytological analysis of X-ray-induced mutations in Drosophila were made. Extensive experiments on the mechanism of radiation recovery of Escherichia coli reported previously were continued. A detailed analysis of yeast extract constituents which would support recovery is in progress. More detailed investigations of effect of oxygen on chromosomes of dry pollen grains of Tradescantia have shown an increase in effect with time up to an hour or more. The effect varies inversely to oxygen solubility, i. e., there is an increase of effect with increase of temperature. It is suggested that the oxygen breakage of chromosomes is due to an acceleration by oxygen of a terminal oxidase enzyme system. As a result of this increased activity, enzymatic production of radicals like OH, HO_2, is greater than that which the cells can normally dispose of, the excess causing the damage to chromosome.

[redacted]

Mammalian Genetics and Development. — A detailed histological study of a spontaneous mutation, which appeared in the course of the mouse genetics experiment, was made. This genetic change has probably come about as a result of the loss of certain metabolic processes in affected mice, the hemopoietic system being, apparently, one of the first tissues to be affected.

Pathology and Physiology. — Further analysis of the effects of thermal neutrons in mice is reported. The latest data verify the findings that as little as 16 r of X radiation will increase the cataract formation, with the effectiveness of thermal neutrons being even higher. The incidence of thymic lymphosarcoma was significantly increased by radiation. A careful study of all this material is now in progress. It was found that shielding of the head prevented cataract formation in spite of the fact that the remainder of the body receives up to 400 r of X radiation, as has also been reported by other investigators. It appears that a cataract is formed only if the eye is exposed directly. Shielding of most of the body, including spleen and bone marrow which are known to increase survival ratio, will not protect against cataract formation. An investigation of the role of platelets in postirradiation hemorrhagic syndrome is under way. A group of investigators at the Naval Medical Research Institute, who found this platelet protection phenomenon, have visited this Laboratory and instructed our staff in the use of their procedures. Data from the continuing leukemia studies indicate that even transient, cortisone-induced thymic atrophy results in a significant and marked reduction in incidence of leukemia. It has been previously reported that a pituitary tumor originating in radiothyroidectomized mice can be transplanted only to mice similarly pretreated and not to normal mice. A late finding reveals that, in the subpassage of one strain, a tumor acquired the ability to grow in normal mice, giving origin to the pituitary tumor line that can be grafted readily to normal mice. The retention of C^{14}, from C^{14}-glycine, by bone tissue in mice has been further analyzed.

Microbiology. — Extensive studies involving the use of C^{14} on the biosynthesis of kojic acid were made.

Biochemistry. — Analysis by means of ion-exchange methods of the production of ribonucleic acid-ribonuclease digest has given further material for an interesting theory about the structure of ribonucleic acid and its breakdown by ribonuclease. This was paralleled by studies to change experimentally the ribose part of nucleotides in nucleic acids. An extension of the direct ion-exchange method has been developed for identification of some biologically important phosphate esters. Further studies of the sensitive firefly-luminescence assay system indicate that, during photosynthesis, light not only forms small amounts of ATP but also promotes its utilization. It has been found that whole-body X irradiation of pigeons immediately affects formate uptake into nucleic acids and proteins. Malononitrile and mecholyl chloride gave results which indicate a protective action on the formate-utilizing enzymes.

Plant Physiology. — The work on the use of ion-exchange methods for the separation of sugars shows very satisfactory progress. The method is now applied to separation of glycosides. By means of scintillation counters, it has been possible to follow the uptake of K^{42} by sunflowers.

Biophysics. — Human erythrocytes have been found to produce a substance which is pharmacologically identical with acetylcholine. In preliminary experiments on the effects of X radiation on bioelectric response of the Venus flytrap, it was found that in some cases, the excitation threshold is lowered and a reduction in size and shape of the wave form takes place. A comparative study of X, γ, and β rays on Tradescantia pollen grains shows consistently that X rays are twice as effective as Co^{60}, γ, or β rays from P^{32} in producing aberrations in Tradescantia pollen tube chromosomes. A preliminary investigation of the effects of deuterium oxide on the frequency of X-ray-induced chromosomal aberrations in onion root tip has been made. No difference in frequency of chromosome deletions was noticed between this material and controls treated similarly with water. The frequency of exchanges in deuterium oxide material was 50 per cent less than that for the control.

It has previously been reported that green plants give out, besides their well-known fluorescence, a phosphorescence which lasts for a few seconds. There is now evidence that this delayed light is closely associated with photosynthesis. An early assumption that chlorophyll emits phosphorescence now seems to be justified.

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications. Thirty-nine articles written by members of the Biology Division have been published during this quarter. This number, composed of 21 full-length papers (of which 4 are Project reports) and 18 abstracts, is almost double that published in the preceding quarter. At this time there are in press 50 additional articles, ranging from abstracts (7) to book-length symposia (2). Manuscripts of the material presented at the 1952 Annual Biology Research Conference are being collected, more than half having been received.

AUTHOR (S)	TITLE OF ARTICLE	PUBLICATION
Billen, Daniel and H. C. Lichstein	The Effect of X Radiation on the Adaptive Formation of Formic Hydrogenlyase in *E. coli*	J. Bact., 63: 533-535, 1952
Burnett, W. T., Jr., R. R. Bigelow, A. W. Kimball, and C. W. Sheppard	Radiomanganese Studies on the Pancreatic Fistula Dog	Am. J. Physiol., 168: 620-625, 1952
Campbell, I. L. and Mary H. Ross	Protection Experiments against Radiation Injury with Lymphocytes	Project Report ORNL-1193
Cohn, W. E. and E. Volkin	Mononucleotides from Ribonucleic Acid by Phosphodiesterase Action: The Isolation of Pyrimidine Diphosphates	Arch. Biochem. Biophys., 35: 465-467, 1952
Conger, A. D. and L. M. Fairchild	Breakage of Chromosomes by Oxygen	Proc. Natl. Acad. Sci. U.S., 38: 289-299, 1952
Doherty, David G. and Fred Vaslow	Thermodynamic Study of an Enzyme-Substrate Complex of Chymotrypsin I	J. Am. Chem. Soc., 74: 931, 1952
Furth, J. and J. B. Kahn	Experimental Radiation-Induced Hormone-Secreting Ovarian Tumors: Adenocarcinoma with Hypervolemia	Acta Unio Intern. contra Cancrum, VII: 827-830, 1952
Gaulden, M. E., C. W. Sheppard, and H. Cember	Techniques for Exposing Grasshopper Neuroblasts to Ionizing Radiation. I. Beta Rays	Nature, 169: 228-231, 1952

AUTHOR (S)	TITLE OF ARTICLE	PUBLICATION
Hollaender, A.	Review of "Advances in Biological and Medical Physics," Vol. II, Ed., J. H. Lawrence and J. G. Hamilton, Academic Press Inc.	Rev. Sci. Instruments, 23: 44, 1952
Hollaender, A. (Ed. E. J. Slaughter)	Biology Division Quarterly Project Report for Period Ending Feb. 10, 1952	Project Report ORNL-1244
Jefferson, W. E., J. W. Foster, E. F. Phares, and S. F. Carson	Formate as a Precursor of Carboxylic Acids in Fungi	J. Am. Chem. Soc., 74: 1477-1478, 1952
Kahn, J. B., Jr. (Asst. by J. J. Lane)	Modification of Sensitivity to X Radiation by Pharmacologic Agents. I. Morphine Sulphate	Proc. Soc. Exptl. Biol. Med., 78: 486-489, 1951
Kahn, J. B., Jr., and J. Furth	The Pathogenesis of Postirradiation Anemia	Blood, 7: 404-416, 1952
Kahn, J. B., Jr., and J. Furth	The Pathogenesis of Postirradiation Anemia	Project Report ORNL-1186
Khym, Joseph X. and Leonard P. Zill	The Separation of Sugars by Ion Exchange	J. Am. Chem. Soc., 74: 2090-2094, 1952
Noggle, G. R. and M. Eleanor Schumacher	The Biosynthesis of Carbon-14-Labeled Compounds. II. The Chromatographic Separation of the Monosaccharides, Disaccharides, and Trisaccharides from Plant Extracts	Plant Physiol., 27: 422-426, 1952
Ross, M. H., J. Furth, and R. R. Bigelow	Capillary Fragility Caused by Ionizing Irradiation. I. Changes in Cellular Composition of the Lymph	Project Report ORNL-1187
Ross, M. H., J. Furth, and R. R. Bigelow	Changes in Cellular Composition of the Lymph Caused by Ionizing Radiation	Blood, 7: 417-428, 1952
Russell, Liane Brauch and W. L. Russell	Radiation Hazards to the Embryo and Fetus	Radiology, 58: 369-377, 1952
Storey, R. H., L. Wish, and J. Furth (Asst. of E. J. Beale and Mary M. Knoohuizen)	Organ Erythrocyte and Plasma Volumes of Tumor-Bearing Mice; The Oligemia of Neoplasms	Cancer Res., 11: 943-947, 1951
Wish, L., J. Furth, C. W. Sheppard, and R. H. Storey	Disappearance Rate of Tagged Substances from the Circulation of X-Rayed Animals	Am. J. Roentgenol. Radium Theraphy, 67: 628-640, 1952

AUTHOR (S)	ABSTRACTS	PUBLICATION
Billen, Daniel, G. E. Stapleton, and Alexander Hollaender	The Effect of X Radiation on the Respiration of Bacterial Cells	Bacteriol. Proc., 1952, pp. 158-159.
Burnett, W. T., Jr., A. W. Burke, Jr., and A. C. Upton	The Effect of Acetyl-β-Methylcholine, Carbamylcholine, and Atropine on the Survival of X-Irradiated Mice	Federation Proc., 11 (Part 1): 328-239, 1952
Carson, S. F. and Eugene A. Delwiche	Oxidative Reactions in the Propionic Acid Fermentation	Federation Proc., 11 (Part 1): 194-195, 1952
Cohn, Waldo E.	The Application of Ion-Exchange Chromatography to Nucleotides, Amino Acids, Sugars, and Related Substances	Abstracts of the 121st Meeting of the Am. Chem. Soc., 1952, p. 4B
Delwiche, Eugene A. and S. F. Carson	An Oxidative Cycle in the Propionic Acid Bacteria	Bacteriol. Proc., 1952, pp. 139-140
Doherty, D. G.	Protection of Alpha-Chymotrypsin against Gamma Radiation	Federation Proc., 11 (Part 1): 35-36, 1952
Furth, J., W. T. Burnett, Jr., E. Gadsden, and J. N. Dent	Conditions of Transplantation and Hormonal Secretions of Pituitary Tumors Induced by I^{131}	Cancer Res., Scientific Proc. 1952, p. 263
Ginsburg, J.	Potassium Exchange in Rat Tissues	Federation Proc., 11 (Part 1): 54, 1952
Khym, Joseph X.	The Separation and Analysis of Sugars by Ion Exchange	Abstracts of 121st Meeting Am. Chem. Soc., 1952, p. 11C
Pomper, Seymour	The Isolation of Triploid Yeast	Bacteriol. Proc., 1952, pp. 42-43
Schwartz, Drew	1. Gamete Factor on Chromosome 9 2. The Effect of Oxygen Tension on the Radiosensitivity of Chromosomes (Note)	Maize Genetics Cooperation News Letter, Cornell Univ., No. 26, Mar. 17, 1952, pp. 34-35
Sheppard, C. W. and Ward Sangren	Transcapillary Movement of an Isotope from a Nonuniformly Mixed Circulatory Pool	Federation Proc., 11 (Part 1): 147, 1952
Stapleton, G. E., Daniel Billen, and Alexander Hollaender	Recovery of X-Irradiated Bacteria at Suboptimal Incubation Temperatures	Bacteriol. Proc., 1952 p. 158

AUTHOR (S)	ABSTRACTS	PUBLICATION
Strehler, B. L. and J. R. Totter	Firefly Luminescence in the Study of Biological Energy Transfer Systems	Federation Proc., 11 (Part 1): 295, 1952
Upton, A. C. and J. Furth	A Transmissable Disease in Mice Characterized by Anemia, Leukopenia, and Erythropoietic Splenomegaly	Federation Proc., 11 (Part 1): 430-431, 1952
Vaslow, F. and D. G. Doherty	Thermodynamic Studies of Some Enzyme-Inhibitor Complexes	Abstracts of the 121st Meeting of the Am. Chem. Soc., 1952, p. 13C
Volkin, Elliot and Waldo E. Cohn	The Enzymatic Degradation Products of Ribonucleic Acid	Federation Proc., 11 (Part 1): 303, 1952
Wilde, W. S. and C. W. Sheppard	Electrolytes in Muscle of Rat Forelimb after Intense Local X Irradiation	Federation Proc., 11 (Part 1): 173, 1952

Scientific Society Lectures and Traveling Seminars. This quarter has been a season of high activity on the scientific lecture platform. The Traveling Seminar Program has sponsored forty-two lectures by members of the Division. A total of twenty-nine papers have been presented at professional society meetings. All lectures are listed.

Anderson, E. H.	Vanderbilt University Nashville	The Recovery of Bacteria after Exposure to Radiation
Anderson, N. G.	Assoc. S. E. Biol. Atlanta	The Effect of Various Enzymes on Nuclei Isolated from Rat Liver
Arnold, W.A.	Oglethorpe University Oglethorpe	Photosynthesis
Baker, W. K.	Emory University	Position Effects Causing Variegation in the Eye Pigments of *Drosophila virilis*
	Tennessee Polytechnic Institute, Cookeville	The Genetic Effects of Radiation
Billen, D., G. E. Stapleton, and A. Hollaender	Soc. Am. Bact. Boston	The Effect of X Radiation on the Respiration of Bacterial Cells
Burnett, W. T., Jr.	Alabama Polytechnic Inst. Auburn	The Protection against X-ray Damage to Enzymes, Bacteria, and Mammals by Chemical Agents

Burnett, W. T., Jr., A. W. Burke, Jr., and A. C. Upton	Fed. Am. Soc. Exptl. Biol. New York	The Effect of Acetyl-β-Methylcholine, Carbamylcholine, and Atropine on the Survival of X-Irradiated Mice
Carson, S. F.	University of Texas Austin	Comparative Biochemistry of Propionic Acid Fermentation
Carson, S. F. and E. A. Delwiche	Fed. Am. Soc. Exptl. Biol. New York	Tracer Studies on Oxidative Reactions in the Propionic Acid Fermentation
Cohn, W. E.	University of Florida Gainesville	Some Recent Developments in the Chemistry of Nucleic Acids
	Florida State University Tallahassee	Same as above
	Harvard University Cambridge	The Influence of Ion-Exchange Chromatography upon our Concept of the Structure of Nucleic Acids
	Rutgers University New Brunswick	Structure of Nucleic Acids (sponsored by Squibb Inst.)
	National Institutes of Health, Bethesda	Same as above
	Am. Chem. Soc. Buffalo	The Application of Ion-Exchange Chromatography to Nucleotides, Amino Acids, Sugars, and Related Substances
Darden, E. B., Jr., and C. W. Sheppard	Assoc. S. E. Biol. Atlanta	Depth Dose Relation for Biological Materials Exposed to Beta Rays
Delwiche, Eugene and S. F. Carson	Soc. Am. Bact. Boston	An Oxidative Cycle in the Propionic Acid Bacteria
Doherty, D. G.	Fed. Am. Soc. Exptl. Biol. New York	Protection of α-Chymotrypsin against γ Radiation
Dolin, M. I.	Fed. Am. Soc. Exptl. Biol.	A Soluble Pyruvate-Ketobutyrate Dehydrogenase from Strep. faecalis

Furth, J.	University of North Carolina Chapel Hill	Conditioned Neoplasms of Endocrine Organs and their Secretions
	Institute of Medicine of Chicago Chicago	Recent Experimental Studies and Current Concepts on the Etiology and Nature of Leukemia
	Am. Assoc. Path. and Bact. New York	Morphology of Transplantable Pituitary Tumors Induced by Radioactive Iodine and the Associated Secondary Changes
Furth, J. and A. C. Upton	Neutron Research Conf. Washington	Late Effects of Exposure to Thermal Neutrons on Mice
Furth, J., W. T. Burnett, Jr., E. Gadsden, and J. N. Dent	Am. Assoc. Cancer Research New York	Conditions of Transplantation and Hormonal Secretions of Pituitary Tumors Induced by I^{131}
Gamble, Frances	Assoc. S. E. Biol. Atlanta	Gonadotropin in the Blood of the Mouse
Gaulden, M. E.	University of Louisville Louisville	Effects of Radiation and Chemicals on Cell Structures and Mitosis as Observed in Living Cells
	Florida State University Tallahassee	Same as above
	Alabama Polytechnic Institute Auburn	Same as above
	Army Med. Res. Lab. Ft. Knox	Same as above
Gaulden, M. E., N. Nix, and E. B. Darden, Jr.	Assoc. S. E. Biol. Atlanta	Effects of Beta Rays and X Rays on Mitotic Rate of Living Grasshopper Neuroblasts
Gaulden, M. E., K. Loemker, and E. B. Darden, Jr.	Assoc. S. E. Biol. Atlanta	Fragmentation of Chromosomes of Grasshopper Neuroblasts by Beta Rays and X Rays
Ginsburg, J.	Fed. Am. Soc. Exptl. Biol. New York	Potassium Exchange in Rat Tissues
Hollaender, A.	Brown University Providence	Studies in Radiation Protection

Hollaender, A.	Amherst College Amherst	Studies in Radiation Protection
	University of Wisconsin Madison	Same as above
	University of Rochester Rochester	Microbiological Effects of Radiation
Hollaender, A. and G. E. Stapleton	Fed. Am. Soc. Exptl. Biol. New York City	Fundamental Aspects of Radiation Protection from a Microbiological Point of View
Hunt, E. L., and P. B. Huff	Assoc. S. E. Biol. Atlanta	Iodine Distribution in Chick Embryos
Jonas, Herbert	Am. Soc. Plant Physiol. Atlanta	Calcium Nutrition and Ascorbic Acid Formation in Peas
Khym, J. X.	Am. Chem. Soc. Milwaukee	The Separation and Analysis of Sugars
Noggle, G. R.	Florida State University Tallahassee	Ion Exchange as a Quantitative Method of Investigating the Carbohydrates in Plants
	University of Florida Gainsville	Ion Exchange as a Quantitative Method of Investigating the Carbohydrates in Plants
	Am. Soc. Plant Physiol. Atlanta	The Biochemical Effects of Plant Growth Regulators
Pomper, S.	Soc. Am. Bact. Boston	The Isolation of Triploid Yeast
	Tulane University New Orleans	Yeast Genetics
	Louisiana State University Baton Rouge	Same as above
Russell, Liane B.	Duke University Durham	The Effects of Radiation on the Prenatal Development of Mammals
	Agnes Scott College Decatur	Same as above
Russell, W. L.	North Carolina State College Raleigh	Genetic Hazards of Radiation
	University of Georgia Athens	Same as above

Russell, W. L.	University of North Carolina Chapel Hill	X-Ray-Induced Mutations in Mice
Sheppard, C. W.	Florida State University Tallahassee	The Role of Potassium in Circulatory Mixing
	Tulane University Medical School, New Orleans	Isotopes and Circulatory Mixing
	U. T. Medical School Memphis	Same as above
	Brooke Army Hospital Ft. Sam Houston	Same as above
	Alabama Polytechnic Institute Auburn	The Role of Potassium in Cell Physiology
	George Washington Carver Foundation, Tuskegee Institute Tuskegee	Isotopes and Circulatory Mixing
	Neutron Research Conference Washington	Methods of Measuring Gamma-Ray Contaminants in Slow-Neutron Fields
Sheppard, C. W. and Ward Sangred	Fed. Am. Soc. Exptl. Biol. New York	Transcapillary Movement of an Isotope from a Nonuniformly Mixed Circulatory Pool
Stapleton, G. E., D. Billen, and A. Hollaender	Soc. Am. Bact. Boston	Recovery of X-Irradiated Bacteria at Suboptimal Temperatures
Strehler, B. L.	University of Arkansas Med. School, Little Rock	Bioluminescence as a Tool for the Study of Energy Metabolism
	University of Texas Austin	Same as above
	University of Tennessee Medical School, Memphis	Green Plant Luminescence and Photosynthesis
Strehler, B. L. and J. R. Totter	Fed. Am. Soc. Exptl. Biol. New York	Firefly Luminescence in the Study of Biological Energy Transfer Systems
Upton, A. C.	Emory University	Effects of Ionizing Radiations Upon Mammalian Tissues

Upton, A. C. and J. Furth	Fed. Am. Soc. Exptl. Biol. New York	A Transmissable Disease in Mice Characterized by Anemia, Leukopenia, and Erythropoietic Splenomegaly.
Vaslow, F. and D. G. Doherty	Am. Chem. Soc. Milwaukee	Thermodynamic Studies of some Enzyme-Inhibitor Complexes
Volkin, E. and W. E. Cohn	Fed. Am. Soc. Exptl. Biol. New York	The Enzymatic Degradation Products of Ribonucleic Acid
Wilde, W. S. and C. W. Sheppard	Fed. Am. Soc. Exptl. Biol. New York	Electrolytes in Muscle of Rat Forelimb after Intense Local X Irradiation

Visiting Lecturers. The Division has been fortunate in securing the services of the following four prominent investigators for lectures on the Biology Seminar Program.

Dr. Robert L. Sinsheimer	Dept. of Physics Iowa State College Ames, Iowa	Chemical and Enzymatic Studies on Desoxyribonucleic Acid
Dr. George Brecher	National Institutes of Health, Bethesda, Maryland	Platelet Problem of the Hemorrhagic Tendency in Relation to Radiation
Dr. Orland E. White	Blandy Experimental Farm University of Virginia Boyce, Virginia	Genetic Aspects of Fasciation, a Cancerous-Like Plant Growth
Dr. Carl F. Cori	Washington Univ. Sch. of Med., St. Louis, Missouri	Molecular Configuration of Starch and Glycogen

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball (Leader)
A. D. Conger — Mary Kathryne King
K. C. Atwood — F. H. Mukai
A. H. Doermann — Lucile M. Fairchild
Drew Schwartz — Betty B. Hill
Seymour Pomper — Dorothy W. McKee
Nenita Gaither — Rachel C. Cheniae
L. Roberta Lovelace*

The Role of Externally Produced Hydrogen Peroxide in the Action of X Rays Upon Paramecium

(Kimball, Gaither, King)

Evidence has been presented (ORNL-1244) that much of the effect of X rays upon division rate and survival could be accounted for by hydrogen peroxide. This was so when the paramecia were irradiated in 3 per cent culture fluid (D medium) but not when they were irradiated in whole culture fluid. During the present quarter, more exact measurements of both the amount of hydrogen peroxide formed by a given dose of radiation and the amount of effect upon the animals has been made and the conclusion reached that hydrogen peroxide can account for part of but not all the effect of irradiated D medium.

The following procedure was followed. Paramecia were exposed to various concentrations of hydrogen peroxide made up in D medium and to D medium exposed to various doses of X rays. Analytical grade "30 per cent" hydrogen peroxide was checked for concentration before use with 0.1 N potassium permanganate. The amount of hydrogen peroxide in the irradiated medium was measured by the KI-starch colorimetric method of Savage (Analyst, 76: 224, 1951) using the series of peroxide dilutions in D medium as standards. More recently these measurements have been found to check satisfactorily with those made by a modified titanium sulfate colorimetric method.

The results are plotted in Fig. 1. It can be seen that the concentration of hydrogen peroxide which had to be added to the medium to produce a given effect was between 1.3 and 1.5 times that in irradiated medium which produced the same effect. In other words, between four-fifths and two-thirds of the effect of the irradiated medium can be accounted for by hydrogen peroxide. This is confirmed by the fact that catalase destroys the detect-

* Research Participant

able activity of the irradiated medium. The other one-fifth to one-third of the effect may be due to stable substances which are produced by irradiation of D medium but not by treatment with hydrogen peroxide. Thus some of the effects of X rays upon the medium are brought about by mechanisms other than hydrogen peroxide production.

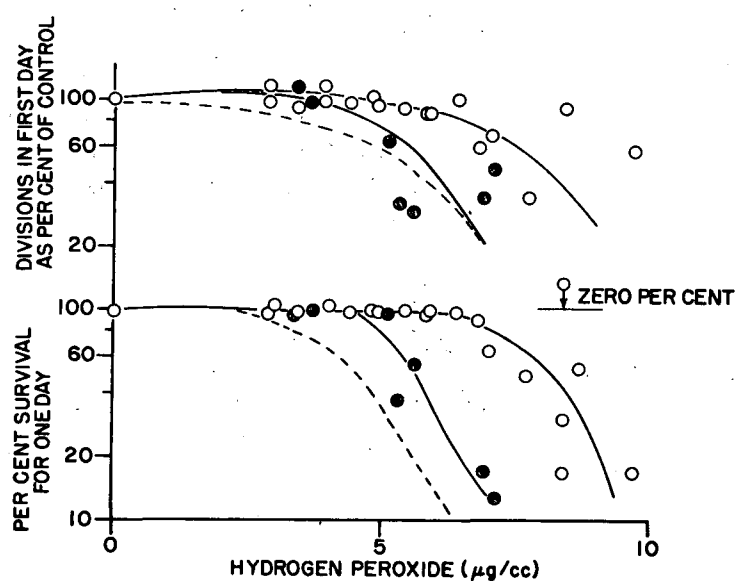


FIG. 1. Effects of hydrogen peroxide, irradiated medium, and direct radiation upon division rate and survival for one day in Paramecium. The doses in kr for direct radiation were converted to μg/cc of peroxide using data from irradiated medium.

O = H_2O_2 in D medium ● = Irradiated D medium

-----Direct irradiation in D medium

In Fig. 1, a comparison is also made with the effects upon paramecia directly irradiated in D medium. The curves are based on data previously obtained and are plotted by converting kiloroentgens to micrograms per cc of hydrogen peroxide using the data from irradiated medium. There are a number of uncertainties in the comparison; and so it can only be concluded that most of the effect, perhaps all, can be accounted for by the action of the irradiated medium.

Effect of X Rays upon Micronuclear Number in Paramecium

(Geckler,* Kimball)

Paramecia were grown in large mass cultures, concentrated by centrifugation and resuspended in culture fluid to reduce, as far as possible, effects of the irradiated medium (ORNL-1244). The paramecia were exposed to the unfiltered beam of the Maxitron at 250 kvp, 30 ma and an intensity of approximately 15 kr/min.

After exposure, the animals were placed in test tubes with excess culture medium and allowed to multiply for 24 hours, after which time they were fixed in Schaudinn's fluid, stained by the Borell procedure, and the number of micronuclei per animal counted.

The results are given in Table 1. The usual number of micronuclei in Paramecium aurelia is two; however, the present data show that unirradiated animals exhibit a small variation from the usual number. In irradiated animals, the fraction with less than two micronuclei increases with increasing dose while the fraction with two decreases. The fraction with more than two showed no such clear change.

TABLE 1

EFFECT OF X RAYS ON MICRONUCLEAR NUMBER IN PARAMECIUM

Dose (kr)	Total animals examined	Per cent with certain numbers of micronuclei		
		More than 2	2	Less than 2
None	435	4	93	3
214	86	5	91	5
275	30	3	87	10
336	118	8	81	11
403	100	5	78	17
470	100	10	79	11
537	83	7	57	36

Loss of micronuclei could occur in several ways. For example, cytoplasmic division might occur while micronuclear division was inhibited, or micronuclei might degenerate or lose their capacity to stain so that they would not be seen. Experiments to determine the mechanism of the loss have not yet been performed.

* Research Participant

The finding that a marked effect on micronuclear number is not produced, except at very high doses, is of importance from two points of view. Firstly, it shows that reduced vigor after autogamy cannot be due, except to a very small extent, to loss of both micronuclei. This is especially convincing since the doses used are sufficient to cause the death of almost all exautogamous progeny. Secondly, it shows that X rays do not inhibit the mitotic divisions of the micronuclei at doses of a few kiloroentgens. The argument is as follows. It has already been established that the division of the cytoplasm and macronucleus of Paramecium is not inhibited by doses of less than about 20 kr. If micronuclear division were appreciably inhibited by lower doses, paramecia with less than two micronuclei should arise frequently as a consequence of division of the cytoplasm without division of the micronuclei. Obviously, this did not happen. In this respect, as in others, Paramecium differs from those organisms, such as the grasshopper, in which mitotic inhibition is brought about by quite low doses.

Breakage of Chromosomes by Oxygen

(Conger, Fairchild)

Enough data have been accumulated to allow indulgence in some speculations on how oxygen breaks chromosomes in the dry pollen grains of Tradescantia. Most of the data pertinent to this discussion are in Fig. 2, which summarizes those features of oxygen dose and environmental variables that have been measured quantitatively. An X-ray curve is included for comparison with the oxygen curves.

From this and other data, we conclude that: (1) Molecular oxygen is not the agent which breaks the chromosomes. (2) Breakage is probably due to some enzymatic reaction which is acted upon by oxygen. (3) The enzyme system is probably a terminal oxidase, but not a cytochrome.

The first conclusion is supported by at least four facts, as follows: (1) Effect increases with time up to an hour or more (Fig. 2B) which is much longer than the relaxation time for oxygen diffusion into the cells. (2) The number of aberrations produced by an oxygen exposure (say an average of ten breaks per cell from one hour in 100 per cent oxygen) is much smaller than the number of collisions of oxygen molecules with chromosomes. (3) No aberrations are produced by air (20 per cent oxygen). (4) Effect versus temperature (Fig. 2C) varies inversely to oxygen solubility.

The second conclusion was reached through the reasoning that (1) since molecular oxygen apparently is not responsible, some cellular mechanism must cause the breaks; and (2) relation of effect to temperature (Fig. 2C) is very similar to the usual enzyme activity-temperature curves, both in slope and in temperature range at which effect varies.

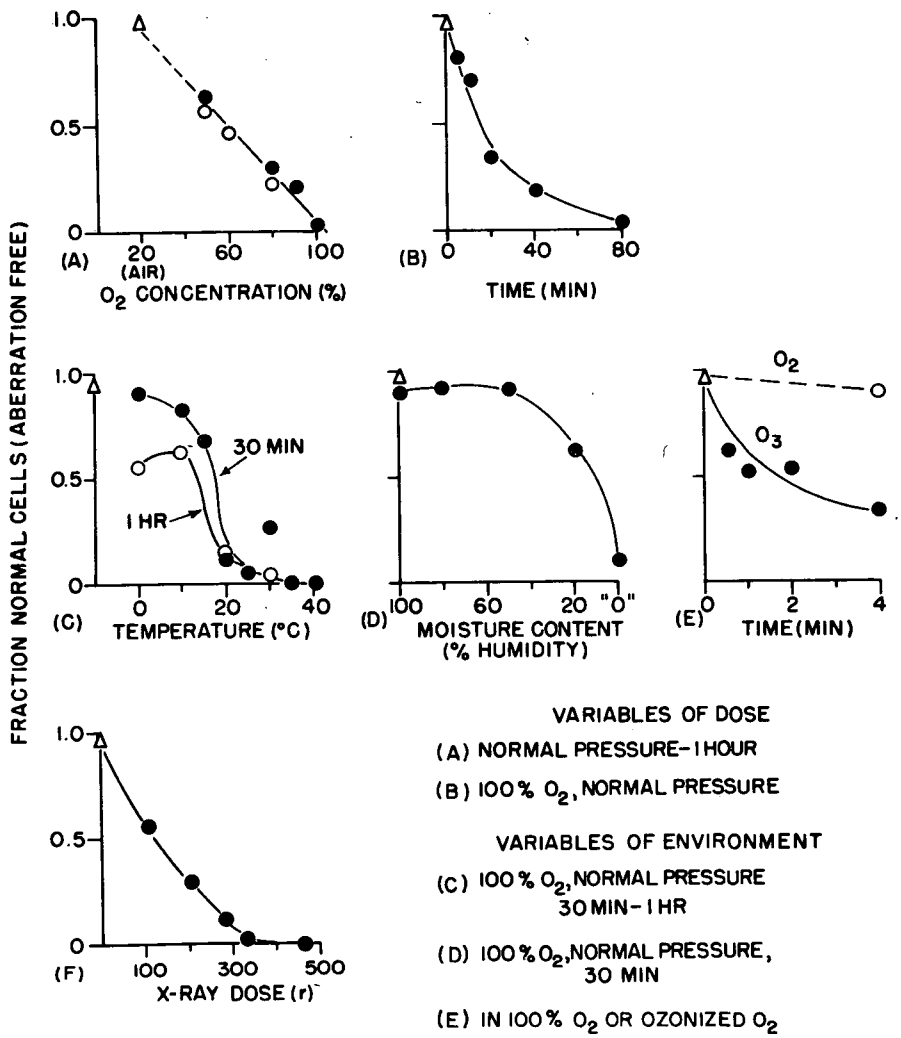


FIG. 2

The conclusion that the enzyme system is probably a terminal oxidase but not a cytochrome finds strength in the following arguments. Granting that chromosomes are broken by enzymatic reactions, the terminal oxidase systems (since they react directly with molecular oxygen) would seem, a priori, to be the most likely causative agents. There are a number of reasons for believing this effect is not due to the cytochromes, since cytochromes are oxygen saturated by air, chromosome breakage does not commence until oxygen concentration exceeds that of air; and chromosome breakage by oxygen is not prevented by having 1-3 per cent hydrocyanic acid, a cytochrome inhibitor, mixed with the oxygen. An experiment just completed established this. There are other reasons, however, for be-

lieving that the reaction may be due to a flavoprotein or some similar enzyme, particularly since the flavoproteins are known to be accelerated (almost linearly) with increasing oxygen concentrations above that of air, and are not inhibited by hydrocyanic acid as are the cytochromes.

On the basis of our present data, we tentatively suggest that the oxygen breakage of chromosomes is due to the acceleration by oxygen of a terminal oxidase enzyme system. As a consequence of this increased activity, enzymatic products or by-products (OH or HO_2 radicals from flavoprotein activity, possibly hydrogen peroxide) are produced in excess of amounts the cell is normally competent to dispose of, and are therefore available to break chromosomes.

It is supposed that chromosomes are ultimately broken by the same agents (active radicals or hydrogen peroxide) from radiation or from oxygen, but they are produced physically by radiation; enzymatically, by oxygen. The clear difference in the effect of environment on chromosome breakage by the two agents is quite compatible with this suggestion. With radiation, change in moisture has no effect (compare Fig. 2D), and change in temperature has a much smaller effect (a ratio of 3:1 between 0° and $35^{o}C$) and in the opposite direction.

Genetic Structure of Bacteriophage T4

(Doermann, Hill)

In three previous quarterly reports (ORNL-727, ORNL-889, and ORNL-989) experiments have been described which were intended to elucidate the genetic structure of bacteriophage T4. Many additional data are now available and certain conclusions may be drawn. The past two quarters have been devoted mainly to procuring the additional data and to consideration of the conclusions which can be drawn.

The mutants used are all plaque-type mutants including (1) several of the r type (see ORNL-727), (2) a large number of the tu type (see ORNL-989), and one of the m type (see Hershey and Rotman, Genetics 34:44-71, 1949). All mutants discussed here were from independent mutations. In addition to those mentioned in previous quarterly reports, twelve tu mutants were isolated, giving a total of 26 independent occurrences of tu. The procedure used was to make crosses of any new isolate with the known genetic markers until it proved to be allelic with one of them, or until crosses with all the known ones showed it to be a new genetic locus.

The resulting data are exclusively from two-factor crosses. Every effort was made to make the experiments uniform in all details, but it

was nevertheless necessary to select the experiments on two counts in order to assure reliable estimates of the recombination values. First, it was necessary to exclude any experiment where the average number of either parent phage adsorbed per bacterium was less than 3. This is because more than 5 per cent of the cells would be infected with only one of the two phage types, and these of course do not yield genetic recombinants. The second criterion in selecting was that approximately equal numbers of the two parents infect the bacteria in any given cross. Experiments were made to determine the effect of unequal multiplicity of the two parents. They indicated that, where the ratio of one parent to the other was 0.67 or greater, a maximum recombination value was obtained, while lower ratios gave consistently low recombination values. Experiments with ratios less than 0.67 were therefore excluded. From the total of 125 experiments made for estimation of recombination values, 4 were eliminated for low multiplicity and 16 for unequal multiplicity.

The results to be considered first are those which give some information on the number of tu loci present in the T4 phage particle. Tests of allelism among the 26 tu mutants are shown in Table 2, which contains the

TABLE 2

RECOMBINATION VALUES WITHIN THE CLUSTERS OF NEAR-ALLELIC tu LOCI

Cluster	Mutant	Cross indicating inclusion in cluster	No. of plaques examined	Per cent recombination*
tu_{41}	41a	—	—	—
	41b	41b x 41a	557	0.0
	41c	41c x 41a	819	0.0
	41d	41d x 41a	489	0.0
	41e	41e x 41c	531	0.0
tu_{42}	42a	—	—	—
	42b	42b x 42a	1137	0.4
	42c	42c x 42a	509	0.0
	42d	42d x 42b	3014	2.3
	42e	42e x 42b	866	0.5
	42e	42e x 42d	4445	1.3
	42f	42f x 42b	686	0.0
	42g	42g x 42f	788	0.2
	42h	42h x 42b	795	2.3
tu_{43}	43a	—	—	—
	43b	43b x 43a	698	0.3
	43c	43c x 43b	426	0.4
	43d	43d x 43b	720	0.0
	43e	43e x 43a	2946	0.1
	43f	43f x 43e	1152	1.6
tu_{44}	44a	—	—	—
	44b	44b x 44a	1313	0.6
	44c	44c x 44b	649	0.0
	44d	44d x 44c	2395	0.7

Table 2 cont.

tu_{45}	45a	—	—	—
	45b	45b x 45a	1835	0.0
	45c	45c x 45a	1064	1.7

* Although the mutation rate from tu to tu^+ has not been measured accurately, it is possible to account for the tu^+ plaques by mutation. Preparation of stock cultures of the tu mutants are the result of multiple infection and, in these, one tu^+ plaque per thousand is rarely found.

results of those crosses which indicate allelism or near-allelism. The 26 clearly fall into five clusters with low intracluster recombination values. (The intercluster recombination values, which are considerably larger, will be discussed presently.) Although all possible intracluster crosses have not been made, it is clear that at least three separable loci are involved in the tu_{42} cluster and two each in the tu_{43}, tu_{44}, and tu_{45} clusters. It is for the present impossible to design an experiment which will decide whether these results are to be interpreted as being due to mutations in separate functional units or whether they indicate mutations at several locations within a single functional unit. The minimum number of tu loci in T4 then appears to be five, but this number must be increased to ten if the members of the clusters are considered to be separate loci. It should be added, however, that many more loci may exist, but that due to a low rate of mutation or due to selective action against them during the isolation procedure, they are not encountered frequently.

The remaining experiments include all the possible intercrosses among nine distinctly separate loci, and form the basis for a genetic map of T4. The nine loci consisted of the five tu loci, three r loci, and one m locus. In considering the recombination values from these crosses, it became apparent that it was desirable to separate them statistically into those which do and do not indicate linkage. The data were analyzed statistically by Dr. A. W. Kimball of the ORNL Mathematics Panel. A weighted mean was obtained for each of the crosses and these were separated into statistically undifferentiable groups. Any pair in the group with the high recombination values was considered as unlinked unless crosses involving intermediate markers were available. Table 3 contains the crosses with linked pairs. Three linkage groups are evident, namely I containing r_{48} only, II comprising r_{47}, r_{51}, and tu_{41}, and III containing tu_{42}, tu_{44}, tu_{43}, m_{41}, and tu_{45}. The remaining locus, r_{48}, is apparently not linked to any of the other markers. The results from crosses between unlinked pairs are shown in Table 4. None of the loci in any one linkage group is linked to any marker in either of the other groups.

TABLE 3

RECOMBINATION VALUES OBSERVED FOR LINKED LOCI IN TWO-FACTOR INTERCLUSTER CROSSES

Cross	Mean recombination value* (Per cent)	95% Confidence limit Lower (Per cent)	Upper (Per cent)
$r_{47} \times r_{51}$	5.4	4.1	6.8
$r_{51} \times tu_{41}$	23.7	20.8	26.7
$r_{47} \times tu_{41}$	25.0	21.3	28.9
$tu_{42} \times tu_{44}$	9.5	8.1	11.1
$tu_{42} \times tu_{43}$	25.5	22.0	29.0
$tu_{42} \times m_{41}$	30.1	25.8	34.7
$tu_{42} \times tu_{45}$	32.6**	27.9	37.4
$tu_{44} \times tu_{43}$	20.5	18.1	22.9
$tu_{44} \times m_{41}$	24.9	14.9	36.4
$tu_{44} \times tu_{45}$	30.3	26.7	34.1
$tu_{43} \times m_{41}$	10.2	8.4	12.0
$tu_{43} \times tu_{45}$	21.8	19.2	24.4
$m_{41} \times tu_{45}$	15.5	13.1	18.1

* The weighted mean

** Statistical analysis would place this value in Table 4. The linkage of these factors is obvious, however, from their linkages with intermediate markers.

TABLE 4

RECOMBINATION VALUES OBSERVED FOR UNLINKED LOCI IN TWO-FACTOR INTERCLUSTER CROSSES

Cross	Mean recombination value* (Per cent)	95% Confidence limit Lower (Per cent)	Upper (Per cent)
$r_{48} \times tu_{45}$	32.9	29.2	36.7
$tu_{41} \times tu_{43}$	34.9	29.2	40.8
$r_{48} \times m_{41}$	35.9	27.5	44.8
$r_{47} \times tu_{44}$	36.1	31.0	41.3
$r_{48} \times tu_{43}$	36.4	31.8	41.2
$r_{48} \times tu_{42}$	36.4	28.9	44.3
$tu_{41} \times tu_{42}$	36.9	34.6	39.3
$tu_{41} \times tu_{44}$	37.0	27.9	46.7
$tu_{41} \times m_{41}$	37.1	31.3	43.0
$r_{48} \times r_{51}$	37.4	30.3	44.8
$r_{47} \times r_{48}$	37.7	29.8	46.0
$r_{51} \times tu_{44}$	38.8	27.3	50.9
$r_{51} \times tu_{42}$	39.1	35.1	43.2
$tu_{41} \times tu_{45}$	39.2	34.7	43.9
$r_{51} \times m_{41}$	39.3	33.4	45.3
$r_{47} \times tu_{42}$	40.3	27.8	53.5
$r_{48} \times tu_{44}$	40.8	31.3	50.7
$r_{47} \times tu_{45}$	41.3	32.9	50.9
$r_{48} \times tu_{41}$	41.6	25.3	58.7
$r_{51} \times tu_{45}$	41.7	39.1	44.3
$r_{47} \times m_{41}$	43.2	37.2	49.3
$r_{47} \times tu_{43}$	43.6	35.2	52.1
$r_{51} \times tu_{43}$	45.5	35.9	55.2

* The weighted mean

Even though three-factor crosses are not yet available with these markers, one can investigate whether the data from two-factor crosses indicate a linear arrangement of the loci. When experiments with low or with unequal multiplicity are discarded, the results are, contrary to a previously made statement (ORNL-989), compatible with a linear distribution. When the loci are arranged as shown in Fig. 3, we see that the recombination value for any pair of nonadjacent factors is always larger than the recombination value for any of the intermediate pairs. For example, the value for $\underline{tu}_{42}$ and $\underline{tu}_{44}$, as well as that for $\underline{tu}_{44}$ and $\underline{tu}_{43}$ is smaller than the value for $\underline{tu}_{42}$ and $\underline{tu}_{43}$; and this is true for any set of three factors in either linkage groups.

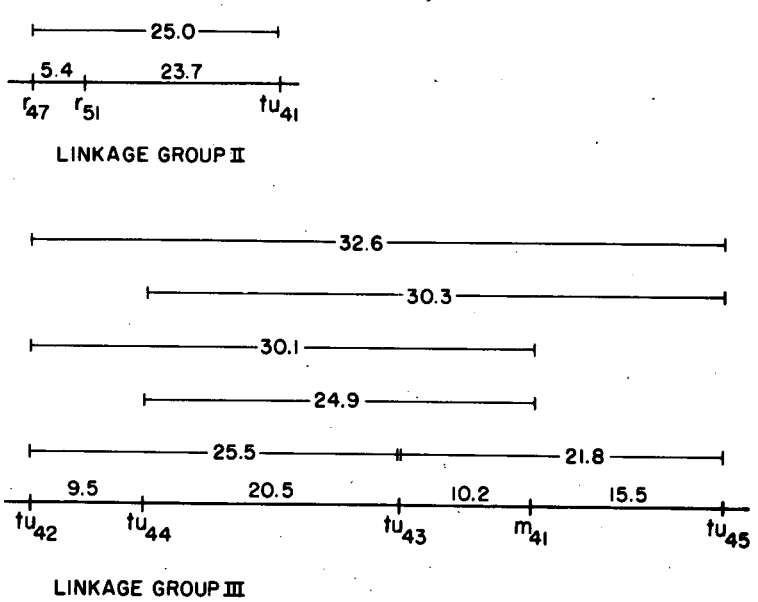


FIG. 3. The linear order of loci in two linkage groups of T4.

One can then proceed to test the question which naturally follows; namely, are the recombination values statistically independent of one another or do they indicate some sort of interference? Assuming independence, one can calculate the two-factor recombination value expected for the terminal members of any set of three linked factors. This can be obtained from observed recombination values for the intermediate pairs. The expected values are given in Table 5, along with those observed. In all cases except one, the calculated values are higher than those observed, indicating a statistical excess of double recombinations which would be unobservable in the two-factor crosses. Thus, a progeny particle showing a recombination in region ab has a greater than average chance of showing a recombination in the adjoining region bc.

TABLE 5

EXAMINATION OF THE OBSERVED RECOMBINATION DATA FOR STATISTICAL INDEPENDENCE OF INDIVIDUAL RECOMBINATION VALUES

Series of factors involved			Recombination value between terminal loci (R_{ac})		Calculated minus observed (Per cent)
a	b	c	Calculated* (Per cent)	Observed (Per cent)	
r_{47}	r_{51}	tu_{41}	26.5	25.0	+1.5
tu_{42}	tu_{44}	tu_{43}	26.2	25.5	+0.7
tu_{42}	tu_{44}	m_{41}	29.7	30.1	-0.4
tu_{42}	tu_{44}	tu_{45}	34.1	32.6	+1.5
tu_{42}	tu_{43}	m_{41}	30.5	30.1	+0.4
tu_{42}	tu_{43}	tu_{45}	36.2	32.6	+3.6
tu_{42}	m_{41}	tu_{45}	36.3	32.6	+3.7
tu_{44}	tu_{43}	m_{41}	26.5	24.9	+1.6
tu_{44}	tu_{43}	tu_{45}	33.0	30.3	+3.0
tu_{44}	m_{41}	tu_{45}	32.7	30.3	+2.4
tu_{43}	m_{41}	tu_{45}	22.5	21.8	+0.7

* $R_{ac} = R_{ab}(1-R_{bc}) + R_{bc}(1-R_{ab})$

Several possible explanations could account for this unexpected negative interference. The most plausible one depends on the following complication: If the individual particles which participate in the over-all process of phage reproduction have varying numbers of opportunity for genetic recombination, the result would be the positive correlation in adjacent recombinations which has been observed. To take an extreme example, suppose that only 50 per cent of the phage population within one bacterium participated in genetic recombination. Then, considering only this half the population, the proportion of double recombinants might be precisely that predicted from the frequencies of the single recombinants. If, however, the whole population is used for estimating the frequencies of single recombinants (as is done in the phage experiments), the predicted number of doubles would be only one half the number observed.

The plausibility of such an interpretation of the positive correlation is indicated by the results of a number of experiments by several investigators. These results have been brought together into a theory, developed by Visconti and Delbrück (Genetics, in press) which treats the phage cross as a population genetics problem. The theory permits calculation of the recombination probability per mating (P_{ik}) from the observed recombination value (R_{ik}) for any pair of markers. Accepting their assumptions, and the parameters which they determined from independent experiments, the values of P were calculated for each linked two-factor cross. The recombination values per mating for the terminal members of each group of three linked loci (P_{ac}) were also calculated on the basis of statistical independence of P_{ab} and P_{bc}. The values of P_{ac} obtained from the data by

both methods are given in Table 6. It is seen that a good fit to the assumption of independence is obtained. The data are thus compatible with the hypothesis that genetic loci are distributed in a linear order and that the recombination values are approximately independent of one another.

TABLE 6

THE RECOMBINATION VALUES OF (P)* FOR INDIVIDUAL MATINGS

Series of factors involved			Recombination values calculated for terminal loci (P_{ac})	
a	b	c	From R_{ab} and R_{bc}** (Per cent)	From R_{ac} (Per cent)
r_{47}	r_{51}	tu_{41}	14.6	13.8
tu_{42}	tu_{44}	tu_{43}	14.0	14.2
tu_{42}	tu_{44}	m_{41}	16.8	18.2
tu_{42}	tu_{44}	tu_{45}	21.1	20.7
tu_{42}	tu_{43}	m_{41}	17.4	18.2
tu_{42}	tu_{43}	tu_{45}	22.4	20.7
tu_{42}	m_{41}	tu_{45}	22.9	20.7
tu_{44}	tu_{43}	m_{41}	14.1	13.7
tu_{44}	tu_{43}	tu_{45}	19.6	18.5
tu_{44}	m_{41}	tu_{45}	19.1	18.5
tu_{43}	m_{41}	tu_{45}	11.2	11.4

* The values of P were calculated according to Visconti and Delbruck (in press, Genetics).

** Assuming statistical independence of P_{ab} and P_{bc}, that is $P_{ac} = P_{ab}(1-P_{bc}) + P_{bc}(1-P_{ab})$

Studies on the Distribution of Genetically Marked Nuclei in *Neurospora* Conidia

(Atwood, Mukai)

In the course of experiments on the effects of X rays on survival and mutation in heterokaryotic conidia (ORNL-1167:29-33), it became necessary to learn more about the distribution of nuclei into the conidia. Accordingly, experiments were done with several cultures of the heterokaryon (methionineless-amycelial)-(ornithineless) in which the relative frequencies of conidia belonging to the heterokaryotic and the two homokaryotic classes were estimated by differential plating, and frequency distributions of conidia with different total numbers of nuclei were determined for each culture by counts of stained preparations. The total nuclei per conidium and the proportions of the three types of conidia were caused to vary from culture to culture by the addition of different amounts of sorbose to the medium.

Superficially, the results suggested that the probability that the inclusion within a conidium of a nucleus of the first or second type may be constant for nuclei of the given type within the same culture. The data were therefore examined with this hypothesis in mind.

Let K_1 represent the proportion of homokaryons of the first type, K_2 of those of the second type, K_3 of heterokaryons, and X_1, X_2, X_3, . . . , X_n the proportion of conidia containing 1, 2, 3, . . . , n nuclei. According to hypothesis,

$$K_1 = X_1 p + X_2 p^2 + X_3 p^3 + \ldots + X_n p^2, \tag{1}$$

where p is the probability that a nucleus of the first type is included within a conidium. Similarly,

$$K_2 = X_1(1-p) + X_2(1-p)^2 + X_3(1-p)^3 + \ldots + X_n(1-p)^n, \tag{2}$$

and

$$K_3 = 1 - (K_1 + K_2). \tag{3}$$

The values of K_1, K_2, K_3, X_1, X_2, etc., were estimated by experiment and p was computed from equation (1) by successive approximation. Expected values of K_2 and K_3 were computed by using this value of p in equations (2) and (3). The frequencies corresponding to the expected values were compared with the observed frequencies by a chi-square test with one degree of freedom. In cases showing significance, a new value of p was found from all three equations which would yield the minimum chi-square for a test of all three classes with two degrees of freedom.

Table 7 shows the distributions of nuclei in conidia in the different ex-

TABLE 7

FREQUENCY DISTRIBUTION OF CONIDIA WITH DIFFERENT NUMBERS OF NUCLEI

Expt. No.	Sorbose in minimal Fries (mg/cc)	Nuclei per cell							Total cells	Av. no. of nuclei/cell
		1	2	3	4	5	6	>6		
a	0.00	92	364	313	130	51	30	20	1000	2.88
b	0.0003	64	329	316	166	61	25	39	"	3.16
c	0.009	99	381	322	131	47	13	7	"	2.71
d	0.039	101	356	308	129	60	29	17	"	2.87
e	0.16	199	448	223	78	28	13	11	"	2.39
f	0.62	208	447	224	77	25	10	9	"	2.34
g	2.5	224	389	212	103	44	13	15	"	2.48

periments and Table 8 shows the observed and expected frequencies of the

TABLE 8

OBSERVED AND EXPECTED FREQUENCIES OF CONIDIAL TYPES

Expt. No.		ZK_1	ZK_2	ZK_3	Z (Total)	p	χ^2	d.f.	Signif. level
a	*	129	87	336	552	0.555	0.531	1	0.47
	**	129	81.1	341.9					
b	*	95	118	213	426	0.468	52.12	2	<0.01
	**	61.3	81.6	283.1					
c	*	222	80	234	536	0.654	36.76	2	<0.01
	**	186.8	51.1	298.1					
d	*	305	16	122	443	0.886	6.38	2	0.02-0.05
	**	298.9	9.2	134.9					
e	*	265	18	92	375	0.858	1.17	1	0.27
	**	265	14.2	95.8					
f	*	161	30	71	262	0.775	12.94	2	< 0.01
	**	149.7	18.8	93.5					
g	*	324	21	98	443	0.876	2.54	1	0.11
	**	324	15.2	103.8					

* = Observed
** = Expected

three types of conidia, with significance levels. In three experiments there is no significant deviation from the hypothesis, one experiment is at the borderline of significance and three are highly significant. In all cases, whether significant or not, there is an excess of homokaryons. This systematic error is consistent with the experimental finding that one can sometimes separate heterokaryons by isolating hyphal tips which contain very large numbers of nuclei. We conclude that our hypothesis holds for regions of a culture where the nuclei are mixed, and that the departures of the observed from the expected frequencies of conidial types are caused by the presence of homokaryotic regions capable of forming large conidia. The size of these regions cannot exceed the limits of cytoplasmic carryover of growth factors, thus the agreement with hypothesis will be fair in forced heterokaryons, poor in unforced.

These results have two further consequences. First, the ratio p/(1-p) is about as good an estimate of the nuclear ratio in macroconidial heterokaryons as can be obtained by any other method. Second, our methods

permit a reasonable minimal estimate of the proportion of heterokaryotic cells having exactly one of the first type of nucleus:

$$Q = \sum_{i=2}^{n} \frac{iX_i\, p(1-p)^{(i-1)}}{1-p^i-(1-p)^i} \qquad (4)$$

It is important that this proportion be estimable, since cells of this description are the only ones in which 100 per cent recognition of recessive lethal mutations is possible.

We acknowledge with pleasure many helpful discussions with Dr. A. W. Kimball.

Radiation Studies on Yeast

(Pomper, McKee)

In ORNL-1244, preliminary experiments on the modification of X-ray lethal effects by low-temperature incubation were reported. A series of experiments have been carried out in which the effect of low temperature (12°C) and of the plating medium (complete versus minimal agar) have been studied. These experiments have been performed on yeast cultures of varying ploidy, from haploid (M) to tetraploid (4 M). The results, with regard only to survival, are summarized briefly in Table 9.

TABLE 9

EFFECT ON SURVIVAL AFTER X IRRADIATION OF MEDIUM AND TEMPERATURE

Ploidy	Culture	Fraction surviving on minimal	Relative survival at 30°C on		Relative survival on complete at	
			Minimal	Complete	30°C	12°C
M	A93.2	0.015	1.0	1.0	1.0	0.73
M	A555.1	0.17	1.0	0.82		
M	A558.1	0.057	1.0	0.93		
M	A558.2	0.14	1.0	0.86		
M	A558.3	0.035	1.0	1.0		
M	A558.4	0.11	1.0	1.1		
2 M	P-4(62-20-194 x 67)	0.12	1.0	1.1	1.0	0.62
2 M	P-4(A92.2 x A93.2)	0.083	1.0	1.0	1.0	2.7
2 M	A555.4	0.0013	1.0	7.4	1.0	3.7
3 M	A220.2 x p-4(A92.2 x A93.2)	0.106	1.0	1.0	1.0	0.44
3 M	P-1(A176.1 x A555.4)	0.0061	1.0	2.1		
4 M	P-3(A541.4 x A555.3)	0.044	1.0	1.4	1.0	0.60
4 M	A555.4 x p-4(A92.2 x A93.2)	0.0054	1.0	3.7		
4 M	A555.4 x p-4(A92.2 x A93.2)	0.0057	1.0	2.2	1.0	1.1

In computing the relative survival at 30° and 12°C, the survival on complete at 30°C was taken as 1, even though, as in the case of A555.4, it might be greater than the survival on minimal.

From the data in Table 9, it may be concluded that a knowledge of the state of ploidy of the cell is not an adequate basis for predicting the occurrence of a medium or temperature effect. However, the uniform lack of response to the medium of six genetically different haploids suggests that such responses may depend on the presence of more than one chromosome set.

An analysis of the irradiated cultures was carried out in terms of the genetic factors originally heterozygous in each. As an example of such a study, tetraploid A555.4 x P-4(A92.2 x A93.2) was analyzed with the following results obtained under the given conditions.

Number of colonies isolated from agar plate	—30
Number of isolates able to grow on minimal	—20
Number of isolates able to grow on supplemented minimal	—10

Original exposure: 200 kr X rays
Original plate counts: minimal 19×10^4; complete 50×10^4
Supplemented minimal contained glucose, salts, vitamins, trytophan, methionine, adenine, and uracil. The last four compounds are those for which the tetraploid is heterozygous, e.g., $Tr^+/Tr^-/Tr^-/Tr^-$.

It can be seen that no new mutant types requiring factors not present in the original cross are found. The survivors may or may not require the heterozygous factors. However, it should be emphasized that there is no significant difference between the survival on minimal and supplemented minimal plates. Thus the effect of the complete agar is not exerted solely through supplying these factors. Further, it should be noted that in the example cited, the expected number of isolates able to grow on minimal was ten $\left(\frac{19}{50} \times 30\right)$, but the observed number was twenty. Hence, plating on complete agar has not only permitted the deficient types to appear, but has also increased the survival of independent types. Our tentative working hypothesis is that the complete agar contains factors which permit the cell to recover from irradiation damage or shock, and that the mechanism of action is not simply the supplying of factors required for continued growth. Rather, it seems more likely that the action of the complete agar is of a priming nature, supplying factors required to initiate synthesis after irradiation.

EFFECTS OF RADIATION ON RATE OF MITOSIS

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

Fragmentation of Chromosomes in Grasshopper Neuroblasts by β and X Rays

(Gaulden, Loemker; Darden - Biophysics)

Fourteen-day-old embryos of Chortophaga viridifasciata were removed from eggs and dissected as for hanging-drop preparations. Some were exposed to 1.7 Mev β rays from P^{32} and some to 125 kvp X rays, conditions for exposure being identical in both cases (Gaulden, Sheppard, and Cember, Nature, 169: 228, 1952). A dose of 64 r of X rays and 70.4 rep of β rays was used; dose rates were adjusted so that the amount of energy dissipated in the tissue at a given dose level would be the same for both types of radiation. The embryos were made into hanging-drop preparations following irradiation and placed at 38°C. At intervals of 44, 220, 264, and 352 minutes after irradiation the embryos were fixed and stained. Cells in late anaphase and very early telophase were examined for chromosome fragments. Since it is not possible to distinguish between chromosome and chromatid breaks in the neuroblast, acentric fragments (exchanges are rare at this dose level) were scored simply as single or double. A "double" fragment is a pair of fragments equal in size, lying close together and obviously the result of one event. (Frequency of acentric fragments has been shown to be directly proportional to dose.)

The results are shown in Table 10. The number of cells with fragments as well as the number of single fragments in X-rayed cells did not differ in a statistically significant manner from those in β-irradiated cells at comparable intervals after irradiation. The number of β-ray-induced double fragments observed in cells fixed 352 minutes after irradiation, however, was greater than the number observed in X-rayed cells fixed at the same interval. This difference was statistically significant at the 0.02 level. Since it has been shown that the mitotic rate of β-irradiated cells

---

* Consultant
** AEC Postdoctoral Fellow
† ORINS Fellow

TABLE 10

FRAGMENTATION IN CELLS TREATED WITH 64 r X RAYS AND 70.4 rep β RAYS

Time after irradiation (minutes)	Total cells analyzed		Cells with fragments		Single fragments		Double fragments	
	X rays	β rays	X rays	β rays	X rays	β rays	X rays	β rays
44	221	162	14	8	14	6	1	4
220	77	142	27	42	27	45	11	12
264	104	112	35	34	32	27	17	14
352	115	197	36	75	35	64	17*	58*

* Significant difference at 0.0005 probability level.

differs from that of X-rayed cells (ORNL-1167), the difference observed in frequency of double fragments in X- and β-irradiated cells may be due to the fact that cells analyzed 352 minutes after irradiation were treated in different stages of mitosis, and not due to a difference in effectiveness of the two types of radiation in producing fragments at one stage of mitosis. A study of X and β rays on mitotic response of individual stages is in progress, the results of which should help in interpreting the difference in fragment frequency observed at 352 minutes after irradiation.

Study of Mitosis in the Presence of Homogenates of Irradiated Embryos

(Gaulden; Wilbur-Biophysics; Loemker)

Grasshopper embryos were separated from membranes and yolk and irradiated in small tubes, with a minimum of culture medium, with approximately 260,000 r (130,000 r/hr) or 300,000 r (17,000 r/hr) of γ rays. Following irradiation a volume of medium about equal to that of the embryos was added and the embryos thoroughly homogenized. Untreated embryos were placed in the homogenate and the neuroblasts were studied. No detectable effects on mitotic rate or cell structures were produced by homogenates from either irradiated or unirradiated embryos.

Effect of Dinitrophenol on Respiration of the Grasshopper Embryo

(Tipton, St. Amand)

This investigation was undertaken to determine whether oxygen consumption of embryos is increased by dinitrophenol (DNP), and, if so, whether the stimulation of respiratory mechanisms can alter the response of embryonic respiration to X radiation. Forty embryos (14 days old at 26°C) were dissected from the eggs and placed in a glucose-salt culture medium in a 5-cc Warburg flask. Respiration was measured at 38°C. DNP in the side arm was tipped over after the first hour. Two concentrations were used -5 x 10^{-7} M, which is the approximate threshold dose for cytological aberrations in the neuroblasts, and 2 x 10^{-5} M, which causes pronounced alterations in mitotic behavior (ORNL-969). Table 11 shows that the respiration of embryos is increased by both concentrations of DNP.

TABLE 11

Overnight temp. (°C)	Without DNP (μl of O_2/embryo)	With DNP (μl of O_2/embryo)	Change (Per cent)
17	0.230	0.385	+67.5
26	0.240	0.282	+17.4

To facilitate handling of the large numbers of embryos used, an experiment was run to determine whether the respiration of embryos separated from yolk and membranes and held overnight (17 hours) in culture medium would be affected by such treatment. The respiration of 40 embryos (one flask) kept overnight at 17°C and of 40 embryos (one flask) kept overnight at 26°C was determined at 38°C with a final concentration of 2 x 10^{-5} M DNP.

As can be seen from Table 12, in the absence of DNP the respiration

TABLE 12

EFFECTS OF DNP ON RESPIRATION OF GRASSHOPPER EMBRYOS

Concentration DNP	No. of flasks (40 embryos/flask)	Without DNP (μl O_2/embryo)	With DNP (μl O_2/embryo)	Per cent change
5 x 10^{-7}	4	0.237	0.293	+ 23.6
2 x 10^{-5}	4	0.255	0.662	+160.0

of embryos dissected overnight did not differ from the respiration of those freshly dissected. The response to DNP, however, was lower in embryos kept overnight at either 17° or 26°C than in embryos freshly dissected.

Measurement of respiration of 80 embryos (40 per flask), kept overnight in culture medium at 26°C prior to irradiation, revealed that 5467 r of X rays decreased the rate of oxygen consumption about 30 per cent below that of control embryos (80). Eighty embryos exposed to DNP immediately after irradiation showed the same percentage of decrease in respiration when compared with embryos exposed to DNP only. In other words, the percentage of decrease in oxygen consumption following X radiation is not affected by DNP — apparently the respiratory mechanisms stimulated by DNP are not affected by X rays.

Effect of X rays on respiration of individual embryos. - Use was made of the Gregg ultramicrorespirometer for studies on respiration of single embryos. This instrument was found to be reliable for measuring respiration of small amounts of tissue as determined by extensive tests made on aliquots of bacterial suspensions. The wide variation observed in respiration of 132 untreated embryos measured separately in the ultramicrorespirometer could not, therefore, be attributed to instrumental error. In view of this great variation among embryos, the effects of X rays on respiration were examined in the following way. The respiration of a given embryo was measured for 40 minutes. The respirometer was then removed from the water bath, X-rayed, and returned to the bath. The respiration of these X-rayed embryos was then measured for 40 minutes. Respiration of control embryos was measured in the same way, the respirometers being removed from the water bath during the time in which the experimentals were being irradiated. The rate of oxygen consumption in the second period (after return to bath) is, in general, lower in most controls and experimental embryos. The doses of X ray used were 5000, 10,000, 15,000, and 42,000 r. More data were obtained for the 5000-r dose, and results were statistically analyzed.

The respiration and the respiratory reaction of embryos to 5000 r of X rays showed large variations. Although the mean difference between the two respiration periods (before and after removal from bath) is slightly greater in the irradiated embryos than in the controls, it does not differ in a statistically significant manner from the difference between the two periods in the controls. We find no evidence, therefore, of a respiratory effect of low doses of X rays on individual embryos.

Mutation Rates at Specific Loci in the Third Chromosome of *Drosophila melanogaster*

(Alexander)

The mean X-ray-induced mutation rate per locus, for seven specific autosomal loci, from irradiated spermatogonia in the mouse (Russell, ORNL-989) is considerably higher than the rates obtained by various investigators from similar but not exactly comparable experiments on *Drosophila*. The need for an investigation in *Drosophila* that would closely parallel the mouse experiment has been discussed by Russell (ORNL-1244). The present investigation was designed to meet this need by determining the X-ray-induced mutation rates, at specific autosomal loci, in spermatogonia. The mutation rate in sperm was also determined for comparison with the rate in spermatogonia.

Since moderate doses of X radiation do not produce a sterile period which will allow the detection of treated spermatogonia in *Drosophila* as in mice, the early larval stage which contains only spermatogonial cells was irradiated. A dose of only 900 r was employed for the spermatogonial test because higher doses produced a high percentage of sterility. The adult males from treated larvae were mated individually to homozygous *ru h th st* p^p *cu sr* e^s females. Most of the males that produced clusters of mutations were remated to marker females for a period of from 6 to 30 days. The recurrence of mutant offspring over this whole period showed that the spermatogonia and not later stages were being affected.

In the mature sperm test, the general procedure of treating adult Oregon-R males with 3000 r of X radiation and mating to homozygous marker females was modified in several ways to decrease the chance of unnecessary error in the experimental technique. The use of individual adult males makes possible the detection of "spontaneous cluster" mutations. The remating of one male to a different female every 24 to 36 hours for a 4-day period limits the test to the later stages of spermatogenesis—probably only mature sperm. In both the sperm and spermatogonial test, those F_1 variants which phenotypically resembled mutations at any of the eight loci were tested for genetic inheritance and loci determined by crossover tests.

Table 13 summarizes the results obtained from treated sperm and spermatogonia. The "Genetic mutations" category includes only the variants that were proven to involve the loci concerned; the numbers in parentheses are probably mutations. Even though the number of "Genetic mutations" and "Sterile variants" is almost equal, nearly half the sterile variants resembled the *ru* mutations and could have been produced by various

TABLE 13

VARIANTS RECOVERED FROM X-IRRADIATED SPERM AND SPERMATOGONIA

Mature sperm tested - 49,521 at 3000 r	Loci tested								
	ru	h	th	st	p^p	cu	sr	e^s	Total
Genetic mutations	2 (2)	6	4	9 (1)	13	8	9 (1)	13	64 (4)
Phenocopies and mimics	82 (1)	0	2	59	3	10	10	19	185 (1)
Sterile variants	30	3	0	9	9	8	2	7	68
Dead before test completed	53	6	3	13	3	3	6	4	91
Partials	27	2	0	0	2	1	13	0	45
Spermatogonia - at 900 r	0	2	0	0	0	0	0	2	4

25,268 controls were maintained in these tests.

other changes such as small deletions at any place in the genome. The "Sterile variants" classification may, therefore, include some true mutations at the loci tested, but it is difficult to assign the sterility effect to the loci which are seemingly involved or to count the sterile variants as true mutations. Variants which involve some other locus in the genome and those produced by physiological effects were placed in the "Phenocopies and mimics" category. Unilateral and partial expressions of variants could not be recovered among the progeny, and may be somatic mutations or physiological effects. Some variants died before sufficient test could be completed to determine a classification.

The X-ray-induced mutations at the specific loci may involve more severe radiation damage to the chromosome than indicated by the recovery of a genetic mutation. Some of the mutations may be associated with gross chromosome rearrangements or damage, whereas others are not. A correlative cytological and genetic study is contained in the following section of this report.

The data given for spermatogonia represent only the "Genetic mutations" category. Tests of the other categories are not yet complete. The total number of four mutations listed for spermatogonia in Table 13 represents three clusters and one single mutation. Two of the clusters were mutations at the hairy locus. These gave, respectively, four mutants in 598 offspring and 17 mutants in 770 offspring. The other cluster and single mutation were mutations at the ebony locus and were observed as 31 mutants in 469 offspring and one mutant in 110 offspring.

The mutation rates are shown in Table 14. The rates for sperma-

TABLE 14

MUTATION RATES AT SPECIFIC LOCI AND AVERAGE RATE FOR THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER

Stage of spermatogenesis	Dose and sample size	Rates at specific loci per roentgen (x 10^{-8})								Av. rate/r (x 10^{-8})
		ru	h	th	st	p^p	cu	sr	e^s	
Mature sperm	3000 r; 49,521	1.35 (2.69)	4.04	2.69	6.06 (6.73)	8.75	5.38	6.06 (6.73)	8.75	5.38 - 5.72
Spermatogonia	900 r; 101,282	-	9.32	-	-	-	-	-	11.33	2.58*

* See text for the method of estimating a rate for spermatogonia.

togonia were calculated by weighting the number of mutants according to the mean number of offspring per irradiated male that gave no mutants. The number of offspring per male for males that gave clusters was much higher than the mean number because these males were mated repeatedly to determine the ratio of mutant to normal sperm as accurately as possible. The data on spermatogonial mutations were collected in three separate experiments. In the first experiment, in which the two clusters at the hairy locus were found, the mean number of offspring per male was 295. In the second experiment, which gave the cluster at the ebony locus, the corresponding number was 141. The single mutation was recovered in the third experiment, which had a mean number of 114 offspring per male. In all three experiments a sample size of 101,279 sperm was obtained from 656 males treated as larvae.

Since clusters of specific mutations have not been previously reported for treated larvae of Drosophila, the cluster sizes are interesting. The mutants recovered up to this time show ratios which vary from 1 mutant per 15 normal sperm to 1 mutant sperm per 119.5 normal sperm. The average ratio of 1 mutant to 72 normal sperm gives an estimation of approximately 36 spermatogonia per treated male—a number which is not far from the average number of 22 determined by histological studies of other workers. This ratio of mutant to normal sperm also supports the hypothesis that groups of primary germ cells in the larva form the adult testes, since a different ratio would be expected if only one of the group formed the "spermatogonia-reproducing" cell in the adult.

The average mutation rate for the third chromosome in both sperm and spermatogonia is noticeably lower than the $(25.0 \pm 3.7) \times 10^{-8}$ per r rate reported for the spermatogonia of mice by Russell (ORNL-989). The

sperm rate of 5.38-5.72 x 10^{-8} per r for the third chromosome falls into the general range of established rates for Drosophila. The spermatogonial rate of 2.58 is approximately one-half the mature sperm rate.

The mutation rates at specific loci in the sperm test vary from 1.35 x 10^{-8} to 8.75 x 10^{-8}. The variation is probably significantly different from chance variation, but no exact statistical tests are available. There is no clear correlation of specific rates with the cytological position of the gene on the chromosome. The ebony locus shows as high a rate as the pink or scarlet loci which are nearer the centromere.

About one-half the mutations obtained from irradiated sperm have been tested homozygous. Heterozygotes were established of the mutant to be tested and a test marker mutant. Paired matings of such heterozygotes give a ratio of 1 test marker: 2 normal: 1 mutant, which is being tested. Seventeen of the mutants were lethal, thus producing a 1:2 ratio with the third class missing. Eleven were viable and fertile in a homozygous condition. One mutant falls into the classification of semilethal, since very few homozygotes are recovered, but these soon died and none was fertile. The remaining one of the thirty which have been tested gave ratios of 1:3 and 1:4, thus indicating that the mutant class is phenotypically normal in a homozygous condition. The two Y-3 and one X-3 translocations were not tested homozygous. Only two of the four cluster mutations from spermatogonia have been tested. One lives homozygous but is reduced in viability and fertility; the other is homozygous lethal.

The results obtained in this experiment agree with the tentative conclusion, reached from experiments that afforded less exact comparison, that the X-ray-induced mutation rate in Drosophila is considerably below that reported for the mouse. However, more data are needed on the spermatogonial rate in Drosophila. Additional experiments are in progress.

Cytological Analysis of X-Ray-Induced Mutations in Drosophila melanogaster

(Ward, Alexander)

Since our knowledge of the proportion of point mutations to those mutations associated with various types of structural changes of the chromosome is incomplete, a cytological study of the irradiation-induced mutations in Drosophila melanogaster is being undertaken. Induced mutations at eight specific loci in the third chromosome are being used in this investigation. The mutants, obtained from irradiated sperm and spermatogonia by a method discussed by Alexander (this report), have been tested for lethality in the homozygous condition. If the homozygotes are viable

and fertile, homozygous males are mated to Oregon-R females, and the F_1 larvae are analyzed cytologically. If the homozygotes cannot be obtained because of lethality, the mutant is balanced against the C(3)x inversion chromosome; heterozygous males are then mated to Oregon-R females. The salivary gland chromosomes of the non-C(3)x individuals are examined by the usual squash technique using aceto-orcein as the stain.

Twenty-one of the mutations (twenty from irradiated sperm and one from irradiated spermatogonia) have been analyzed cytologically, and the results are summarized in Table 15. The term "point mutation" is used

TABLE 15

CYTOLOGICAL ANALYSIS OF X-RAY-INDUCED MUTATIONS

Cytological analysis	Loci							Total
	h	th	st	p^p	cu	sr	e^s	
Point mutation	1*	1	1	3	2		2	10
Deletion		1**	2**					3
Inversion	2			2			1	5
Translocation			1		1	1		3
Total tested	3	2	4	5	3	1	3	21

* From irradiated spermatogonium

** One deletion involving both the th and st loci

to describe a mutation when it is not possible to demonstrate a chromosomal aberration in the salivary gland chromosome. The three remaining classes of mutants are those which are associated with some visible structural change of the chromosome.

Of the twenty-one mutants, ten have been classed as point mutations, three are associated with deletions, five with inversions of various sizes, and three with translocations. All inversions were large rearrangements except for one at the pink locus which involved a maximum of fifteen bands in the regions 85C and D of the salivary gland chromosome map (C. B. Bridges, J. Heredity, 26:60-64, 1935). Those mutations associated with inversions and translocations, and possibly those associated with deletions, would be classed as position effect mutants.

In Table 16 the types of mutations are classed according to the viability in the homozygous condition. It is interesting to note the proportions of viable to lethal mutations in the different categories. Seven of the ten point mutations are viable in the homozygous condition while three are lethal or semilethal. The two deletions are lethal in the homozygous state and four of the five inversions live as homozygotes.

TABLE 16

TEST OF VIABILITY OF THE HOMOZYGOTES

Cytological analysis	Viability of homozygotes			Total
	Viable	Semilethal	Lethal	
Point mutation	7	1	2	10
Deletion			2	2
Inversion	4	1		5
Translocation	Not tested			

Chromosomal aberrations are used in the localization of the genes on the salivary gland chromosomes; therefore any deletion may prove useful for this purpose. The appearance of an individual showing two mutations, thread and scarlet, was an indication that one of three possibilities had occurred: (1) simultaneous point mutations at the two loci, (2) a mutation at one locus and a deletion of the other, (3) a deletion of both loci. A cytological analysis of this chromosome revealed a deletion of approximately twenty-five bands in regions 72 and 73A of the left arm of the third chromosome. The deletion included the region which had been assigned to thread by an earlier investigator (P. N. Bridges, J. Heredity, 32:64-65, 1941) but not that which had been assigned to scarlet. Analyses of other scarlet mutations showed a deletion of the regions 72F and 73A and a translocation with the break in region 73A. These three chromosomal abnormalities are evidence that the scarlet gene is located in either 72F or 73A of the third chromosome.

RADIATION PROTECTION

Alexander Hollaender (Leader)
G. E. Stapleton
E. H. Anderson
Daniel Billen
W. T. Burnett, Jr.
R. B. Grayson
Ruth W. Whittle
A. W. Burke, Jr.

Recovery of Bacterial Cells from X Rays

(Stapleton, Billen, Grayson)

It has been reported (ORNL-1167; ORNL-1244) that E. coli B/r can recover from the lethal effects of X rays if plated on a solid complete medium (nutrient agar) at temperatures between 6° and 37°C. The optimum temperature for survival was observed to be about 18°C. The data suggest that the ability of irradiated bacteria to survive a dose of X rays is dependent to some extent on the relative rates of anabolic and catabolic processes, which are very temperature dependent.

It seemed important to test the ability of irradiated cells to recover in liquid media, both minimal and complete, in an effort to establish the nutritive requirements for the process, as well as to correlate this process with normal processes such as growth and cell division. A minimal medium composed of inorganic salts and glucose, as described by Billen and Lichstein (J. Bact., 62:793-797, 1951) was found to support growth and cell division of nonirradiated E. coli; however, no recovery of irradiated cells was observed in the medium. Recovery did occur in a complete medium (nutrient broth) and was similar to that obtained on nutrient agar, both in regard to rate and to the final recovery obtained at any temperature studied.

A finding of utmost importance was that, although no recovery occurred in the minimal medium, the addition of yeast extract (Difco) at concentrations of 1 mg/ml or greater was sufficient to stimulate recovery. It would appear that a special factor or factors present in both nutrient broth and yeast extract is required for recovery. Addition of a mixture of seventeen amino acids to minimal media does not result in any stimulation of recovery. A mixture of the known vitamins to minimal medium does not stimulate recovery under those conditions in which yeast extract was found to stimulate the process, suggesting that either an unknown factor is required or that there are multiple requirements. As yet the proper combination has not occurred in any synthetic mixture prepared.

The resolution of possible factors required is being attempted by extraction and fractionation by means of both paper and ion-exchange chromotography. Simultaneously, we are assaying synthetic mixtures for activity in stimulating recovery. The results of these assays will be reported at a later date.

Radiation Protection Studies — Sulfhydryl Compounds

(Burnett, Burke)

Cysteine hydrochloride and BAL(2, 3-dimercaptropropanol) oxidize in phosphate buffer that contains dissolved oxygen, the rate of oxidation being faster with BAL than with cysteine. The protection which these compounds afford to Escherichia coli B/r during X irradiation at 2°C can be attributed, in part, to the ability of these compounds to decrease the concentration of dissolved oxygen of the cell environs. However, a protection is afforded to E. coli over and above that which has been found when oxygen is removed mechanically by sweeping with nitrogen, or, chemically, by a treatment with sodium hydrosulfite. It may be that the nonoxidized sulfhydryl groups compete with the cell constituents for the radicals produced in the aqueous solution by the X radiation. This is a mitigation of the indirect effect of the radiation. It is suggested that the "target" would be less radiosensitive if there is some type of "complex" formed by the cells and the sulfhydryl groups. This mechanism represents a modification of the radiosensitivity of the cell.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL 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

Histological Study of a Semilethal in the Mouse

(Oakberg, Crowell)

An analysis of histological effects of genetic changes resulting in lethal or semilethal effects in mice has been undertaken. The mutation chosen for initial study is important both because of its unusual phenotypic effect and because of its genetic nature. A brief description of the phenotype and semilethal effect is given here; the genetic aspects will be presented by W. L. Russell in a later report. The mutation arose spontaneously in a stock of mice homozygous for the genes aa bb pp dd ch ch se se and has been maintained by repeated backcrossing to the 129 inbred strain. The number of backcrosses now is ample to assure a uniform genetic background for expression of the mutant, with the possible exception of genes closely linked with the mutation.

Fifty-three mice ranging from 2 to 24 days old have been autopsied. Normal litter mates of affected animals have been used as controls. Cover-slip blood smears were made from the tail; blood for total leucocyte and erythrocyte counts was obtained by severing the left foreleg close to the body and allowing the blood to drip into a depression slide previously prepared with a dried film of concentrated heparin. This procedure prevents both the clumping of erythrocytes and the difficulty of obtaining sufficient blood in young mice. Pieces of skin, ear, liver, spleen, thymus, kidney, mesenteric lymph node, and bone were fixed in Zenker-formol, sectioned at 7μ, and stained with hematoxylin azure-II eosin. Blood smears were stained with brilliant cresyl blue, the peroxidase reaction, and the Wright-Giemsa technique.

Separation of normal from abnormal mice has not been possible until clinical symptoms appear. Therefore, only the older age groups are considered here, and only those litters where accurate classification of the phenotypes was possible.

Affected mice are normal at birth, and cannot be distinguished until 7, or more frequently, 9 to 10 days of age. The initial changes are bright pink, swollen ears and a tightening of skin so that it is not as loose and freely movable as in normal mice. A scaly appearance of the feet and tail is apparent, with the blood vessels in these regions appearing more prominent than normal. Alterations in the skin progress rapidly and result in a dry, scaly appearance over the entire body, the pelage becomes ruffled, and the eyes frequently become closed. Emaciation, dehydration, and diarrhea develop rapidly, and death usually occurs between the 15th and 25th day postpartum. Other genetic backgrounds have considerable effect on survival, for in the outcross to 101xC_3H hybrids several individuals have lived 30 to 40 days, and rare cases have survived over 60 days. These older animals lose their dorsal hair, and scabs appear wherever the skin is subject to continued creasing, as on the neck, in the axillary region, at the base of the tail, and under the chin.

Autopsy of affected mice reveals a greatly enlarged spleen which is firm, edematous, and frequently hemorrhagic. The liver usually is enlarged slightly and has a dark red, turgid, and glossy appearance. The thymus is atrophic, often markedly so. The mesenteric lymph nodes show variable enlargement, and lymphatics of the viscera often present a "beaded" appearance. Ascites and excess pleural and pericardial fluid frequently occur in individuals over 20 days old.

Skin sections reveal a mild leucocytic infiltration of the basal portions of the dermis and of the panniculus muscles at 7 to 10 days. Lymphoid and neutrophilic cells predominate, with scattered eosinophils. Following these initial changes there is a rapid loss of fat, the dermis becomes thinner, leucocytic infiltration becomes moderate to heavy, and there appears to be an increase in connective tissue. By 20 days, degeneration of sebaceous glands and hair follicles is apparent, with almost complete loss of these structures from the dorsum by 30 to 35 days. The panniculus muscle appears poorly developed, though this may be due to general emaciation. Changes in the ear follow a similar pattern, with more severe leucocytic infiltration, edema, and a larger proportion of eosinophils. By 20 to 24 days the degree of infiltration of skin frequently subsides, though this reaction varies considerably.

Internal organs show infiltration with lymphoid, myeloid, and erythroid cells. An increase of extramedullary erythropoiesis is apparent both in liver and spleen. In the kidney accumulations of lymphoid cells occur around blood vessels of the hilus, with smaller infiltrations extending to the cortex. Myeloid cells occasionally appear at the hilar region, but rarely are seen in the cortical lesions. The mesenteric lymph node is hyperplastic with loss of normal structure, scattered myelocytes, and increased erythrophagocytosis. The spleen shows a variable loss of normal

architecture with diminution of white pulp, reticulum cell hyperplasia, myeloid metaplasia, and extramedullary erythropoiesis. Pathology of the liver is the most striking of the tissue changes. Large periportal infiltrations of lymphoid cells occur without exception. Occasionally myelocytes and juvenile neutrophils are associated with these areas, but the lymphoid cells invariably predominate. Frequently crowding of liver parenchyma and infiltration of surrounding sinusoids by these cells is apparent. Small foci of lymphoid (stem cells ?), myeloid, neutrophilic, and erythroid cells occur in the sinusoids. These smaller areas rarely are of a "mixed" type. The normal occurrence of hemopoiesis in the liver until 10 or 12 days postpartum hinders separation of normal from abnormal tissue in young animals. Extent of periportal infiltrations and cell type involved are useful criteria, and indicate that pathological changes in the liver do not lag behind, but possibly precede changes in the skin.

A summary of erythrocyte and leucocyte counts for 16 control and 20 abnormal mice is given in Table 17. Additional data are necessary before

TABLE 17

MEAN ERYTHROCYTE AND LEUCOCYTE COUNTS OF NORMAL AND ABNORMAL MICE

	Normal			Abnormal		
Age (days)	No. of mice	Erythrocyte counts*	Leucocyte counts	No. of mice	Erythrocyte counts*	Leucocyte counts
7	2	5.43	5,400	1	5.92	6,260
9	4	5.76	5,590	5	5.80	8,930
11	1	6.09	7,700	1	6.64	22,900
12	2	6.74	5,970	3	6.36	24,000
13	1	8.25	5,880	1	5.40	12,380
14	2	7.56	5,860	3	6.16	17,970
15	1	6.68	4,300	3	6.16	13,290
↕						
22	1	8.93	—	1	4.30	5,700
23	1	8.66	7,520	1	3.18	1,340
24	1	8.78	9,160	1	1.62	10,800

* Values given to be multiplied by 10^6

statistical analyses would be feasible, particularly in studies of this nature, where the time-development curve is based on a different animal for each observation. There appears to be considerable variation both in time of onset and in rate of progression of pathogenesis which results in great variation within any one age group. However, it appears as if the slight decrease in erythrocyte number which appears at 12 to 14 days, with progression to the severe anemia manifest just before death, would be substantiated by further data. With the anemic condition, number of reticulocytes is increased. Likewise, the high leucocyte counts at 11 to 15 days appear too large to be due to sampling errors. Differential counts of 200 leucocytes from each mouse indicate an increase in mature neutrophils and the appearance of myelocytes and myeloblasts in the blood. Marked irregularity in size of myeloblasts, myelocytes, and mature neutrophils occurs, and in some mice most of the myeloid series is of the micromyeloblastic type.

Many additional experiments will be needed to elucidate the changes involved in these animals. However, the most plausible hypothesis is that a genetic change (either gene or chromosome mutation) has resulted in a loss of certain metabolic processes in affected mice. Data are not sufficient to indicate the nature of this deficiency (or deficiencies), but the hemopoietic system appears to be one of the first tissues to be affected.

PATHOLOGY AND PHYSIOLOGY

PATHOLOGY AND PHYSIOLOGY

Jacob Furth - Section Chief

M. C. Woods
A. C. Upton
R. H. Storey
J. R. Thomson
Evelyn Gadsden
R. R. Bigelow*
K. W. Christenberry**
W. D. Gude
Frances Gamble
Peggy Ledford

Late Effects in Mice of Exposure to Thermal Neutrons

(Upton, Christenberry, Thomson)

These experiments, begun in 1949 and mentioned in previous quarterly reports, are still in progress. 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 250 kvp X radiation or set aside as nonirradiated controls. The 30-day LD_{50} amounted to 80 minutes' exposure in the reactor, at a thermal neutron flux of 0.9×10^9 per sq. cm per second, with an estimated 4-8 r of high energy γ radiation per minute; the corresponding LD_{50} of X radiation was 80 minutes' exposure at a rate of 6.4 r per minute (512 r). Mice were exposed to both types of radiation for 80, 20, 5, and 2.5 minutes, respectively. Of approximately 2500 mice in this series about 70 per cent have been observed until natural death. The remainder are being sacrificed as they reach the age of 24 months.

The acute radiation-induced injuries, essentially identical in both pile-exposed and correspondingly X-irradiated mice, have been reported earlier. The earliest chronic lesions observed were cataracts, first noted about 70-80 days after exposure. The latent period varied inversely, and the rate of progression directly, with the dose (Table 18). Senile cataracts began to appear at about one year of age, tending to obscure the radiation-induced opacities thereafter; however, there was a definite increase in the incidence of cataracts even following 16 r of X radiation. Thermal neutron γ radiation is more effective than X radiation in inducing cataracts, when the comparison is made on basis of equal effect as concerns midlethal and hemopoiesis-depressing doses.

* Consultant, surgery
** Consultant, ophthamology

TABLE 18

PRELIMINARY TABULATION OF CATARACTS IN PILE-EXPOSED MICE

Months after exposure:	1-3		4-6			7-10				11-14				15-18			
Degree of opacity[a]:	0	+	0	+	++	0	+	++	+++	0	+	++	+++	0	+	++	+++
Number of mice examined																	
N 80[b]	6	37			3		2	17	2		1		6			5	7
X 80[c]	12	16	1	4	7	1		5	1			5	4		1	4	2
N 20	16	10		13	1		8	5			1	4			1	14	3
X 20	29	2	1	11		1	7					2			13	5	1
N 5	20	4		3			13	1		1	9			1	12	5	
X 5	23		1	3		6	7				4			4	20	2	
N 2.5	22			4		6	7			1	10			4	12	3	
X 2.5	16		1	3		10	3			4	7			1	10	4	
Controls	18		15	1		26	9			9	2			27	22		

[a] For a description of degree of opacity, see Proc. Soc. Exptl. Biol. Med., 77: 559-560, 1951.
[b] N 80: exposure to thermal neutron-γ radiation for 80 minutes in the thermal column of the Oak Ridge reactor.
[c] X 80: exposure for 80 minutes to 250 kvp X radiation at 6.4 r/minute.

The incidence of thymic lymphosarcoma was significantly increased after median lethal doses of either neutron-γ or X radiation (Table 19).

TABLE 19

MEDIASTINAL LEUKEMIA IN MICE EXPOSED TO SLOW NEUTRONS AND X RAYS

Exposure	No. of mice	Dead (%)	Percentage of leukemia incidence/months				Total leukemia (%)
			5-8	9-12	13-16	16+	
N 80[a]	244	79	6	8	3	4	21
N 80[b] (512 r)	163	83	8	9	3	4	24
N 20	277	76	1	3	3	2	9
X 20 (128 r)	221	60	2	3	2	3	10
N 5	263	65	0	2	2	4	8
X 5 (32 r)	246	55	1	1	1	4	7
N 2.5	197	46	0	4	4	6	14
X 2.5 (16 r)	135	36	1	1	4	0	6
Nonexposed	468	54	1	2	2	3	8

[a] N 80: Exposure for 80 minutes in the thermal column of the reactor.
[b] X 80: Exposure for 80 minutes to X radiation at 6.4 r/minute.

The peak incidence was observed at 6-12 months of age. Irradiation did not appear to have altered the total incidence of nonmediastinal leukemia, although the data suggest that it hastened its onset (Table 20). Typing of these leukemias is in progress. They include lymphoid (not mediastinal), myeloid, and monocytic leukemias.

TABLE 20

NONMEDIASTINAL LEUKEMIA IN MICE EXPOSED TO SLOW NEUTRONS AND X RAYS

Exposure	No. of mice	Dead (%)	Percentage of leukemia incidence/months				Total leukemia (%)
			5-8	9-12	13-16	16+	
N 80[a]	244	79	2	5	6	12	25
X 80[b] (512 r)	163	83	3	5	5	11	24
N 20	277	76	1	5	10	15	31
X 20 (128 r)	221	60	2	5	10	13	30
N 5	263	65	2	1	4	20	26
X 5 (32 r)	246	55	1	2	5	16	24
N 2.5	197	46	1	1	3	15	20
X 2.5 (16 r)	135	36	0	2	5	14	21
Nonexposed	468	54	0	1	4	19	24

[a] N 80: Exposure for 80 minutes in the thermal column of the reactor.
[b] X 80: Exposure for 80 minutes to X radiation at 6.4 r/minute.

Other neoplasms of various types have been encountered, such as soft tissue sarcomas, skeletal tumors, squamous cell and adenocarcinomas, and neoplasms of endocrine glands. Among the last, ovarian tumors have been most conspicuous. Available data indicate that the threshold dose for induction of ovarian tumors is below 32 r (Table 21). The in-

TABLE 21

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

Duration of exposure	Percentage with ovarian tumor	Percentage with lung tumor		Percent of total dead	
		M	F	M	F
N 80[a]	25.4	5.4	8.8	86.8	71.9
X 80[b] (512 r)	19.4	10.0	7.5	88.6	77.4
N 20	20.4	6.7	9.2	85.2	67.6
X 20 (128 r)	12.1	5.7	8.2	65.7	54.3
N 5	6.3	9.9	10.8	65.1	65.7
X 5 (32 r)	7.8	13.7	5.2	63.4	46.9
N 2.5	2.7	2.4	4.0	43.1	48.6
X 2.5 (16 r)	0	3.5	4.0	31.8	40.0
Nonexposed	1.5	8.2	2.9	58.8	48.8

[a] N 80: Exposure for 80 minutes to thermal neutrons in the reactor.
[b] X 80: Exposure for 80 minutes to X radiation at 6.4 r/minute.

cidence of pulmonary adenomas and of hepatomas also appears to have been increased by irradiation, but, as these tumors appear late in life, the threshold doses for the induction of these neoplasms and induction rates remain to be determined.

Histologic typing and statistical analysis of the leukemias and of other neoplasia must be made before final conclusions may be drawn; however, to date, except for a relatively higher cataract-inducing potency, thermal neutrons have revealed late effects in mice which are not significantly different from those produced by corresponding doses of X radiation.

Studies on the Pathogenesis of Radiation-Induced Cataract

(Christenberry, Upton, Thomson)

Cataract is known to result from a variety of systemic biochemical, metabolic, and endocrine disorders. Hence, in the light of the profound biochemical disturbances which are produced by ionizing radiation, it appeared conceivable that radiation injury of extraocular tissues might contribute to cataract formation. If this were the case, the threshold cataract-inducing dose of radiation would depend to some extent upon the degree of general bodily damage, or upon the volume of extraocular tissue irradiated. Thus, shielding of a portion of the body might confer some measure of protection against cataract induction, as has been observed for radiation mortality and hemopoietic injury. In order to answer these questions, mice were given graded doses of X radiation, exposing the whole body, head, or body only, respectively (Table 22).

The data in the table indicate that opacities of the lens occurred at all dose levels, from 15 to 405 r. The time of onset, progression, and severity of the lesions were identical in the head-irradiated and whole-body-exposed mice. Furthermore, shielding of the head prevented cataract formation, even though the remainder of the body received 405 r. Thus, it is concluded that cataract results from irradiation only when the eye is exposed directly, and that there are no significant indirect effects of radiation in cataract induction. It is also evident that protection is not afforded against cataract formation by shielding most of the body, including the spleen and bone marrow. Further detail of this experiment is included in a report now being prepared for publication.

TABLE 22

CATARACTS IN RELATION TO DOSE OF X RADIATION AND PART OF BODY EXPOSED

No. of mice	Dose (r)	Region exposed	Latent period (wks.)	No. of mice with opacities 33 wks. after exposure[a]		
				0	+	++
9	405	Total body	11		1	8
9	405	Head only	11			9
18	405	Body only	—	16	1[b]	1[b]
14	135	Total body	17		14	
13	135	Head only	17		13	
17	135	Body only	—	17		
18	45	Total body	28	2	16	
19	45	Head only	28	3	16	
21	15	Total body	32	14	6	1
17	15	Head only	32	12	5	
18	0	Nonexposed	--	18		

[a] Grade of opacities: 0 = none, + = early posterior subcapsular polar opacity; ++ = more advanced posterior subcapsular opacity; for illustrations of these lesions see Proc. Soc. Exptl. Biol. Med., 77: 559-560, 1951.

[b] These isolated opacities are attributed to imperfect shielding, due to movement of the mice during exposure, resulting in direct irradiation of the head.

Permeability Problems of Ionizing Radiations

(Storey, Woods, Bigelow)

The experimental work on the physiology of albumin exchange between blood and lymph in normal and X-rayed dogs has been concluded and the data are being written up for analysis by the ORNL Mathematics Panel and for publication.

The plasma volume was found to change after irradiation in a manner similar to that found in rabbits and mice. The metabolism rate of injected iodoalbumin was found to be essentially unchanged in the irradiated host. The ratio of radioactivity per cc of lymph to radioactivity per cc of plasma was found to be increased during the first postirradiation week; thereafter

(+9 - +16 days) the trend appears to be a lowering of this ratio to below the normal. This observation was also confirmed with chemical determinations of the ratio of albumin concentrations between lymph and plasma.

The rate of loss of injected albumin from the circulation was found to be increased during 8-30 hours in dogs in a manner similar to that previously observed in mice and rabbits. Estimation of the lymph space is being done with the aid of the Mathematics Panel. Technical and theoretical difficulties involved in this estimation are many, especially in the irradiated animal; no satisfactory technique is known for precise determination of the lymph volume in normal man or animal.

Role of Platelets in Postradiation Hemorrhagic Syndrome; Platelet Transfusions in Dogs with Exteriorized Lymph-Venous Anastomosis

(Woods, Bigelow, Gamble)

The present study was undertaken to determine the extent to which platelets diminish the hemorrhagic tendency, and the mechanism of platelet action, following massive acute radiation. This study began in cooperation with E. P. Cronkite (Naval Medical Research Institute) and George Brecher (National Institutes of Health, Bethesda, Maryland), using their procedures. These investigators have already established that transfusions of platelets will diminish the hemorrhagic tendency.

The parameters used to determine the therapeutic efficiency of the platelet transfusion were the erythrocyte counts in thoracic lymph of X-rayed dogs before and at various intervals after platelet infusion. All dogs were pretreated (dewormed and vaccinated), the white cell count of the blood established, and then X-rayed with a near-lethal dose of radiation (350-450 r). Antibiotic therapy with 400,000 units of penicillin daily was begun at the time of radiation and continued until death. Dogs having a satisfactory low white blood count (1500 or less) were operated on as described in earlier reports.

The lymph samplings and the intravenous injections were made through exteriorized thoracic lymph-venous anastomoses. It was found desirable to follow the red-cell count in the lymph for about 24 hours after operation in order to establish adequately the character of the lymph and the condition of flow.

The results of platelet infusions into six X-rayed dogs are given in Table 23 and the mean results in Fig. 4. Five such transfusions gave clear-cut results, the red-cell count in the lymph of the X-rayed dogs being rapidly depressed and held low as long as the blood platelet level was above a critical level of approximately 30,000/mm^3. The anomalous behavior of the sixth animal (dog No. 59) is being considered to be due in part to an unexplained technical failure.

TABLE 23

SUMMARY OF EXPERIMENTS ON DOG PLATELETS

	Postinjection of platelets		Blood platelets	Erythrocytes in thoracic lymph	Flow of thoracic lymph
	Time (hr)	Treatment	(thousands)	(thousands)	(cc/min)
Dog #45 Wt., 24 lb X-ray dose, 350 r	-48	Operation	10.0	5.5	—
	-26			5.0	1.0
	- 6		10.0	49.0	2.0
	0	1st inj. + 11 days post-X	95.0*	64.5	2.0
	2	2nd. inj.	135.0 (66%)*	31.0	—
	7			6.2	2.5
	21		47.5	0.8	2.2
	42		17.0	1.25	1.3
	68		10.0	30.0	1.1
Autopsy findings: D + 144 hours postinjection. Few intestinal hemorrhages, but otherwise typical acute hemorrhagic syndrome.					
Dog #46 Wt., 24 lb X-ray dose, 350 r	-24	Operation	10.0	40.0	—
	- 6			71.0	1.0
	0	+ 10 days post-X	85.0 (90%)*	80.0	—
	1			25.7	—
	3		65.0	16.0	1.2
	18	Cannulas obstructed	12.5	21.0	—
	40		22.5		
Autopsy findings: D + 70 hours postinjection. No intestinal hemorrhages. Diffuse generalized thoracic purpera.					
Dog #51 Wt., 21 lb X-ray dose, 400 r	-72		170.0		
	-36	Operation	35.0	18.0	0.5
	- 6		10.0	60.0	1.4
	- 1			400.0	0.9
	0	+10 days post-X	125.0 (55%)*	900.0	1.1
	0.5			160.0	2.1
	5		115.0	18.8	1.0
	18		25.0	25.0	1.2
	20			150.0 (postmortem)	
Sutopsy findings: D + 20 hours postinjection. Definite hemorrhagic tendency, but without intestinal hemorrhage; nodes brown instead of red.					

Table 23 cont.

Dog #59 Wt., 17 lb X-ray dose, 350 r	-48		115.0		
	- 6	Operation	30.0	100.0	0.25
	0	+ 9 days post-X	95.0 (50%)*	8.0	0.5
	18		58.5	227.0	0.3
	40		30.0	1700.0	

Autopsy findings: K + 40 hours postinjection. Lymphatics and lymph nodes red; serosal, but not mucosal, intestinal hemorrhages. Lymph cannula out of duct.

Dog #61 Wt., 18 lb X-ray dose, 350 r	-72		47.5		
	-24	Operation		11.2	0.2
	- 5		12.5	46.0	0.4
	0	+ 10 days post-X	77.5 (46%)*	73.0	0.3
	1			25.0	0.2
	6		75.0	11.0	0.4
	23		57.5	10.0	0.5
	44		20.0	145.0	0.3

Autopsy findings: D + 4 days postinjection. Typical acute hemorrhagic tendency.

Dog #70 Wt., 27 lb X-ray dose, 350 r	-30	Operation		760.0	1.4
	-24		10.0	215.0	0.3
	0	+ 14 days post-X	152.0 (90%)*	413.0	1.1
	1			91.0	---
	6		65.0	14.0	1.4
	18		72.5	21.0	0.7
	40		32.5		

Autopsy findings: K + 40 hours postinjection. Hemorrhagic tendency not completely blocked. Hepatic lymph nodes and lymphatics intensely red; mesenteric nodes brown, mesenteric lymphatics pink.

* Count taken 30 minutes after infusion; per cent is of expected platelet rise.

At autopsy, in no case was the typical hemorrhagic tendency found to be completely abolished by one platelet transfusion, but reduction in the tendency was usually easily discernible even 48 hours after transfusion. Cronkite, Brecher, and Dillard have already shown that the hemorrhagic manifestation of radiation death may be prevented by repeated platelet transfusions.

The mechanism by which the platelet repairs endothelial damage is being studied.

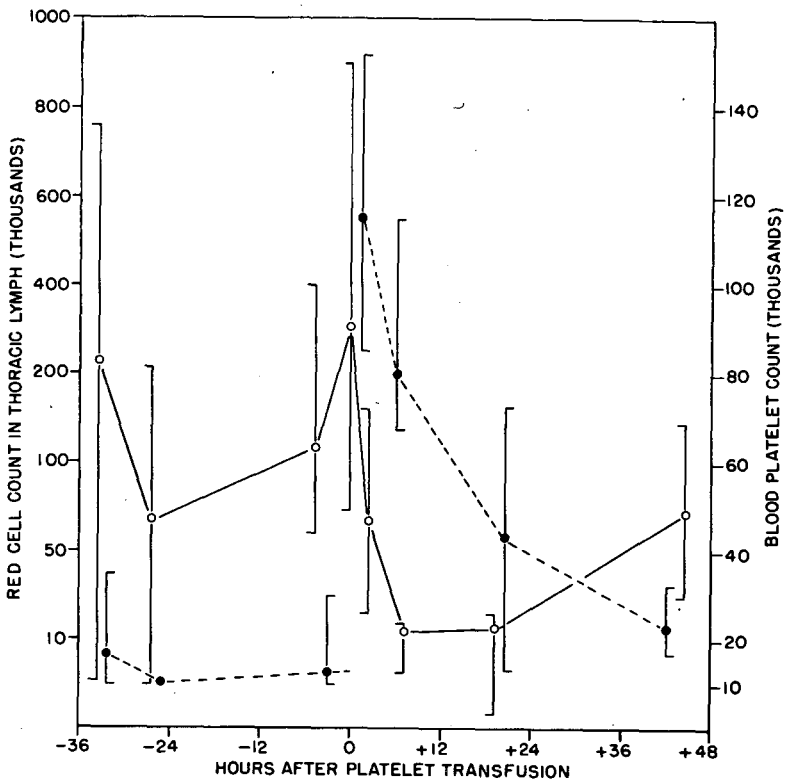


FIG. 4. Blood platelet levels and red blood cells in thoracic lymph of platelet-transfused irradiated dogs
(Composite of 5 dogs, + 10 to 11 postirradiation, 350-450 r)
● = platelets o = red cells
The ordinate scale is arbitrary, showing only the thousands of units.

Studies of the Mechanism of Leukemogenesis by Radiation

(Upton, Thomson)

An earlier quarterly report described experiments which were designed to study the effects of cortisone-induced thymic atrophy upon radiation-induced, as well as spontaneous, leukemia. These investigations are still in progress; the following is a preliminary analysis of the existing data.

Cortisone brings about atrophy of lymphoid tissues, and therefore pretreatment with cortisone might be expected to influence the induction of leukemia by radiation. In fact, shortly after our preliminary work, Kaplan reported that this was the case. The results of our first experiments, summarized in Table 24, indicate that cortisone inhibits slightly the development

TABLE 24

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

Treatment*	Total no. of mice in group	No. of mice dead	No. dead with leukemia	Per cent of leukemia
Cortisone only	139	14	5	3.7
Cortisone vehicle only	152	15	8	5.3
Irradiation only	125	52	51	40.8
Cortisone after irradiation	132	57	46	34.1
Cortisone before irradiation	135	62	47	34.8

* 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 in subcutaneous injections of 1.0 mg each on 3 successive days, beginning either 1 week before or 2-3 weeks after irradiation. Cortisone vehicle (1.5% benzyl alcohol in physiological saline) was injected subcutaneously in comparable amounts (0.04 cc) on 3 successive days at 8-12 weeks of age.

of leukemia following whole-body irradiation in the low-leukemia strain Rf; furthermore, it appears that the effect is approximately the same whether the hormone is administered before or after irradiation, under the conditions of this study. It is also evident that neither cortisone nor its vehicle, given alone, exert a leukemogenic action. These animals are being followed until natural death, and the leukemias are being typed histologically; other effects, if any, will be analyzed. It is noteworthy that cortisone alone is not leukemogenic.

For comparison, the effects of cortisone on the development of spontaneous leukemia are being studied in the high leukemia strain Ak, which is highly susceptible to lymphoid leukemia. The results to date of the experiment in which cortisone was given at 2-3 months of age are summarized in Fig. 5. The data show that cortisone reduced the incidence of leukemia during the first 15 months of life. This is the period during which mice of this strain characteristically die of this disease. It remains to be seen whether this effect is one of retardation or of prevention of leukemia; i. e., whether, after a longer period of observation, the incidence of leukemia in the cortisone-treated mice will equal that in the vehicle-treated group.

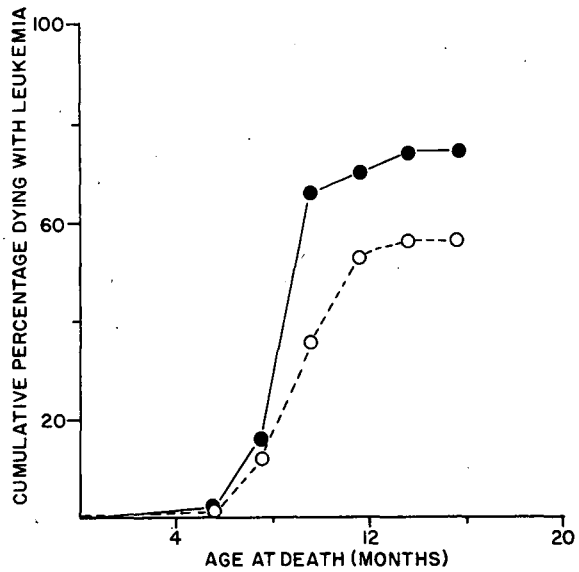


FIG. 5. Cumulative mortality due to spontaneous leukemia in Ak male mice treated with cortisone and cortisone vehicle.
——— = vehicle-treated
----- = cortisone-treated

In a second experiment in progress three sets of cortisone treatments were given and the effects thus far appear even more marked than those shown in Fig. 5. The incidence of leukemia following cortisone treatment is thus far 6.3 per cent in the males and 19 per cent in the females, as compared with 34 per cent in male controls and 52 per cent in female controls.

It appears highly significant that even transient, cortisone-induced thymic atrophy should result in such a marked reduction in the incidence of leukemia. Other studies are being carried out to determine the effects of stress-induced lymphoid atrophy upon leukemogenesis as will be reported later.

In recent experimental studies on the etiology and nature of leukemia the concept is evolved that leukemia, like neoplasias in general, is a heterogenous group of diseases in which the change leading to seemingly unrestrained growth may reside not only in the hemopoietic cell but also in the host.

Characteristics and Secretions of Pituitary Tumors Originating in Radiothyroidectomized Mice

(Furth, Burnett, Gadsden)

Earlier studies have shown that destruction of the thyroid by I^{131}(250-300 μc) is followed by the development of pituitary growths 10-14 months later. These growths can be grafted readily in the muscle of mice similarly radiothyroidectomized but not in normal mice. They grow progressively in the former until the death of the host and almost invariably metastasize to the draining lymph nodes.

In more recent studies all of seven pituitary growths induced by thyroid destructive doses of I^{131} proved transplantable to mice similarly pretreated but not to normal mice. In a subpassage of one strain (B3) a tumor acquired the ability to grow in normal mice, giving origin to a pituitary tumor line which can be grafted as readily, if not better, in normal mice than in radiothyroidectomized mice.

In normal hosts of this autonomous tumor there is a tremendous hyperplasia of the thyroid gland with multiple adenoma-like nodules. There is also some gonadal stimulation of the hosts but no evidence of ACTH secretion by these tumors. Thus a gain in autonomy occurred with retention of the ability of these pituitary (tumor) cells to secrete thyroid- and gonad-stimulating hormones.

These studies indicate the uncertainty and relativity of the term "autonomy" in relation to neoplastic growth. Regional metastases do not indicate "autonomy" as they are occurring also with conditioned neoplasms.

Hyperplasia and cystic dilatation of the extrahepatic biliary ducts is a usual secondary change in I^{131}-pretreated mice bearing large grafts of pituitary tumors which secrete TSH and GSH. Occasionally, rupture of the common duct cyst is the immediate cause of death. The available evidence suggests that this change is due to a pituitary hormone yet to be identified.

Comparison of the Long-Term Distribution of C^{14} from Glycine-2-C^{14} and $NaHC^{14}O_3$ in Mice

(Woods, Gamble)

One phase of the study on the possible pathological effects of C^{14} in mice has been the distribution analysis for C^{14} in selected tissues over the period from exposure until death. Studies by others of the retention of C^{14}

from glycine-2-C^{14} have included only the first of 6-12 weeks. This compound showed the highest early retention of the organic compounds which have been surveyed.

The retention of C^{14} from glycine-2-C^{14} administered in two injections is shown in Table 25. The tissue specific activities are expressed as the

TABLE 25

RETENTION OF C^{14} FROM GLYCINE-2-C^{14} (100 μc)

Months after exposure	6.2	8.7	9.5	12.0	17.0
Percentage retained:	1.15	0.64	0.67	0.34	0.29
"Total-body" specific activity (mμc/g)[a]	33.61	18.69	17.61	9.08	7.69
"Tissue" specific activity:					
Plasma	0.00	(0.09)[b]	(0.03)[b]	0.00	—
Red cells	0.60			0.06	
Spleen	1.13	1.92	1.04	1.38	0.51
Liver	0.25	0.15	0.25	0.25	0.13
Kidneys	0.53	0.52	0.47	0.38	0.55
Lungs	0.72	2.03	2.06	3.26	1.21
Thymus	0.13	0.16	—	1.69	0.00
Submaxillary glands	0.34	0.45	—	0.25	—
Mesenteric lymph nodes	—	1.03	—	—	—
Muscle, femoral	0.84	0.62	1.71	—	1.17
Fat depot	0.37	0.79	0.02	0.32	0.46
Femur	2.60[c]	7.59[c]	5.98[c]	6.09[c]	6.42[c]
Bones of thoracic cage	1.58[c]	4.34	8.41[c]	6.57[c]	0.70
Skeletal remains	0.50	2.88	1.88	1.07	0.99
Residue	1.07	0.99	0.93	1.10	1.03

[a] Ratio of mμc/g of tissue to "total-body" specific activity.
[b] Per cc of blood.
[c] Activity in excess of 15 mμc/g.

ratio of mμc of C^{14} per gram of tissue to the total body specific activity. The corresponding tissue analysis for $NaHC^{14}O_3$ administered in five injections is given in Table 26. Analyses for each time period are for individual animals.

TABLE 26

RETENTION OF C^{14} FROM $NaHC^{14}O_3$ (100 μc, INTRAPERITONEAL)

Months after exposure:	0.5		1.0		2.0	7.0		10.5	
Age at exposure (wks)	3.5-5.0	9.0-12.0	3.5-5.0	9.0-12.0	3.5-5.0	3.5-5.0	9.0-12.0	3.5-5.0	9.0-12.0
Percentage retained	0.07	0.15	0.08	0.12	0.06	0.010	0.014	0.012	0.013
"Total-body" specific activity (mμc/g)	3.17	5.04	2.47	4.04	1.64	0.33	0.43	0.36	0.25
"Tissue" specific activity:*									
Plasma	0.53	0.08	0.53	0.02	0.00	0.00	0.00	—	—
Red cells	2.25	0.83	5.09	2.49	0.00	0.00	0.00	—	—
Spleen	1.81	0.59	0.70	0.53	0.18	0.00	0.00	—	—
Liver	0.28	0.25	0.26	0.26	0.09	0.23	0.00	—	—
Kidneys	0.67	0.26	0.20	0.44	0.00	0.69	0.00	0.00	0.00
Lungs	0.18	0.60	0.49	0.47	0.25	0.97	0.00	0.00	0.00
Thymus	0.65	0.36	0.34	0.29	0.80	0.00	0.00	—	—
Submaxillary glands	0.68	0.49	0.18	0.53	0.36	1.36	0.00	—	—
Mesenteric lumph nodes	0.46	—	0.60	0.74	—	2.47	0.00	—	—
Muscle, femoral	0.72	0.69	0.91	0.41	0.73	0.56	0.00	—	—
Femur	3.32	4.74	2.13	1.06	2.99	18.61	7.60	12.58	7.68
Bones of thoracic cage	4.08	4.72	1.91	1.47	2.78	5.95	4.79	7.75	5.88
Skeletal remains	4.10	1.16	3.07	1.22	3.09	11.18	4.56	10.89	8.24
Residue	0.97	1.09	0.90	1.00	1.04	0.55	0.93	0.64	0.80

* Ratio of tissue specific activity in mμc/g to "total-body" specific activity.

The following generalizations are evident on comparing the data for retention of C^{14} from glycine with those from bicarbonate animals.

1. After exposure to glycine, radioactivity in liver, kidneys, submaxillary gland, and fat depot is low in comparison to total body specific activity, and loss of C^{14} from these tissues is at about the same rate as the decrease in total body specific activity. Spleen and muscle retain about the same activity as the total body. The activity in thymus is variable but shows no preferential uptake or retention. Plasma at 6 months is without excess activity, while the red blood cells retain slight activity up to 12 months.

2. In the glycine-exposed animals, the lung at 6 months has a relatively low activity, but subsequently either picks up activity or retains the initially acquired C^{14}.

3. Retention in bone from glycine-2-C^{14} is high at 4 months and the activity is either retained or accumulated up to 17 months. Using 15 mμc of C^{14} per gram of tissue as equivalent to the recommended tolerance 0.3 rep per week, the femur, and often the bones of the thoracic cage, receive radiation in excess of 0.3 rep per week during 17 months. Assuming uniform tissue distribution, an exposure to 33 μc [100 mc man (70 kg) equivalents] would provide femoral exposure in excess of 0.3 rep per week during this period. Retention of C^{14} from $NaHC^{14}O_3$ in both groups of test animals did not exceed 0.3 rep per week beyond 2 weeks when the retention was expressed in terms of uniform bone distribution. The distribution of C^{14} is certainly not uniform. Radioautography may give some clue as to the maximum concentration of C^{14} in localized areas.

4. Retention of C^{14} from glycine at 17 months (0.29 per cent) is about twice the retention from bicarbonate at 2 weeks in the 9-12 week animals. According to the data of others, about 10 per cent of an injected dose of glycine C^{14} is retained at the end of 1 month.

Table 27 gives a comparison of the bone retention of C^{14} from glycine and sodium bicarbonate. Activities for inorganic bone were obtained by measuring the $C^{14}O_2$ evolved from bone with inorganic acid, and the C^{14} from "organic" sources was obtained by collecting the $C^{14}O_2$ obtained by subsequent wet oxidation of the sample. The total C^{14} retention in the femurs of the glycine animals is about tenfold greater during 4 to 17 months after exposure than in the period 0.5 to 10.5 months in the animals which were exposed to bicarbonate when 4 weeks old. After about 12 months, C^{14} in the bones of the thoracic cage and skeletal remainder is approximately the same as that of the bicarbonate animals in the periods beyond 6 months.

TABLE 27

C^{14} RETAINED (mμc/g OF BONE) FROM 100 μc OF GLYCINE-2-C^{14} AND FROM 100 μc $NaHC^{14}O_3$

		Months after exposure:	3.6	6.2	8.5	9.5	12.0	17.0		
Glycine-2-C^{14}	Femur	Inorganic	113.2	87.3	126.9	105.8	55.3	4.2		
		Organic						45.1		
	Thoracic cage	Inorganic	98.5	52.6	32.9	148.1	59.7	3.3		
		Organic						2.1		
	Skeletal remains	Inorganic	—	16.7	34.1	33.1	9.7	3.4		
		Organic						4.2		
		Months after exposure:	0.5	1.0	2.0	2.6	6.4	7.4	8.2	10.8
$NaHC^{14}O_3$ 3.5-5.0-weeks'-old mice	Femur	Inorganic	9.13	4.44	2.13	1.09		3.75		4.20
		Organic	2.59	2.15	4.01	1.78		1.21		0.83
	Thoracic cage	Inorganic	14.37	5.84	5.71	—		1.94		2.73
		Organic						0.76		0.37
	Skeletal remains	Inorganic	14.13	9.15	6.24	12.20		3.58		3.89
		Organic	0.32	0.32	0.08	0.43		0.49		0.47
	Bone lipid		0.00	0.18	0.07	—		0.06		—
$NaHC^{14}O_3$ 9.0-12.0-weeks'-old mice	Femur	Inorganic	2.68	3.02			9.13		4.07	6.60
		Organic	0.00	2.33			0.00		1.29	0.00
	Thoracic cage	Inorganic	0.68*	7.41			3.36		2.12	2.77
		Organic					1.01		0.00	0.76
	Skeletal remains	Inorganic	4.80	5.74	4.46		2.41	4.25		
		Organic	0.23	0.40	0.06		0.12	0.31		
	Bone lipid		0.05	0.25	0.00		0.00	—		

* Probable artefact

While the significance of the findings of high bone retention of C^{14} from glycine is not clear, it is evident that the current assumption is unfounded that skeletal concentration of C^{14} from organic compounds is low as compared to that from $C^{14}O_2$ or $NaHC^{14}O_3$. It is possible that the glycine-retained activity does not represent a static deposit, but rather a continuous loss with replacement from other body deposits which are losing C^{14} at a slow rate. This would indicate a superior metabolic utilization of endogenous C^{14} as opposed to exogenous C^{14}.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson (Leader)

M. I. Dolin
E. F. Phares
F. W. Denison, Jr.*
J. M. Adams*
Mary V. Long
Suzanne H. Berger

Biosynthesis of Kojic Acid

(Denison, Carson)

Summary

Physiological studies on the mold, Aspergillus flavus NRRL 484, showed a zinc requirement for kojic acid production, an iron requirement for growth, and a lowered phosphate concentration for maximum kojic acid production. Conditions were obtained for a kojic acid production higher than that previously reported. It was shown that resting cells of A. flavus metabolizing various sugars have a markedly different metabolic pattern for each sugar. The mold was shown to grow well with kojic acid as the sole source of carbon and to produce an abundance of yellow pigment under these conditions.

Various chemical studies were made on kojic acid, and a degradation scheme was modified to isolate the various individual carbon atoms (except carbons 2 and 3) separately and to determine their specific radioactivities accurately.

Studies were made on the mechanism of kojic acid biosynthesis, using various C^{14}-labeled compounds. Kojic acid was formed from 3, 4-C^{14}-glucose as the substrate; it was also formed from both nonradioactive glucose and xylose, each in the presence of minute amounts of various low molecular weight C^{14} tracers. The data are subject to at least two main interpretations: (1) glucose carbon chain does not cleave before forming kojie acid, the latter arising via dehydrogenation and dehydration of the intact glucose carbon chain; (2) the glucose chain splits into smaller fragments which are subsequently resynthesized to kojic acid. The evidence is more consistent with (1) and indicates that compounds smaller than hexose are first synthesized to a hexose by a reversal of glycolysis, and thence to kojic acid.

* ORINS Predoctoral Fellow

Experimental Methods and Results

Tracer studies were performed with a number of C^{14}-labeled compounds. The following listing (Table 28) gives the amount and the total

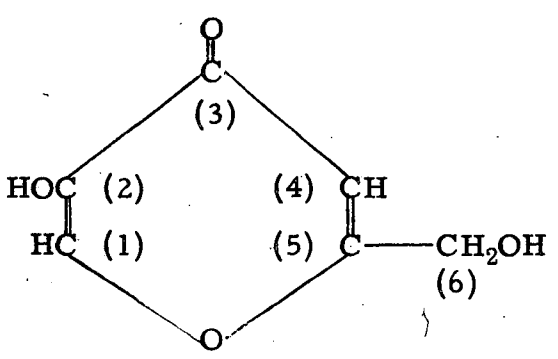
TABLE 28

Substrate	Tracer	Amount of Tracer (mM)	Total activity of tracer* (μc)
Glucose	$C^{14}O_2$	0.2	520
Glucose	$HC^{14}OOH$	0.07	210
Glucose	$CH_3C^{14}OOH$	0.08	250
Glucose	$C^{14}H_3CH_2OH$	0.4	600
Glucose	$HC^{14}HO$	0.4	500
Xylose	$C^{14}O_2$	0.2	500
Xylose	$HC^{14}OOH$	0.2	600
Xylose	$CH_3C^{14}OOH$	0.3	1000
Xylose	$C^{14}H_3COOH$	1.0	1000
Xylose	$C^{14}H_3CH_2OH$	0.4	600
Xylose	$C^{14}H_3OH$	0.12	500
Xylose	$HC^{14}HO$	0.04	500
Xylose	$CH_3C^{14}OCOOH$	1.0	100
Xylose	$COOHC^{14}H_2C^{14}H_2COOH$	0.25	100

* 1 μc = 36,000 counts/minute with the particular counting equipment used.

initial radioactivity of the tracer compounds (except data on 3, 4-C^{14}-glucose which is given in the following section). In each case the tracer was added to 75 ml of regular growth when kojic acid production was first detectable.

To simplify subsequent comparison with glucose (obtained from polysaccharide) the carbon atoms of kojic acid have been numbered as follows:



Kojic acid from 3, 4-C^{14}-glucose as substrate. Two experiments were performed using two different batches of 3, 4-C^{14}-glucose. Each batch represented hydrolyzed glycogen from two pooled rat livers. The glucose was made up to 1 g with carrier and placed in 10 ml of glucose-free regular medium in a 50-ml Erlenmeyer flask. Spores were added and allowed to grow for 14 days in experiment A and 9 days in experiment B. The kojic acid obtained had the same specific radioactivity as the starting glucose, and the labeling of the kojic acid is shown in Table 29. Carbon atoms 2

TABLE 29

RADIOACTIVE KOJIC ACID PRODUCED FROM 3,4-C^{14}-GLUCOSE

Experiment	Percentage of recovered radioactivity in carbon atoms of kojic acid			
	1	2 + 3	4	5 + 6
A*	4.5	41.5	49.5	4.5
	2.4	42.3	49.7	5.3
B	1	46	51	4

* Duplicate degradations

and 3 of kojic acid are indistinguishable since they are obtained as oxalic acid in the kojic acid degradation. In the case of 3, 4-C^{14}-glucose it was unnecessary to degrade glycolic acid containing carbons 5 and 6 of the kojic acid, since it possessed only a small portion of the total radioactivity of the kojic acid.

Kojic acid from nonlabeled glucose in the presence of various C^{14}-labeled tracers. Arnstein and Bentley reported that insignificant amounts of $C^{14}O_2$ and $HC^{14}OOH$ were incorporated into kojic acid when the former were used as tracers in glucose experiments. The present experiments confirm their conclusions. Labeled CO_2, $HC^{14}OOH$ and $C^{14}H_3COOH$ each were used separately as tracers with glucose as substrate; the kojic acid produced had less radioactivity than the minimum desirable for accurate counting.

In similar experiments in which $HC^{14}HO$ and $C^{14}H_3CH_2OH$ functioned as tracers, a sufficient amount of radioactivity was incorporated into the kojic acid to render it suitable for degradation studies. In the case of $HC^{14}HO$, 0.1 per cent of the tracer was incorporated into kojate, and in the case of

$C^{14}H_3CH_2OH$, 0.8 per cent. The relative labeling of the carbons of kojic acid with these tracers is shown in Table 30.

TABLE 30

KOJIC ACID PRODUCED FROM GLUCOSE IN THE PRESENCE OF $HC^{14}HO$ AND OF $C^{14}H_3CH_2OH$ AS TRACERS

Tracer	Percentage of radioactivity recovered in carbon atoms of kojic acid				
	1	2 + 3	4	5	6
$HC^{14}HO$	5.5	24.0	43.5	8.4	18.5
$C^{14}H_3CH_2OH$	4.3	17.5	10.0	36.5	31.5

Kojic acid produced from xylose in the presence of various C^{14}-labeled tracers. If kojic acid were formed from glucose by direct transformation not involving cleavage of the hexose carbon chain, the small incorporation of radioactivity from the various tracers used, into kojic acid would be understandable. Since kojic acid is also formed readily from xylose, a 5-carbon sugar, it seemed probable that a greater incorporation of tracers might be possible when this pentose is used as the substrate. However, it was found that labeled carbon dioxide, formate, and methanol were incorporated into kojate in amounts below the level required for accurate counting. Although $CH_3C^{14}OCOOH$ was incorporated to the extent of about 0.1 per cent of its radioactivity, the specific radioactivity of the $CH_3C^{14}OCOOH$ used as a tracer was so low that the radioactivity of the recovered kojic acid was unusable. Several other tracers were, however, incorporated to a satisfactory extent into kojic acid when xylose was employed as the substrate. The following values represented the portion of available tracer which was incorporated into kojate produced from xylose: $HC^{14}HO$, 0.13 per cent; $CH_3C^{14}OOH$ and $C^{14}H_3COOH$, 0.3 per cent each; $C^{14}H_3CH_2OH$, 2.8 per cent; and $COOHC^{14}H_2C^{14}H_2COOH$, 0.5 per cent. The kojic acid produced in each of these experiments was degraded and the relative labeling is shown in Table 31. Several of the degradations were repeated with very close checks.

Table 32 compares the per cent of the total radioactivity of kojic acid found in positions 1, 2, and 3 with that in positions 4, 5, and 6. In every case the latter carbons contain more than twice the radioactivity of the former.

TABLE 31

DISTRIBUTION OF RADIOACTIVITY IN KOJIC ACID PRODUCED FROM XYLOSE IN THE PRESENCE OF VARIOUS C^{14} TRACERS

Tracer	Percentage of distribution of radioactivity of kojic acid in carbon atoms				
	1	2 + 3	4	5	6
$HC^{14}HO$	12	19	36	3	29
$CH_3C^{14}OOH$	4	27	62	7	
$C^{14}H_3COOH$	8.7	20.5	13.2	33	25
$C^{14}H_3CH_2OH$	12.7	18.9	11.4	31.5	25.5
$COOHC^{14}H_2C^{14}H_2COOH$	8.6	14.6	12.3	37.3	27.0

TABLE 32

RELATIVE RADIOACTIVITIES OF THE TWO HALVES OF THE KOJIC ACID MOLECULE PRODUCED IN THE PRESENCE OF VARIOUS TRACERS

Substrate	Tracer	Percentage of total radioactivity in carbon atoms of kojic acid	
		1 + 2 + 3	4 + 5 + 6
Glucose	$HC^{14}HO$	29	71
Glucose	$C^{14}H_3CH_2OH$	22	78
Xylose	$HC^{14}HO$	31	69
Xylose	$CH_3C^{14}OOH$	31	69
Xylose	$C^{14}H_3COOH$	29	71
Xylose	$C^{14}H_3CH_2OH$	31	69
Xylose	$COOHC^{14}H_2C^{14}H_2COOH$	23	77

Glucose fraction of polysaccharide synthesized in glucose media in the presence of various C^{14} tracers. The glucose obtained by hydrolysis of the polysaccharide formed during growth of the fungus in a glucose medium containing $CH_3C^{14}OOH$ and $C^{14}H_3CH_2OH$ was relatively low in specific radioactivity. However, large samples were taken for each determination and all C^{14} measurements were made with the vibrating reed electrometer. Thus, even the specimen containing the lowest radioactivity showed a count in this instrument which was eight times higher than the background count.

Table 33 compares the radioactivities of the various carbons in polysac-

TABLE 33

COMPARISON OF THE LABELING OF GLUCOSE (FROM POLYSACCHARIDE) WITH LABELING OF KOJIC ACID, BOTH PRODUCED IN THE PRESENCE OF THE SAME TRACERS

Compound degraded	Tracer	Percentage of radioactivity in carbons							
		1	2	3	1 + 2 + 3	4	5	6	4 + 5 + 6
Glucose	$C^{14}H_3CH_2OH$	14	19	7	40	8	27	25	60
Kojic	$C^{14}H_3CH_2OH$	12	19		31	11	32	26	69
Glucose	$CH_3C^{14}OOH$	6	10	25	41	47	6	6	59
Kojic	$CH_3C^{14}OOH$	4	27		31	62	3	4	69

charide glucose with those of the corresponding carbons in kojic acid produced in the presence of the same tracers. Irrespective of the tracer used the polysaccharide glucose contained approximately 60 per cent of its radioactivity in carbons 4, 5, and 6, compared to approximately 70 per cent in the corresponding carbons of kojic acid.

BIOCHEMISTRY

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

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

Studies on Nucleic Acids and Enzymes

(Cohn, Volkin, Jones)

As stated briefly in the previous report, we have been able recently to isolate many of the products of the ribonucleic acid-ribonuclease digest. This has afforded us an opportunity to postulate a theory for the specificity of ribonuclease action and, at the same time, to define some of the actual nucleotide sequences as they exist in ribonucleic acid (RNA).

Ribonuclease hydrolysis liberates about 60 per cent of the pyrimidines as mononucleotides while the remaining pyrimidines and all the purines are released as more complex polynucleotides. With the use of a low cross-linked anion-exchange resin of fine mesh size, we have had considerable success in isolating these various polynucleotide fragments. The identification of the polynucleotides was carried out as indicated in Fig. 6. If

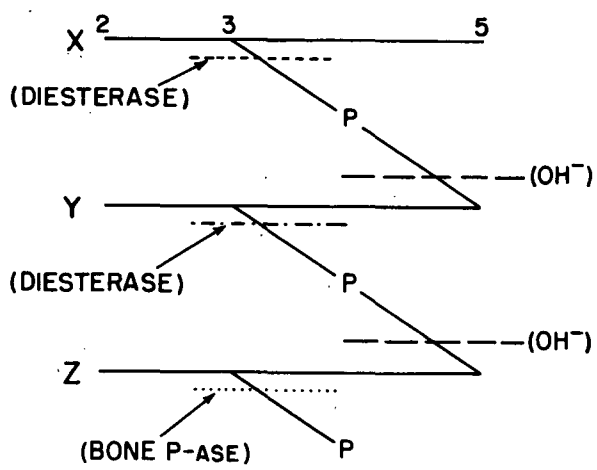


FIG. 6

we consider a hypothetical trinucleotide (XYZ), alkaline hydrolysis will hydrolyze as indicated and, on subsequent ion-exchange analysis, the nucleotide composition can be obtained. X and Y will appear as a and b nucleotides; Z, as a or b only. If instead, the trinucleotide is digested with an end-group phosphatase, such as bone phosphatase, then hydrolyzed with alkali, the terminal position (Z) will be obtained as the nucleoside, X and Y again appearing as a and b nucleotides. On the other hand, if bone phosphatase action is followed by diesterase hydrolysis, the other terminal position (X) will be removed as the nucleoside, while X and Z will appear as 5' nucleotides. Thus di- and trinucleotides are amenable, as far as their detailed structures are concerned, to this approach.

We have applied ion-exchange separation to ribonuclease-hydrolyzed yeast and calf-liver RNA; Fig. 7 shows the ion-exchange elution curve for the digested yeast RNA. In this figure, optical density at 260 mμ is plotted against volume of eluant, the eluant consisting of increasing concentrations of chloride ion. The dashed lines represent the 280/260 optical ratios, their constancy being a measure of purity. The fractions thus far identified are labeled with the appropriate nucleotide abbreviations (C = cytidylic, U = uridylic, A = adenylic, G = guanylic) and are shown above the corresponding elution peak. Fractions shown in parentheses have been only tentatively identified thus far.

When this type of experiment was carried out with calf-liver RNA, a nucleic acid of quite different nucleotide composition from yeast RNA, it became evident that there was no qualitative difference between the two elution patterns. Thus, the difference between these two nucleic acids of widely different sources resides in a quantitative variance of certain specific nucleotide sequences.

There are certain general features about the polynucleotide products thus far identified which warrant consideration. First, it should be stated that, in all cases, the end group containing the terminal phosphorus is always a pyrimidine nucleotide. Second, all these end-group pyrimidine nucleotides are of the b type; thus, alkaline hydrolysis of the polynucleotides yields these only as the b nucleotides, but both a and b isomers of the other moities. The mononucleotides, cytidylic and uridylic acids, obtained in these digests, are likewise only the b types. From this data, the specificity of the enxyme, ribonuclease, apparently can be established. Thus all the products are compatible with a pyrimidine specificity such as is shown in Fig. 8. As indicated in the figure, the enzyme is required to hydrolyze the phosphoryl linkage distal to the pyrimidine b linkage. Thus, hydrolysis at the positions indicated would yield such polynucleotides as we have encountered, and likewise would yield pyrimidine mononucleotides where these occur adjacent in the molecule.

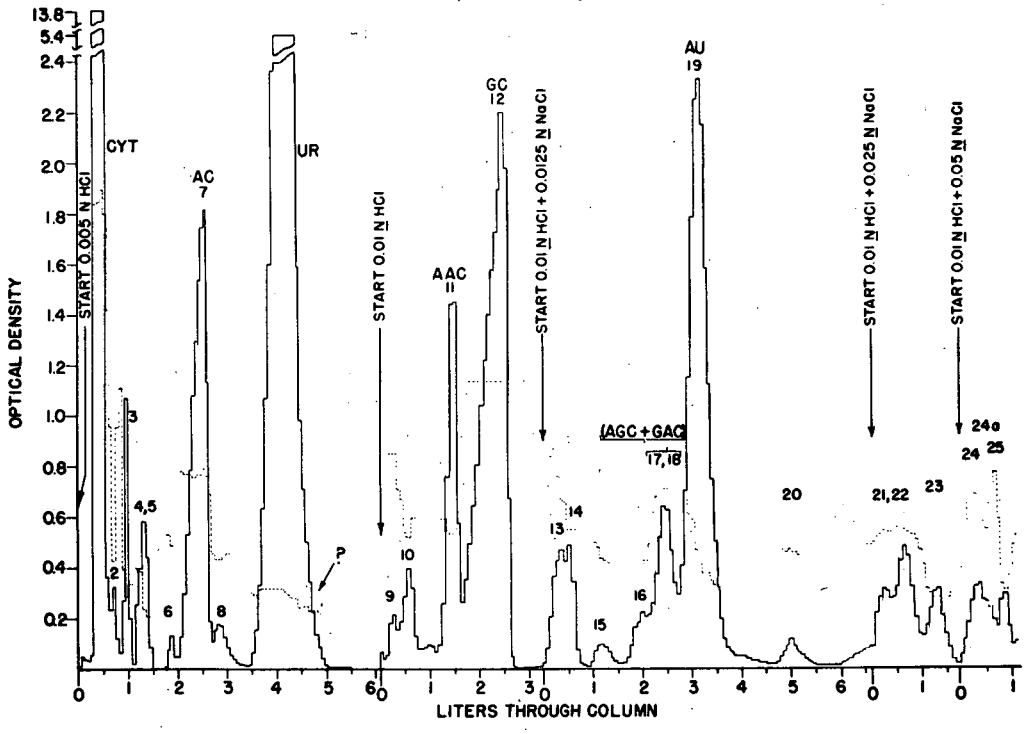


FIG. 7a. Separation of acid-soluble components of ribonuclease-digested yeast ribonucleic acid on column 3.7 sq cm x 16 cm of 400 mesh, 2% cross-linked Dowex-1 resin; flow rate 80 ml/hour.

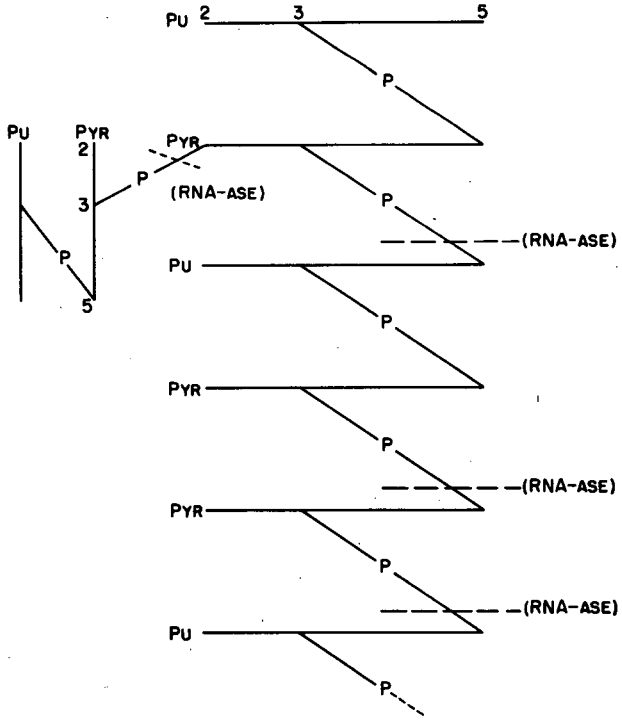


FIG. 8

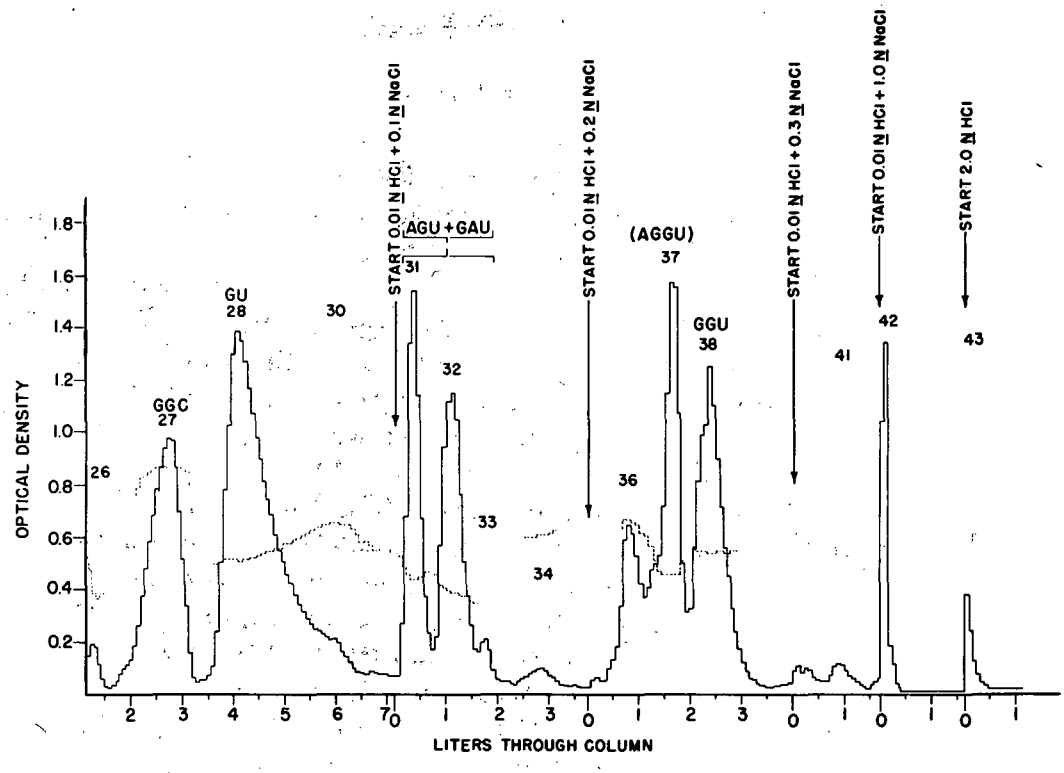


FIG. 7b

Table 34 shows the nucleotide recovery in all fractions which have thus far been identified. With both yeast and calf-liver RNA, this recovery amounts to about 60-65 per cent of the total.

TABLE 34

	Yeast μM				Liver μM			
Fractions	A	G	C	U	A	G	C	U
Intact RNA	366	654	494	506	378	837	680	396
C			282				405	
U				286				175
AC	33		33		33		33	
AAC	18		9		14		7	
GC		59	59			110	110	
(AGC, GAC)	27	27	27		25	25	25	
GU	18			18	17			17
(AGU, GAU)		44	22			96	48	
AGGU	13	26		13	13	26		13
GGU		36		18		36		18
μM recovered	136	267	432	410	120	351	628	281
Percentage recovered	37	41	88	73	32	42	92	71

Nucleotide Studies

(Cheniae, Doherty)

Attempts have been made to substitute the hydroxyl groups of the ribose moiety in nucleotides and nucleic acid under conditions which would not permit isomerization and with a group which would not migrate on subsequent degradation. Adenylic acid "b" was treated in a nonaqueous system with toluene, benzene, methane, and ethane sulfonylchlorides. Each of the above reactions yielded a mixture of products which have been difficult to separate. Ion-exchange procedures have been the most promising and some separation on a small scale has been obtained. Larger scale runs are in preparation so that sufficient quantities of the products can be isolated for identification and analysis. Calf-liver ribonucleic acid (RNA) was treated with methane sulfonylchloride and a sulfonated product obtained in good yield. Ultracentrifugal studies revealed no degradation of the nucleic acid. Hydrolysis of this preparation should give some chemical evidence as to the nature of the internucleotide linkage and also the position of the phosphate group on the ribose chain. Classic methylation of calf liver RNA with silver oxide and methyl iodide gave a small yield of methylated product. Ultracentrifugal analysis of this product showed that the methylation process was accompanied by extensive degradation, as no intact nucleic acid remained.

Enzyme Substrate Equilibria

(Doherty, Vaslow)

This study has been continued by preparing compounds with varying charges on different parts of the molecule and measuring the thermodynamic properties of their binding on the chymotrypsin molecule. This has involved the preparation of p-methoxy-N-acetyl-3, 5-dibromo-L-tyrosine, p-nitro-N-acetyl-3,5-dibromo-L-phenylalanine, p-amino-α, N-acetyl-3,5-dibromo-L-phenylalanine. The thermodynamic properties of two of these compounds have been measured. The first compound with the hydrogen atom of the phenolic hydroxyl replaced by a methyl group to give an uncharged ether was bound about 50 per cent stronger than acetyl-L-dibromotyrosine as shown by a more negative ΔH. The other catalytic properties were similar to those of the original substrate. This increased binding may be explained by the decreased attraction of the methyl ether for water compared to the phenolic hydroxyl group. The second compound where the phenolic hydroxyl was replaced by an amino group — p-amino-α, N-acetyl-L-phenylalanine — gave binding properties at 5° C that were very similar to those of the original substrate, indicating that a charge in the para position is of importance in the binding to obtain catalytic properties.

A Direct Ion-Exchange Method for the Quantitative Analysis of Some Biologically Important Phosphate Esters

(Khym)

Techniques for the analysis of sugars through their borate complexes on ion-exchange resins have been previously described. This report concerns an extension of this method developed for the identification of some phosphate esters. Glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, ribose-5-phosphate, and inorganic phosphate were quantitatively analyzed by the procedure developed.

The exchanger used was Dowex-1 in the chloride form. The test material in a small volume at pH ~ 8.5 was adsorbed on the resin. Then selective deadsorption of the phosphate esters was accomplished by varying the composition of the eluting solution as shown in Fig. 9. The concen-

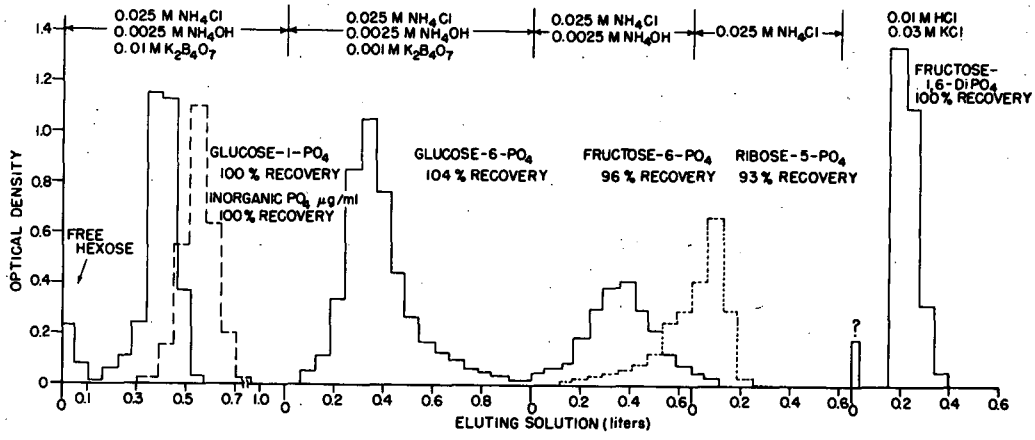


FIG. 9. Separation of phosphate ester
Exchanger: Dowex-1, 0.86 sq cm x 11 cm, chloride form, 300 mesh
Rate: 2.4 ml/minute
Test material: ~ 10 mg each of phosphate ester as hexose - 0.04 mM of K_2HPO_4 in 10-ml volume at pH~8.5.
Ordinate values: —— = anthrone test at 620 mμ.
--- = inorganic phosphate at 660 mμ (ordinate x 10 - μg/ml).
----- = orcinol test at 660 mμ.

trations of the components eluted were determined by standard colorimetric procedures (three tests: hexose, pentose, inorganic phosphate) specific enough in these instances so that the overlapping of glucose-1-phosphate with inorganic phosphate and fructose-6-phosphate with ribose-5-phosphate is not a factor in quantitative recovery.

If borate is not present, all the phosphate esters are eluted in the same position as that shown for glucose-1-phosphate using 0.025 M NH_4Cl + 0.0025 M NH_4OH as the eluting solution. Glucose-1-phosphate does not form a strong borate complex, hence is unaffected by borate concentration. Glucose-6-phosphate has a pair of cis hydroxyls available on carbons 1 and 2; hence the borate concentration is a factor in its elution position. Fructose-6-phosphate and ribose-5-phosphate have cis hydroxyls available, and moreover these compounds exist in a fixed furanose ring form which lends to their strong affinity for the exchanger. The phosphate ester fructose-1, 6-diphosphate has cis hydroxyls, furanose ring form, and two phosphate groupings which explain its very strong affinity for the exchanger.

Elution order has been determined thus far by individual column runs and by material balance based on intensity of color developed per unit weight of diphosphate esters at a given wave length.

Biological Energy-Transfer Systems

(Strehler, Brigham)

ATP Changes During Photosynthesis

Using the sensitive firefly assay system, an intensive study has been made of the effect of light on the ATP (adenosinetriphosphate) level of green plants.

In general the results obtained indicate that light has a great effect on the level of ATP in plants and strongly suggests that ATP is involved in the transformation of the split products of water into reducing material at the carbohydrate level. Stated briefly, it appears likely that (1) part of the reducing material produced by the action of light on plants is reoxidized, (2) the energy liberated by this reaction is converted into phosphate bond energy which then (3) is used to raise the potential of the remaining reductant to the potential of carbohydrate (DPN). The evidence on which this mechanism is postulated is as follows. Following a period of dark anaerobiosis, the time course of ATP on the admission of air in the dark and on illumination anaerobically is illustrated in Fig. 10. It should be noted that the time course in the presence of light shows an induction phenomenon reminescent of the time course of the luminescence of the plants themselves

and also of photosynthesis and fluorescence, while aeration in the dark not only shows no such effect but actually goes to a steady state ATP level some 60 per cent higher than that attainable with light. In conjunction with Fig. 11, in which the steady-state level of ATP at different light intensities is illustrated, this evidence indicates that light not only forms ATP but also promotes its utilization. In Fig. 12, the effect of light and subsequent dark in the absence of CO_2 is illustrated. It should be noted that a rapid increase in the level of ATP is manifest immediately after the light is turned off, indicating perhaps that the light-driven system suppresses the ATP level by furnishing reductant which not only forms ATP but utilizes it in further reactions. Further evidence indicates that the rate of ATP formation following dark anaerobiosis, saturates with increasing light intensity (Fig. 13.)

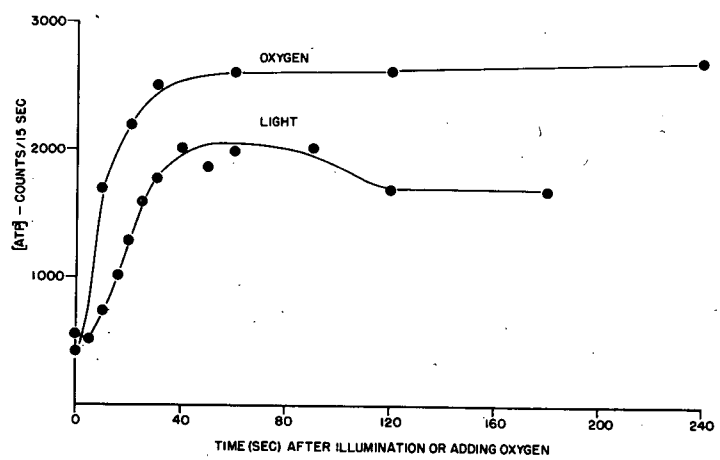


FIG. 10. Effect of light and oxygen addition on ATP level in plants kept anaerobic for 2-3 hours.

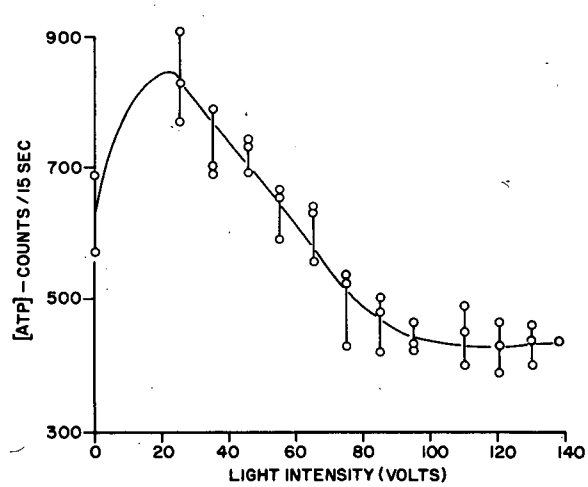


FIG. 11. Light intensity versus ATP - 10 minutes - carbon dioxide present.

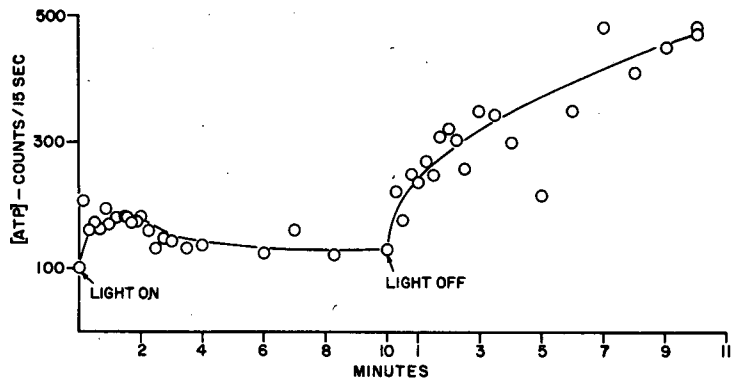


FIG. 12. Effect of light and dark without carbon dioxide.

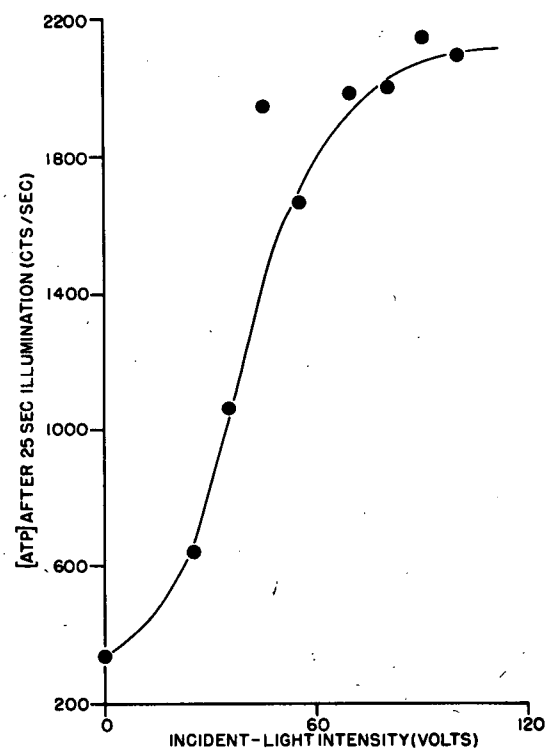


FIG. 13. Effect of light intensity on rate of ATP formation.

Studies on the Formate Pigeon Bone Marrow System

(J. R. Totter, Consultant)

Work on the formate pigeon bone marrow system has been continued. Known protective agents including malononitrile, mecholyl chloride, cysteine, and saline solution have been tested for their effect on formate uptake. Malononitrile and mecholyl chloride gave results which indicate a protective action on the formate-utilizing enzymes. Surprisingly, cysteine appeared to have the opposite effect.

The studies to date appear to demonstrate conclusively that whole-body X irradiation of pigeons immediately affects formate uptake into nucleic acids and proteins. The results are sufficiently uniform that a survey of protective agents using two birds per test should give reasonably reliable results.

Table 35 shows results obtained since the last quarterly report.

TABLE 35

UPTAKE OF RADIOFORMATE BY BONE MARROW SUSPENDED IN SALINE

(Given as percentage of controls)

	Supernatant	Phospholipids	Nucleic acids	Proteins
Average controls	100	100	100	100
Average X-rayed	51	83.5	37.5	19.2
X-rayed + 20 mg malononitrile	84.7	101	48.5	65.5
X-rayed + 0.5 g betaine	75	70	74	45
X-rayed + 18 mg mecholyl chloride	104	122	58.5	78.5
X-rayed + saline (1 ml of 0.9%)	62	105	40	47.1
X-rayed + 0.5 g cysteine	41	17.7	20	7.9

PLANT PHYSIOLOGY

G. R. Noggle (Leader)
L. P. Zill Eleanor Schumacher
M. A. Chambers* H. Jonas**

Separation of Labeled Sugars by Ion Exchange

(Noggle, Schumacher)

When separating and identifying labeled sugars from the ion-exchange columns, it is essential to have a simple method for determining the radioactivity of each collected fraction. It has been our practice to pipette 1-ml aliquots into small aluminum discs and, after drying the samples in an oven, to count them with a methane-flow proportional counter. It seemed desirable to run some standards to test the accuracy of this procedure.

Two C^{14}-labeled sucrose samples were made up to known concentrations and 1-ml aliquots were pipetted into aluminum discs (area, 5.725 sq cm). The samples were dried in an oven and then counted in a methane-flow proportional counter. The results, shown in Fig. 14, indicate that, at the levels of sugar concentration used, the samples are infinitely thin and the

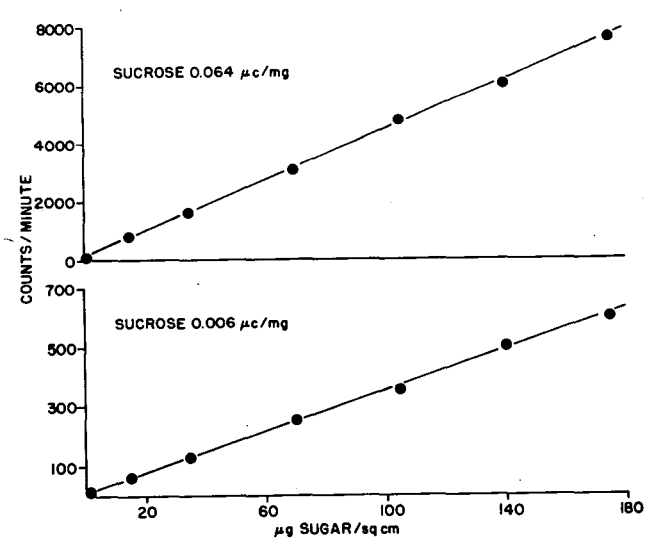


FIG. 14

* Research Participant
** AEC-NRC Postdoctoral Fellow

counts are directly proportional to the concentration of sugar. From the data found with the ion-exchange columns, the concentrations of sugar to be counted will fall in the same range as indicated in Fig. 14 and no correction need be made for self adsorption.

Ion-Exchange Separation of Sugars

(Zill)

Work which has been carried out on this project during the last quarter has been directed toward the conclusion of experimental work concerning the fundamental aspects of the method. It is felt that the establishment of the conditions necessary for the separation of sugar mixtures likely to be encountered by various investigators can be effectively predicted on the basis of the work already presented.

In order to determine the maximum pH range over which sugars could be separated, several runs were made with eluting solutions ranging from boric acid through boric acid-tetraborate buffers to tetraborate solutions, the last of which has been the most common eluting agent thus far. Comparison of the results from the various runs demonstrates that the volume-to-peak values are shifted differently for different sugars; therefore this provides another condition which may be varied in order to effect the separation of certain sugars. On the basis of these results it was found possible to separate turanose from fructose by means of elution with a boric acid solution. Such a separation was necessary in order to use the method for the preparative isolation of turanose from melezitose.

The removal of borate from the isolated sugars (ORNL-1167) is being studied further to determine the extent of removal and optimum conditions for removal. Ordinary chemical methods of analysis are not applicable to the determination of boron in the isolated sugars; therefore, spectrographic analysis will be used to determine any boron remaining in the samples. Removal of borate as determined by analysis of the distillate obtained in the method mentioned demonstrates that borate is removed very rapidly. Whether borate removal is quantitative cannot be stated until the spectrographic analyses have been completed.

The Behavior of Glycosides on Anion-Exchange Columns

(Zill, Chambers)

Since a glycoside contains a sugar residue as part of its molecule it was of interest to determine whether such compounds might be separable

by the borate technique in a manner similar to that of the sugars. The chromatography of salicin, arbutin, and a saponin (Quillaja saponaria) on a strong-base anion-exchange column demonstrated that these compounds were retained by such a column and could subsequently be eluted with borate solutions. Digitoxin and gitoxin were not retained under such conditions. Inspection of the structural formulas of the glycosides which were retained by the column indicated that, if a borate complex were formed, it would probably be a weekly ionized complex such as that formed by sucrose. However, the three glycosides studied were found to be retained by the column much more firmly than could be accounted for on this basis. In order to determine whether a nonexchange type adsorption was taking place, two synthetic glycosides were studied. Methyl-β-D-arabinopyranoside was found to be eluted very easily from a column in the borate form while benzyl-β-D-arabinopyranoside was found to be very strongly retained by the column. It thus appears that the presence of an aromatic moiety in the glycoside molecule contributes to the retention of the molecule by an anion-exchange column by a nonexchange type adsorption. That such an adsorption may play a useful role in the analysis and isolation of certain glycosides has been demonstrated by the assay of arbutin in bearberry leaves by this method (M. A. Chambers, unpublished work).

A complete and detailed report concerning this work has been submitted for publication.

Some Effects of the Concentration of Potassium Bicarbonate and 2, 4-D on the Uptake of K^{42} by Sunflowers as Determined by a Localizing Scintillation Count-rate Meter

(Jonas)

A negative linear proportionality exists between the fresh weight of a plant and the osmotic pressure of its nutrient solution (Magistad, et al., Plant Physiol., 18:151, 1943). There is also evidence that the rate of ion uptake by various plants is independent of the ionic concentration, (Olsen, Lab. Carlsberg, Sér. chim., 27:291, 1950; Broyer and Overstreet, Am. J. Botany, 27:425, 1940). It was the purpose of this series of experiments to follow the pattern of K^{42} uptake from solutions of $K^{42}HCO_3$ by young sunflower plants during several K^{42} half lives. For this, a recording and localizing scintillation count-rate meter was used. An analysis of the time sequence of the number of disintegrations of K^{42} in a particular tissue location should yield evidence about the dependence of potassium uptake on its osmotic pressure in the nutrient solution and on the presence of stimulants and inhibitors in it.

Sunflowers were grown in vermiculite with #2 Hoagland's solution of an osmotic pressure of 0.77 atmospheres. When the fourth pair of leaves was sufficiently large, a series of plants was transferred to a concentration series of potassium bicarbonate of 0.3-28.2 atmospheres osmotic pressure, each containing 43.4 mg of $K^{42}HCO_3$ from a 16-hour pile bombardment. The scintillation crystal was placed 1.6 mm above the lower quarter of the center vein of a leaf of the fourth pair, and the isotope uptake was recorded.

The relation between period of uptake and total amount of potassium in the leaf is composed of three functions: (1) the initial fast active accumulation, (2) the period of salt saturation, and (3) the final period of salt accumulation by the dominance of ion exchange. The initial rate of accumulation varies inversely with the salt concentration and extends for not more than 200 minutes. The mid-point of the period of saturation occurs around 530 minutes in all cases. The final period of salt accumulation commences abruptly around 1000 minutes and coincides with the appearance of salt damage on the leaves.

A second series of plants was transferred to $K^{42}HCO_3$ solutions of 0.8 atmospheres osmotic pressure with additions of 2,4-D. Typical 2,4-D contortions were observed at concentrations of 15 and 30 ppm and wilting at lower ones. The uptake of K^{42} seems to have been increased by the presence of 30 ppm of 2,4-D, but decreased by 7.5 ppm.

BIOPHYSICS

C. W. Sheppard (Leader)
J. S. Kirby-Smith E. B. Darden, Jr.
N. G. Anderson* D. S. Daniels
K. M. Wilbur** Patty Jean Mathias

GENERAL PHYSIOLOGY

Effect of X Radiation on the Bioelectric Response in Dionaea muscipula

(Darden)

Preliminary experiments indicate that the characteristic bioelectric transient potential obtained when an appropriate stimulus is applied to the excitable tissue of Venus's flytrap (Dionaea muscipula) is considerably affected by exposure to X rays. In some cases an apparent lowering of the excitation threshold was observed; in others a reduction in size and change of shape of the wave-form appeared to characterize the response after irradiation.

Erythrocytes

(Mathias, Sheppard)

By the use of the rectus abdominis procedure, aided by electromechanical recording and ion-exchange purification of extracts, discussed in previous reports, we have established the production in human erythrocytes of a substance which is pharmacologically identical with acetylcholine, as shown by the following properties.

1. It is produced only in the presence of a cholinesterase inhibitor.

2. Its effect on the frog muscle is greatly attenuated by atropine.

3. It is readily hydrolyzed by heat at alkaline pH.

4. The contraction curve of the muscle is identical with that of acetylcholine, and also the muscle relaxation shows no detectable difference.

* AEC Postdoctoral Fellow

** Research Participant

5. It is not eliminated by ether extraction.

6. It is not readily hydrolyzed by strong trichloroacetic acid.

Experiments are now in progress to investigate whether this finding is a true example of a natural process, and whether there are optimum conditions, such as the best concentration of cholinesterase inhibitor, time, and temperature of equilibration.

Ionizing Radiation on Rat-Liver Homogenates

(Anderson, Wilbur)

An attempt has been made to study the distribution of protein, nucleic acids, and acid-soluble nucleotides between the sedimentable and unsedimentable phases of rat-liver homogenates after high intensity γ irradiation (10 minutes exposure, 170,000 r, Co^{60}). Homogenates were made in a phosphate-bicarbonate buffer containing sucrose at pH 7.1, and were held at 0° C throughout preparation and irradiation. Sedimentable and nonsedimentable fractions were separated by 20 minutes centrifugation at 21,000 x g.

It has been found necessary to modify both the Ogur-Rosen and Schneider methods for extracting nucleic acids because of large losses of ribonucleic acid in certain steps of the procedures. Further studies on analytical techniques adapted to rat-liver homogenates are in progress.

Comparison of the Effects of X rays, γ rays, and β rays on *Tradescantia*

(Kirby-Smith, Daniels)

Experimental work on the comparisons of these radiations is essentially complete and an analysis of the data is in progress. The results of five independent experiments scored to date consistently show X rays to be approximately twice as effective as Co^{60} γ rays or P^{32} β rays in producing aberrations in *Tradescantia* pollen-tube chromosomes. A recently completed study of the relative effects of 250 kvp X rays and Co^{60} γ rays on *Tradescantia* microspores also shows a considerably greater dependence of aberration frequency on wave length than has been reported previously in the literature. It appears highly unlikely that any possible errors in dosimetry in the present work are sufficient to explain these findings.

A relation between isochromatid breaks and dosage of the form $y = aX + bX^2$ has been repeatedly observed in the pollen-tube divisions. This is in general agreement with some results of Catcheside, Lea, and Thoday on Tradescantia microspores.

Effects of Deuterium Oxide on Aberration Frequency

(Kirby-Smith, Daniels)

An exploratory investigation of the effects of deuterium oxide on the frequency of X-ray-induced chromosomal aberrations in onion root tips has been completed. Material treated with deuterium oxide for 2 hours prior to and during irradiation and controls treated similarly with water show no difference in the frequency of chromosome deletions. The frequency of exchanges in the deuterium oxide-treated material was 50 per cent less than that for the control material.

Radiological Physics

Gamma Radiation Work

(Darden, Sheppard)

A low-intensity Co^{60} distributed type source made up originally for use in this Division has been turned over to USDA Experiment Station at Kerrville, Texas, for use in connection with their screwworm population control studies. The Kerrville representative was instructed in the operation of the source, and for their information, Victoreen calibration plot was made of the dose intensity on and off the axis.

Beta-Ray Depth Dose Studies

(Darden)

The results of these measurements show that the energy adsorbed within thin sheets of low atomic number plastic material may be expressed by the following empirical formula

$$\log_e I/I_0 = 9.50\,t + 1.45\,t^2 - 8.80\,t^3$$

in which the dose rate of I after passing through t grams per square cm of intervening material is expressed as a fraction of the dose rate I_0 at the

surface of the absorber. Because of the uniformity of results in various plastics containing 8 weight atoms, particularly C, H, and O, the relation should hold accurately for tissue also. The unexpected agreement has prompted an investigation of the possibility of explaining the adsorption of β-ray energies in tissue in teams of fundamental parameters such as the transport mean free path of electrons in a given material.

Energy Distribution of P^{32} Beta Rays

(Darden, Kirby-Smith)

Recent results on the wave-length dependence of aberration frequency in Tradescantia have clearly pointed out the need for a more precise knowledge of the energy distribution in the Biology laboratory radiation sources. A scintillation spectrometer to obtain such information has been ordered and is under construction in the Instrument Department. Arrangements have been made with P. R. Bell of the Physics Division to have a scintillation spectrographic analysis run on the energy distribution of the electrons emitted by P^{32} bakelite sources. For this purpose, reduced-size plaques have been specially activated in the pile to extremely low intensities.

Photosynthesis

(Arnold, Thompson)

Together with Strehler (Biochemistry) we have shown that green plants have, in addition to their well-known fluorescence, a phosphorescence that lasts for some seconds. We have shown that this delayed light is closely connected with the process of photosynthesis. The assumption that the phosphorescence came from chlorophyll was stated in our publication (Strehler and Arnold, J. Gen. Physiol., 34:809-820, 1951). At that time we were unable to determine the emission spectrum of the delayed light because of its very low intensity.

During the last 4 months, with the help of Mr. Davidson of the instrument section, we have devised a red-sensitive light detector, consisting of CV-148 image tube and a 5819 photomultiplier, all at the temperature of dry ice. This new detector, when used with the Farrand 4 monochromator, now enables us to make emission spectra of the phosphorescence.

The data for Chlorella are given in Fig. 15. Curve B shows the intensity of the phosphorescent light as a function of the wave length; curve A gives the intensity of the fluorescence as excited by blue light. Both

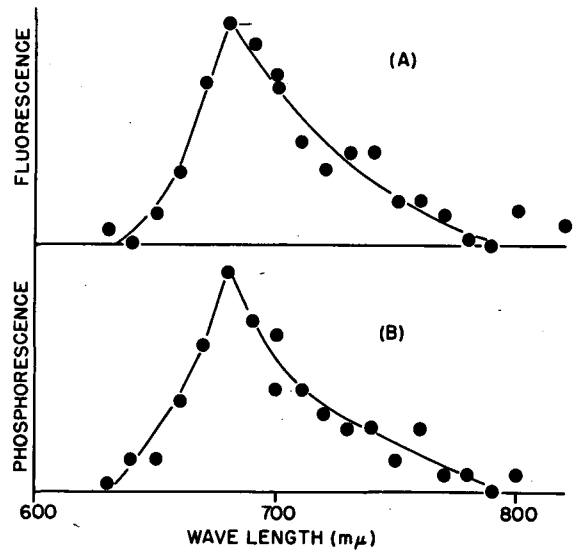


FIG. 15

determinations were made with the same slit width (0.5 mm); the intensity of blue exciting light was adjusted to give approximately equal signals. The two curves are so similar as to leave little doubt that our assumption of emission of delayed light by chlorophyll was justified.

At the moment, we are extending the observation to blue-green and red algae and to the purple bacteria.