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BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT

for Period Ending May 15, 1950

Alexander Hollaender, Director

DATE ISSUED: JUL 7 1950

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INTRODUCTION

Review of research progress. The study of the effect of oxygen tension on the X-ray sensitivity of living cells has continued to occupy the interest of several groups in the Cytogenetics Section. Increasing the pressure during the irradiation has increased the effectivity of oxygen in sensitizing chromosomes of *Tradescantia* inflorescences to X rays. The pressure relationship is almost linear. In an attempt to find whether there is an indirect effect, female *Drosophila* flies were X-irradiated in pure oxygen before insemination. These irradiated females were immediately permitted to mate with nonirradiated males in an effort to detect any residual agent in the female that might affect the sperm, which lies in the female for a considerable time before use in fertilization. No effect was found. A more detailed discussion of the effect of oxygen tension on rate of mitosis in the grasshopper neuroblast is given, in which it is shown that irradiation of cells in higher concentrations of oxygen result in greater mitotic inhibition. Bacteria maintain their excellent standing as material for studying oxygen effect. *Escherichia coli* grown and irradiated anaerobically showed extreme resistance to X rays, far outside the usual range. The same bacteria grown anaerobically and irradiated aerobically showed a threshold before responding to X rays. It was mentioned previously that amino acids added to the irradiation medium have a protective action against X rays. Prompted by this finding, a number of amino acids were tested. Glutamic acid and cysteine demonstrated high protective power, the latter being much more effective than the former. This clue is now being investigated systematically.

The studies on the sensitivity of chromosomes to different types of particulate radiation are pursued intensively. The present work deals with the effect of alpha particles on *Tradescantia* chromosomes.

Mammalian artificial insemination technique has been developed to the point of about 70 per cent efficiency, thus providing an excellent tool for studying different problems connected with radiation effect. The effect of X rays on embryological development has been extended to lower energy levels. It appears that energy levels of X rays which are not uncommon in examination of pregnant women show highly significant effects on embryonic development in mice.

The study of the mechanism and formation of 4-carbon compounds has been continued with interesting results.

The new techniques developed by the Biochemistry group are yielding consequential data in regard to new breakdown products of ribose nucleic acids, interesting information being derived also from the identification of these products. Following this study of ribose nucleic acids, a similar one has been initiated with desoxyribonucleic acid, including investigations on the structure of the desoxyribonucleotides. Characteristics of ribonucleic acids from different sources are under study.

Cooperative work on gold colloids in the Radiobiochemistry and Pathology groups is now reaching the publication stage. Achievements in this effort are being put on a very substantial basis for further studies and possible use for treatments. The mode and action of manganese in biological systems are being probed. The liver seems to be a distribution center for manganese that is utilized by other tissues, as well as being the principal organ for ridding the body of an excess of the element.

Further techniques for the isolation and purification of C-14-labeled sugars have been developed and are described in a paper now in review for publication. Radiosugars (C^{14}) have been made available to the Isotope Division for sale.

As mentioned in the previous quarterly report, the exposure of mice to slow neutrons has given striking results in regard to cataract formation and induction of leukemias. The percentage of cataract formation after six months is 90 per cent. A much higher rate of leukemias has been observed than in the mice irradiated with equivalent intensities of X rays. Blood-volume studies constitute a very important supplement to the neutron studies because they serve as tools for detecting radiation effects at a fairly early period. Several of these projects were studied in cooperation with the Radiobiochemistry group.

The third annual Biology Conference was held in Oak Ridge on April 13-15. Approximately 130 out-of-town visitors came from many states; several visitors were from Southern Universities. This symposium gave an excellent opportunity for discussion of the present status of nucleic acid chemistry. Not the least

part of the success of the conference was attributable to the excellent cooperation received from all those involved in the management of this meeting, at Oak Ridge National Laboratory as well as A.E.C. It is planned to publish the proceedings in book form. The program follows.

Thursday morning, April 13

Chairman, Charles E. Carter

Erwin Chargaff	Composition and Structure of Nucleic Acids
Herbert S. Loring	Absorption Spectrophotometry in the Analysis of Purine and Pyrimidine Components of Ribonucleic Acid
A. M. Michelson	Organic Chemistry of Nucleic Acids

Thursday afternoon

Chairman, George H. Hitchings

Gerhard Schmidt	Enzymatic Degradation of Nucleic Acids
John M. Buchanan	Biosynthesis of Purines
George B. Brown	Precursors of Nucleic Acid
Dwight B. M. Scott (and Seymour Cohen)	Origin and Metabolism of Ribose

Friday morning, April 14

Chairman, Daniel Mazia

Arthur W. Pollister	Nucleic Acid Content of Nuclei
Alfred E. Mirsky	Some Aspects of the Chemistry of Chromosomes
Berwind P. Kaufmann (and M. R. McDonald)	The Distribution and Interrelation of Nucleic Acids in Fixed Cells as Shown by Enzymatic Hydrolysis

Friday afternoon

Chairman, Jackson W. Foster

Helen R. Skeggs	Microbiological Systems Involving Nucleic Acid Derivatives
William Shive	Some Functions of B Vitamins and the Synthesis of Purines and Pyrimidines

Traveling seminars and scientific society lectures. A very busy quarter has produced a total of fifty-one seminars and lectures given on Biology Division research. A list of participants and their work in this project follows.

W. A. Arnold	Yale University, New Haven	Photosynthesis
W. A. Arnold	Knoxville Science Club, Knoxville	Atmospheres of the Planets
W. A. Arnold	Purdue University, Lafayette	Photosynthesis
W. A. Arnold	Wake Forest College, Wake Forest	Photosynthesis
W. A. Arnold	North Carolina State Col., Raleigh	Photosynthesis
W. A. Arnold	University of Florida, Gainesville	Photosynthesis
W. A. Arnold	University of Georgia, Athens	Photosynthesis
W. A. Arnold	Clemson College, Clemson	Photosynthesis
A. V. Beatty	University of Tennessee, Knoxville	Normal Mitotic Frequencies in Leaves of Plants
*Oscar M. Bizzell	A.C.S. Meeting, Detroit	Radioactive Decontamination Properties of Laboratory Surfaces: I. Glass, Stainless Steel and Lead
S. F. Carson	Am. Soc. Biol. Chem., Atlantic City	The Genesis of C_4 Dicarboxylic Acids in the Tricarboxylic Acid Cycle
S. F. Carson	Indiana University, Bloomington	Organic Acid Synthesis by C_2 Condensation and Reversible CO_2 Exchange Reactions
S. F. Carson	University of N. C., Chapel Hill	Use of C^{14} and C^{13} in Biochemical Studies
S. F. Carson	Duke University, Durham	Respiratory Cycles
S. F. Carson	University of Tennessee, Knoxville	Hydrogen Transfer and Other Problems in Fermentation Chemistry

* Report on work done in Radiochemistry Section under direction of P. C. Tompkins.

C. E. Carter	University of Texas, Austin	Composition of Ribose Nucleic Acids
C. E. Carter	University of Va., Charlottesville	1. Fundamentals in Radioactivity 2. Current Problems in Nucleic Acid Chemistry
C. E. Carter	M.I.T., Cambridge	Current Problems in Nucleic Acid Chemistry
W. E. Cohn	Am. Soc. Biol. Chem., Atlantic City	The Separation of Adenosine Polyphosphates by Ion Exchange and Paper Chromatography
W. E. Cohn	Am. Chem. Soc., Houston	The Ion-Exchange Separation of Organic Electrolytes Derived from Nucleic Acid
W. E. Cohn	Sloan-Kettering Inst., New York	Ion-Exchange Separation of Nucleic Acid Constituents
W. E. Cohn	Tulane University, New Orleans	The Application of Ion Exchange to the Chemistry and Enzymology of Nucleic Acids
W. E. Cohn	Rice Institute, Houston	Same Subject
W. E. Cohn	Miss. State Col., Starkville	Same Subject
W. E. Cohn	La. State Univ. Med. Sc., New Orleans	Same Subject
W. E. Cohn	Southern Res. Inst., Birmingham	Same Subject
Alan D. Conger	Georgia Acad. Sci., Oglethorpe	Experimental Study of the Effects of Slow Neutrons on Biological Materials
Mary E. Gaulden	Assoc. S.E. Biologists, Charlottesville	The Effect of Oxygen on X-Ray Induced Mitotic Inhibition
N. H. Giles, Jr.	Univ. of N. C., Chapel Hill	The Effects of Ionizing Radiation on Chromosomes
N. H. Giles, Jr.	N. C. State College, Raleigh	Cytogenetical Mechanism of Reversion in Biochemical Mutants of *Neurospora*

N. H. Giles, Jr.	La. State Univ., Baton Rouge	Effects of Ionizing Radiation
N. H. Giles, Jr.	Vanderbilt Univ., Nashville	Reverse Mutations in *Neurospora*
N. H. Giles, Jr.	Atlanta University, Atlanta	Cytogenetical Studies on the Mechanism of Reversion in Biochemical Mutants of *Neurospora*
A. Hollaender	Am. Physiol. Soc., Atlantic City	The Effect of Oxygen on the Response of Living Cells to X rays, with Special Emphasis on Bacteria
A. Hollaender	Duke University, Durham	Secondary Effects of Radiation
A. Hollaender	Univ. of N. C., Chapel Hill	Factors Modifying the Effect of Ionizing Radiation
A. Hollaender	Davidson College, Davidson	Biological Effects of Radiation
A. Hollaender	Univ. of Ky., Lexington	The Effect of Oxygen on the Response of Living Cells to X Rays, with Special Emphasis on Bacteria
A. Hollaender	Berea College, Berea	Biological Effects of Radiation
R. F. Kimball	Indiana University, Bloomington	(1) Delay in Cell Division by Ultraviolet Radiation (2) Structure of the Macronucleus as Seen Under the Phase Contract Microscope
R. F. Kimball	Vanderbilt University, Nashville	Photoreactivation in *Paramecium*
R. F. Kimball	University of Tennessee, Knoxville	Effects of Radiation on *Paramecium*
E. F. Phares	Am. Chem. Soc., Houston	Degradation of Labeled Acetic and Propionic Acids
Seymour Pomper	University of Tennessee	Some Aspects of Microbial Genetics
W. L. Russell	Assoc. S.E. Biologists, Charlottesville	Genetic Effects on Environment and Environmental Effects on Genes

C. W. Sheppard	Biometrics Society, Oak Ridge	The Mathematics of Isotope Experiments in Multicompartment Systems
C. W. Sheppard	Am. Soc. for Exp. Path., Atlantic City	The Fate of Gold Colloids Introduced into the Circulation of the Mouse
Leon Wish	Am. Soc. for Exp. Path., Atlantic City	Rate of Clearance from Blood of ^{131}I-Tagged Plasma; Determinations of Cell and Plasma Volumes With Radioisotopes and Evans Blue
*Clyde Watson	A.C.S. Meeting, Detroit	Radioactive Decontamination of Laboratory Surface: II. Paints, Plastics and Floor Materials

Biology and Medicine Directors' Meeting. The last quarterly convention of the Directors of Biology and Medicine of the Atomic Energy Commission was held in Oak Ridge on May 8 and 9, sessions taking place in the Biology Division conference room. On Monday, May 8, the greater part of the day was devoted to presentation of the work of the Division. The following program was given.

Monday Morning, May 8

Introduction	Alexander Hollaender
Studies on Oxygen Effect	
Rate of Mitosis	Mary Esther Gaulden
Chromosome Changes	Norman H. Giles, Jr.
Mutation Rate in *Drosophila*	William K. Baker
Bacteria	George E. Stapleton
General Discussion	
Reverse Mutations	Norman H. Giles, Jr.
Chemical Composition and Slow Neutron Effect	Alan D. Conger
Photorecovery in *Paramecium*	Richard F. Kimball
Effects of Radiation on Mammalian Genetics	William L. Russell

*Report on work done in Radiochemistry Section under direction of P. C. Tompkins.

Effects of Radiation on Mammalian Development	Liane B. Russell
Permeability Studies on Erythrocytes	Charles W. Sheppard
Studies of the Pathology-Physiology Group	Jacob Furth

a) Slow Neutron Exposure Series
b) Radiation Induced Ovarian Tumors
c) Plasma and Cell-Volume Changes Following X Irradiation
d) Protection from Acute Radiation Injury

Afternoon

Photosynthesis	William A. Arnold
Studies in Nucleic Acid Chemistry	Charles E. Carter
Metabolism Studies with C^{14}	Stanley F. Carson
Biosynthesis of C^{14} Compounds	Glenn R. Noggle

Publications. Publication information on some papers by members of the Division are listed, as well as prepublication notes on others now in final preparation.

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
W. K. Baker, and Elizabeth Sgourakis	The Effect of Oxygen Concentration on the Rate of X-ray Induced Mutations in *Drosophila*	Proc. Nat. Acad. Sci., 36: 176, 1950	ORNL-575
S. F. Carson, J. W. Foster, D. S. Anthony and E. F. Phares	Genesis of C_4 Dicarboxylic Acids in the Tricarboxylic Acid Cycle (Abstract)	Fed. Proc. 9: 159, 1950	
C. E. Carter	The Decomposition of DPN and ATP by Ultraviolet Light	J. Am. Chem. Soc., 72: 1835, 1950	ORNL-326
C. E. Carter	Paper Chromatography of Purine and Pyrimidine Derivatives of Yeast Nucleic Acid	J. Am. Chem. Soc., 72: 1466, 1950	ORNL-313
W. E. Cohn	The Anion-Exchange Separation of Ribonucleotides	J. Am. Chem. Soc., 72: 1471, 1950	ORNL-285

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
W. E. Cohn	The Ion-Exchange Separation of Organic Electrolytes Derived from Nucleic Acid (Abstract)	Published in Abstracts of the A.C.S. Meeting, March, 1950, Houston, Texas	
W. E. Cohn	Heterogeneity in Pyrimidine Nucleotides	Communication to Editor, J. Am. Chem. Soc. (Accepted)	ORNL-658
W. E. Cohn and C. E. Carter	The Separation of Adenosine Polyphosphates by Ion Exchange and Paper Chromatography (Abstract)	Fed. Proc., 9: 161, 1950	
J. W. Foster, S. F. Carson and W. E. Jefferson	The $2C_2$ Condensation Reaction in Citric Acid Formation by a Strain of *Aspergillus niger* (Abstract)	Fed. Proc., 9: 172, 1950	
J. W. Foster, and S. F. Carson	Metabolic Exchange of Carbon Dioxide with Carboxyls and Oxidative Synthesis of C_4 Dicarboxylic Acids	Proc. Nat. Acad. Sci.,36: 219, 1950	ORNL-615
J. W. Foster, and S. F. Carson	Citric Acid Formation by *Aspergillus niger* through Condensation of $3C_2$ Moieties (Communication to Editor)	J. Am. Chem. Soc., 72:1865, 1950	
N. H. Giles, Jr. and H. P. Riley	Studies on the Mechanism of the Oxygen Effect on the Radiosensitivity of *Tradescantia* Chromosomes	Accepted by Proc. Nat. Acad. Sci.	ORNL-688
A. S. Holt, and W. A. Arnold	The Effect of Ultraviolet on Green Algae and Isolated Chloroplasts (Abstract)	The Biol. Bull., 97:268, 1949	
A. Hollaender, G. E. Stapleton, and F. L. Martin	The Effect of Oxygen on the Response of Living Cells to X Rays, with Special Emphasis on Bacteria (Abstract)	Fed. Proc. 9: 61, 1950	
H. I. Kohn	Chemical Changes in the Blood of the Rat During Ether and Barbiturate Anesthesia	Am. J. Physiol., 160:277, 1950	

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
H. I. Kohn	Changes in the Plasma of the Rat During Fasting; The Influence of Genetic Factors Upon the Sugar and Cholesterol Levels	Submitted to Am. J. Physiol.	ORNL-414
M. L. Morse	Resistance to Ultraviolet Radiation in *Escherichia coli*, Strains B and B/r (Abstract)	Bact. Proc., 1950, page 135	
S. Pomper	Recent Developments in Yeast Genetics	Submitted to Wallerstein Laboratories Communication	ORNL-696
C. W. Sheppard, and W. R. Martin	Cation Exchange Between Cells and Plasma of Mammalian Blood: I. Methods and Application to Potassium Exchange in the Human	Accepted by J. Gen. Physiol.	ORNL-458
C. W. Sheppard, J. Furth and Leon Wish	The Fate of Gold Colloids Introduced into the Circulation of the Mouse (Abstract)	Fed. Proc., 9: 343, 1950	
R. H. Storey, L. Wish and J. Furth	Changes in Cell and Plasma Volumes Produced by Total Body X Radiation	Submitted to Proc. Soc. Exper. Biol. & Med.	ORNL-687
H. J. Teas	Canavanine Inhibition of Some Normal and Amino Acid Requiring Strains of *Neurospora* and *Bacillus subtilis*	Submitted to J. Biol. Chem.	ORNL-616
Leon Wish, R. H. Storey and J. Furth	Rate of Clearance from Blood of ^{131}I-Tagged Plasma; Determination of Cell and Plasma Volumes with Radioisotopes and Evans Blue (Abstract)	Fed. Proc., 9: 349, 1950	

Visiting speakers. The Division has been fortunate in securing the services of the important speakers heard this quarter on the Biology Seminar program.

Dr. Tracy M. Sonneborn - Indiana University - "Antigenic Types in *Paramecium* in Relation to Cytoplasmic Inheritance and Plasmagenes".

Dr. P. R. Burkholder - Dept. Botany, Yale University - "Some Patterns of Plant Nutrition".

Dr. John W. Tukey - Princeton University - "Simpler (Statistical) Analyses by Ranking Methods".

Dr. Joshua Lederberg - University of Wisconsin - "An Approach to the Genetic Mechanisms of the Bactericidal Effects of Radiation".

Dr. Kenneth V. Thiman - Harvard University - "The Biochemistry of the Growth and Inhibition of Plant Tissues".

Dr. Jesse P. Greenstein - National Cancer Institute - "Biochemistry of Cancer".

Dr. J. G. Linn, Jr. - Philadelphia, Pennsylvania - "Cataract Problems in Relation to Radiation".

Dr. Eric Ponder - Nassau Hospital - "Red-Cell Structure".

Dr. Jack Schultz - Institute for Cancer Research - "Chemical Genetics in *Drosophila*".

Dr. Duncan A. MacInnes - Rockefeller Institute - "Effect of Centrifugal Force on Electromotive Force of Galvanic Cells".

CYTOGENETICS

CYTOGENETICAL EFFECTS OF RADIATIONS

N. H. Giles, Jr. (Leader)
A. H. Doermann
A. D. Conger
Seymour Pomper
A. V. Beatty (Res. Participant)
H. E. Wheeler (Visit. Investigator)
Dorothy L. Giles
Lucille M. Fairchild
S. R. Suskind
Betty B. Hill
Kathryn Meyer
Mary E. Case

The effect of oxygen on X-ray induced chromosomal rearrangements in Tradescantia. (Giles, Beatty and Fairchild) Additional experiments have been performed to determine the yield of interchanges when exposures to a constant X-ray dose are made in atmospheres containing different percentages of oxygen. A particular effort was made to free the helium gas used of any oxygen, by passing the gas through liquid nitrogen and over hot copper foil before it was introduced into the exposure chamber. In addition, the plants in pure helium were allowed to respire in the dark for approximately two hours before the X-ray exposure in an attempt to remove any remaining oxygen. The statistical reliability of exposures in varying percentages of oxygen was increased by examining a larger number of cells. The data obtained in the exposures in 0, 2, 5 and 10 per cent oxygen are given in Table 1. These data, together with the average of previous results in higher percentages of oxygen are plotted in Fig. 1.

Additional tests of the oxygen effect have been made in experiments in which inflorescences in a constant percentage of oxygen were irradiated under various pressures. The data obtained in a series of such exposures are given in Table 2, and the values for 10 per cent oxygen are indicated on the graph (Fig. 1) in relation to the expected results on the assumption that the amount of oxygen dissolved in the cells is directly related to the pressure and that the interchange yield depends on this percentage of oxygen present during irradiation. Only in the exposure in 5 per cent oxygen at three atmospheres is the yield considerably lower than would be expected. This exposure is being repeated.

Preliminary experiments have been carried out to test the effect of temperature during irradiation and these tests will be continued in the coming

FIG. 1

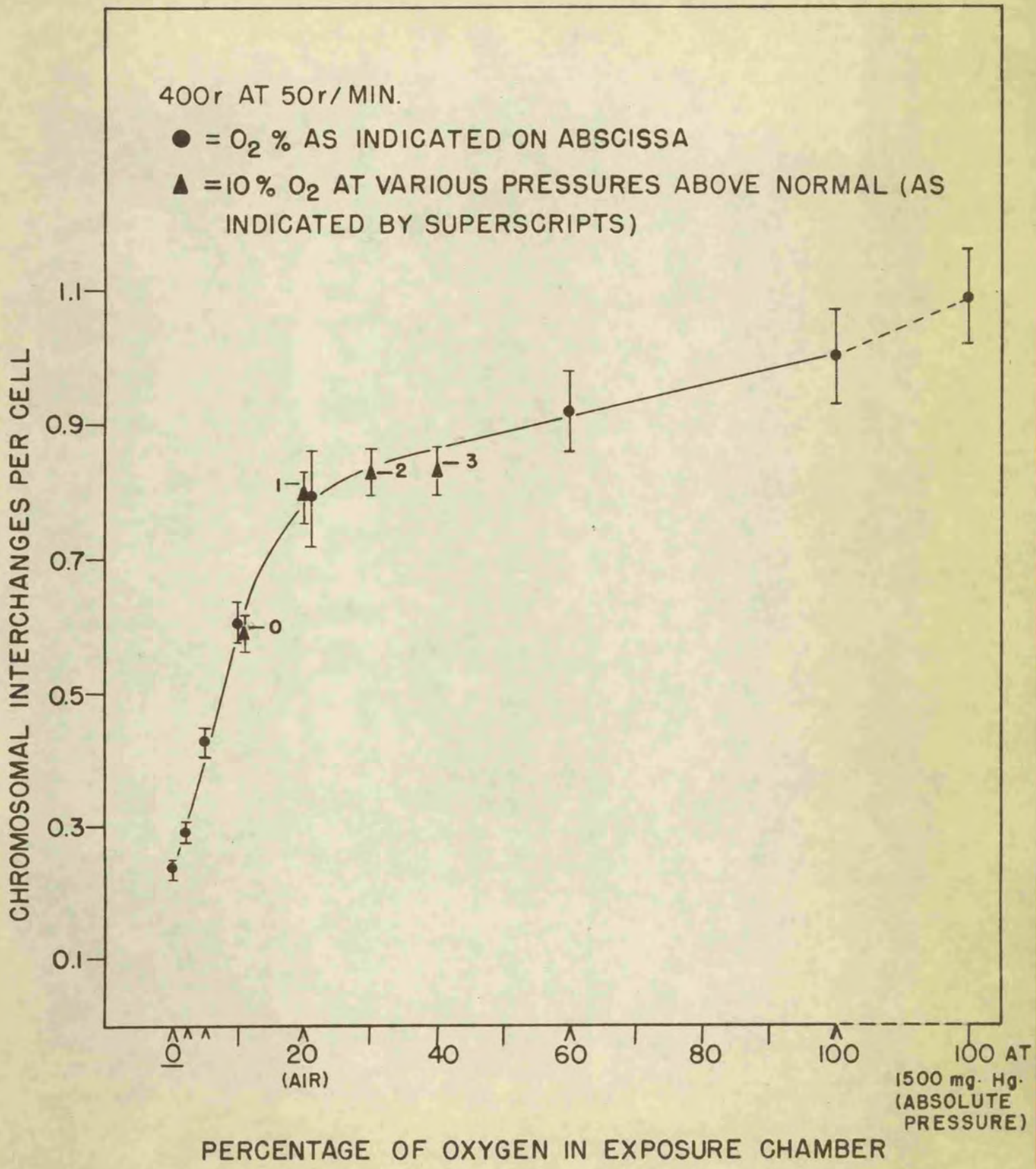

400r AT 50r/MIN.
● = O_2 % AS INDICATED ON ABSCISSA
▲ =10% O_2 AT VARIOUS PRESSURES ABOVE NORMAL (AS INDICATED BY SUPERSCRIPTS)
CHROMOSOMAL INTERCHANGES PER CELL
1.1
0.9
0.7
0.5
0.3
0.1
1
2
3
0
0
20
(AIR)
40
60
80
100
100 AT
1500 mg. Hg.
(ABSOLUTE PRESSURE)
PERCENTAGE OF OXYGEN IN EXPOSURE CHAMBER

TABLE 1

Effect of various percentages of oxygen on the frequency of chromosomal aberrations in Tradescantia microspores. All X-ray exposures of 400 r at 50 r/min. Clone 5 Expt. 50-VIII.

OXYGEN PERCENTAGE	NO. CELLS	NO. INTERCHANGES	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS	I.D. PER CELL
0	1000	234	0.234 ± 0.015	287	0.287 ± 0.017
2	1200	347	0.289 ± 0.016	394	0.328 ± 0.016
5	1000	427	0.427 ± 0.021	501	0.501 ± 0.022
10	600	364	0.607 ± 0.032	453	0.755 ± 0.035

quarter. In addition exposures to fast neutrons in the presence and absence of oxygen are planned.

Photoreactivation effects in Neurospora. (Giles, Beatty and Fairchild) Several experiments have been performed to study the effect of photoreactivation on survival and mutation with the microconidial, inositolless mutant 37401. A general graphical summary of these results to date is presented in Figs. 2 and 3. A more detailed discussion of the methods used and an interpretation of the results will be deferred until additional exposures have been completed. It is clear, however, that there is a marked effect of photoreactivation on both survival and mutation. The results to date are interpretable on the basis of some single effect of visible light which affects equally these two processes.

Production of chromosomal aberrations by alpha rays. (Conger and Suskind) The production of chromatid aberrations in dry *Tradescantia* pollen grains by polonium alpha rays has been investigated during this quarter. The irradiation apparatus consisted of a 1-millicurie polonium alpha-ray source on the end of a small rod inside an evacuated brass cylinder. At one end of the cylinder was a 0.9 mg/cm^2 mica window through which the alpha rays emerged to fall on a monocellular layer of pollen grains placed 1 mm below the window. The apparatus was calibrated before and after the pollen grain exposures. It

TABLE 2

The effect of pressure on the frequency of X-ray induced chromosomal interchanges in Tradescantia microspores irradiated in atmospheres containing varying percentages of oxygen. All exposures of 400 r at 50 r/min.

PRESSURE IN EXPOSURE CHAMBER	NO. CELLS	NO. INTER-CHANGES	INTERCHANGES PER CELL	NO. INTERSTITIAL DELETIONS	INTERSTITIAL DELETIONS PER CELL
Irradiated in 10 per cent oxygen (plus 90 per cent helium)					
Normal atmospheric (ca 740 mm Hg)	800	474	0.59 ± 0.027	599	0.75 ± 0.031
One atmosphere above normal	550	436	0.79 ± 0.038	563	1.02 ± 0.043
Two atmospheres above normal	700	584	0.83 ± 0.034	875	1.25 ± 0.042
Three atmospheres above normal	700	584	0.83 ± 0.034	732	1.05 ± 0.039
Irradiated in pure helium (~99.8 per cent)					
Normal atmospheric (ca 740 mm Hg)	950	229	0.24 ± 0.016	256	0.27 ± 0.017
Three atmospheres above normal	1000	223	0.22 ± 0.015	259	0.26 ± 0.016
Irradiated in air					
Normal atmospheric (ca 740 mm Hg)	1000	713	0.71 ± 0.027	780	0.78 ± 0.028
Three atmospheres above normal	950	786	0.83 ± 0.030	994	1.05 ± 0.033
Irradiated in 5 per cent oxygen (plus 95 per cent helium)					
Normal atmospheric (ca 740 mm Hg)	800	365	0.46 ± 0.024	444	0.56 ± 0.026
One atmosphere above normal	950	583	0.61 ± 0.025	671	0.71 ± 0.027
Two atmospheres above normal	1000	683	0.68 ± 0.026	802	0.80 ± 0.028
Three atmospheres above normal	950	634	0.67 ± 0.027	740	0.78 ± 0.029

FIG. 2 UNCLASSIFIED DWG. #8997

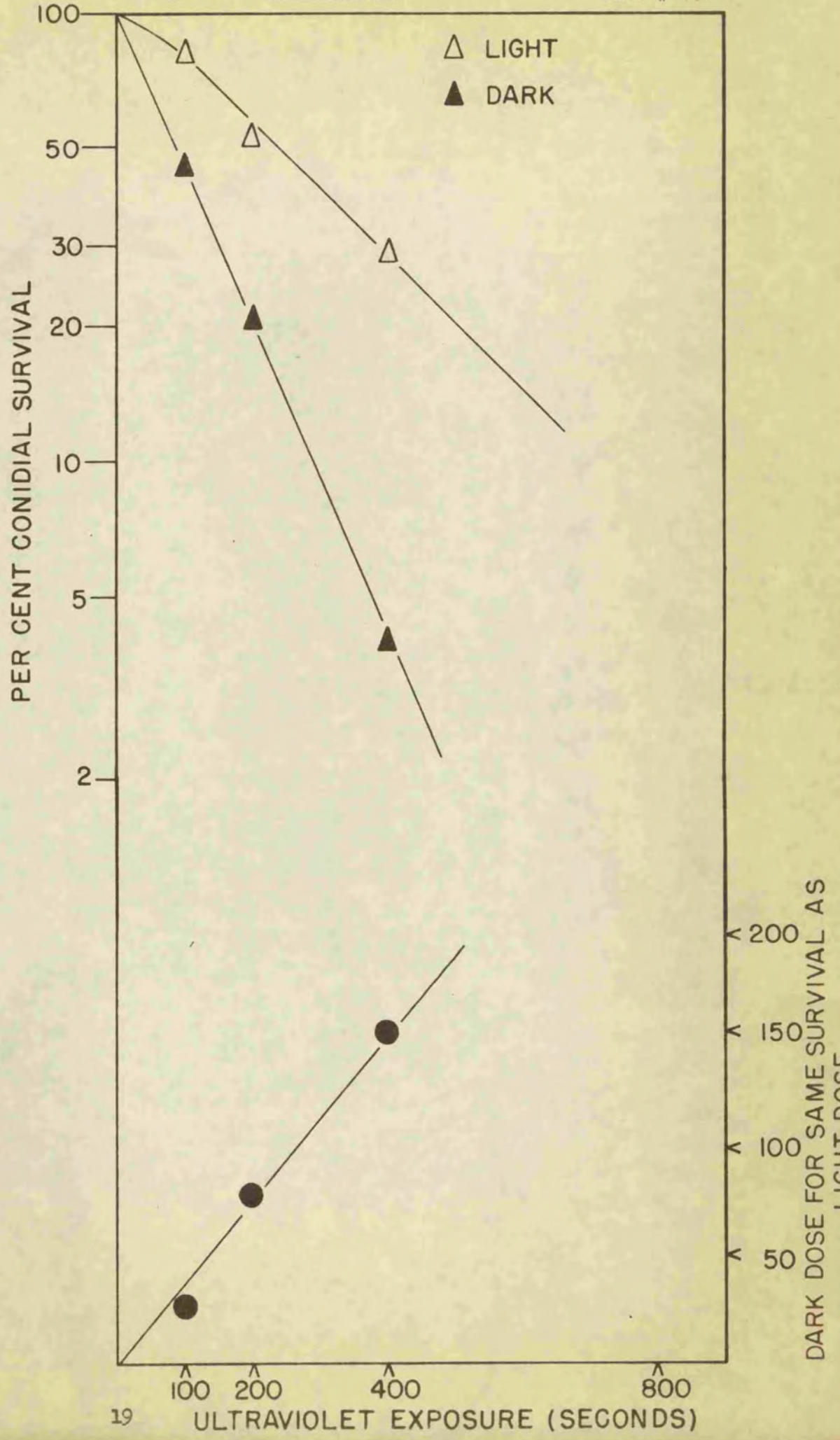
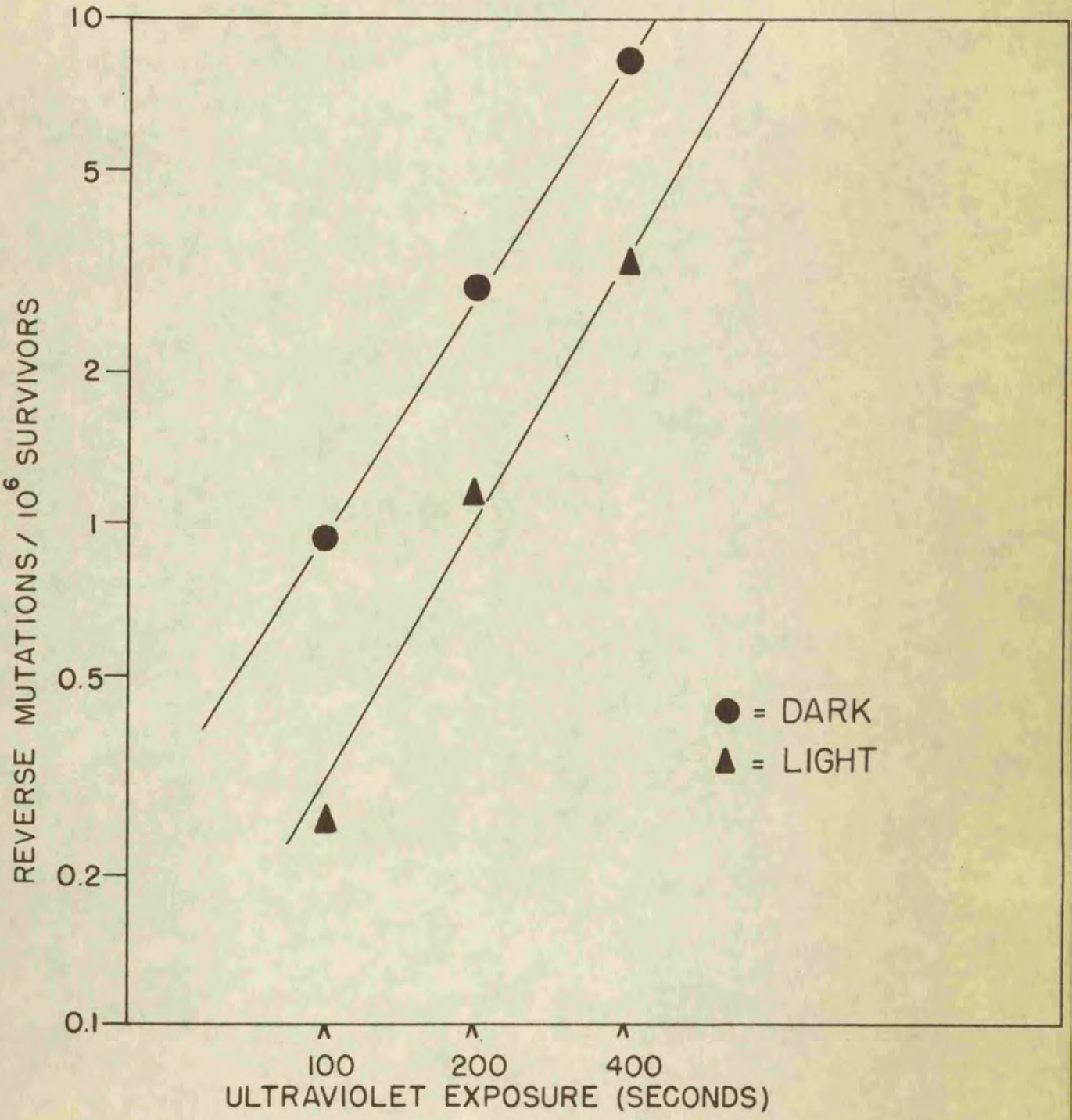


FIG. 3


10
5
2
1
0.5
0.2
0.1
REVERSE MUTATIONS / 10^6 SURVIVORS
● = DARK
▲ = LIGHT
100
200
400
ULTRAVIOLET EXPOSURE (SECONDS)

was found that during the period of use a considerable portion of the polonium had crept off the end of the source and distributed itself around inside the chamber; new sources, to be prepared by the volatilization of polonium onto palladium, should eliminate this creepage trouble.

Irradiated pollen grains were grown on a sucrose-agar medium for 18-19 hours and the frequency of chromatid aberrations observed at the pollen tube mitosis from Feulgen preparations. Only a small number of cells have been analyzed since the completion of the most recent irradiations but the data available may be used for some tentative conclusions. The data are shown in the graph (Fig. 4); dose is given as alpha particles per nucleus, as the dosage calculations have not been completed. The frequency of exchange aberrations increases linearly with dose, as is to be expected with a radiation of heavy particles. No quantitative comparisons can be made until more data are available.

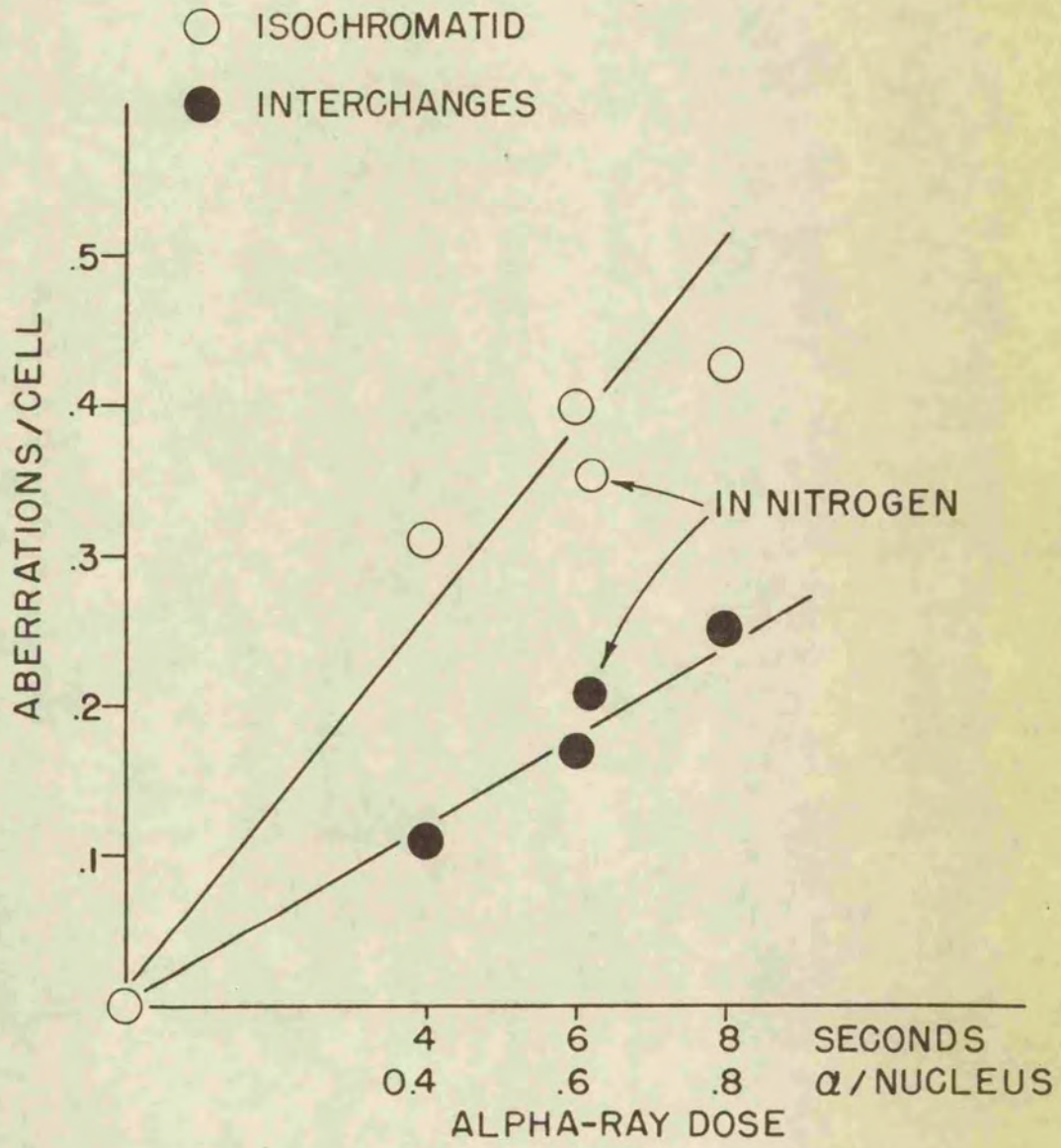
An experiment to compare the frequency of aberrations when the cells are irradiated in air or in nitrogen shows that the nitrogen had little, if any, effect — shown on the graph at the six-second exposure point. This lack of difference in the chromosome aberration frequency when cells are alpha-irradiated in air and in nitrogen is in sharp contrast to the large difference found when pollen grains are X-irradiated in air and in nitrogen (Conger, Quarterly Report, ORNL-537), and suggests that the reduction in chromosome sensitivity to X rays caused by nitrogen will not be found with particulate radiations.

Yeast genetics. (Pomper and Meyer) The genetic analysis of the purine-requiring mutants of *Saccharomyces cerevisiae* has been pursued in the last quarter and is nearing completion. It will be recalled that in the last quarterly report (ORNL-644), a procedure was outlined whereby it was possible by means of genetic tests to arrange the purine mutants in the order of their biochemical relationship to a pigment-accumulating purine mutant. That is to say, by appropriate crosses, dissections, analyses, and back-crosses of the pigment-accumulating mutant by the other four mutants, it is possible to ascertain which block precedes, and which follows, the pigment block. Thus far, it seems safe to conclude, based on this analysis, that two of the nonallelic mutant types precede the pigment-accumulating mutant. This suggests that the pigmented strain is blocked at a point fairly late in the biosynthesis of purine, but it is not yet possible to identify the reaction involved. Since

Figure 4

Chromatid aberrations induced in *Tradescantia* dry pollen grains by alpha irradiation in air (and in nitrogen at 6 seconds).

FIG. 4


ISOCHROMATID
INTERCHANGES
ABERRATIONS/CELL
.5
.4
.3
.2
.1
IN NITROGEN
4
6
8
SECONDS
0.4
.6
.8
α/NUCLEUS
ALPHA-RAY DOSE

the ribosides and ribotides of purines have been inactive in growth tests with the mutants, it suggests that the reactions blocked are somewhere between the free purine and the nucleosides or nucleotides and that the direction is from the more complex to the less complex structure. Another possibility is that there is no direct molecular conversion of purine to riboside or ribotide, and therefore the latter compounds do not serve as metabolites for these mutants. Still a third possibility is that selective permeability is preventing the larger molecules from penetrating the cell to be metabolized. Numerous other speculations could be adduced, but would serve little constructive purpose at this time. In an effort to answer some of these questions, enzymatic studies have been initiated with the purine-requiring mutants. These are, however, in a very preliminary stage, and cannot yet be discussed beyond the point of noting that respiring, dried preparations have been obtained. A more detailed report on these experiments with purine and pyrimidine mutants is now being prepared for publication.

Bacteriophage investigations. (Doermann and Hill) The basic plan pursued has been to extend our knowledge and control of genetic factors available in bacteriophage T4. To this end experiments were continued which are designed to elucidate the genetic properties of a series of plaque type mutants of T4r_{48}. Preliminary results with these types were described in the previous report. The types studied most intensively are #'s 1, 2, 5, and 6. The following hypothesis fits the results so far obtained except in several crosses. Future experiments are expected to clear up these exceptional cases. We propose that the phenotypes of the four strains depend on three hereditary factors whose effects are additive. Thus # 1 can be characterized genetically by three wild type loci, $a^+b^+c^+$, while # 6 contains the three mutant loci, abc. The # 2 type contains any two wild type loci with one mutant locus (*e.g.*, a^+b^+c), while # 5 contains a single wild-type locus with any two mutant loci (*e.g.*, ab^+c). Tentatively it is proposed that recombination occurs among these loci as though they are independent of each other. Under these conditions any two factor cross yields of the order of 65 per-cent parental and 35 per cent recombination types (Cf. Hershey and Rotman, Genetics 33:34, 1949). On the theory proposed and from this result, one can predict the expected percentage of the various plaque types to be expected from the possible types of crosses. These predictions are made in Table 3. In Table 4 are shown results of 22 intercrosses made with these types. The deviation

found can be explained by inadequate differentiation of types #'s 5 and 6. There is also some evidence that a selective factor is operating against one of the mutant loci.

To obtain more genetic markers in T4, crosses were made between T4 and several *r* mutants of T2H obtained from Dr. A. D. Hershey (refer to paper by Hershey and Rotman cited above). In this way two of Hershey and Rotman's unlinked *r* mutants were introduced into T4. Using these markers, the relative location of six other T4 *r* loci were determined. The strains involved are *r*1 and *r*7 from T2H, and r_{43}, r_{47}, r_{48}, r_{49}, r_{51}, and r_{52} from independent occurrences in T4. The results show that three groups embrace the eight types. Group I consists of r_1, r_{48}, and r_{52}. These are all genetically identical or very closely linked since less than 1 per cent recombination was found in any of the possible intercrosses between them. Group II consists of r_{43}, r_{49}, and r_{51}. These are genetically similar or very closely linked by the same criteria. The same is true of Group III, which comprises r_7 and r_{47}. Crosses between members of different groups indicate that Group I is independent of both Group II and Group III. Group II and Group III are linked, giving of the order of 4 per cent wild type in intercrosses (indicating 8 per cent recombination).

Glomerella genetics. (Wheeler) This investigation has been designed to make available groups of genetic markers for use in future studies concerning sexuality and mutability in this fungus. Some of the mutant stocks in use were produced in this laboratory by ultraviolet irradiation and the remainder were supplied by Dr. C. L. Markert of the California Institute of Technology. The latter includes mutants induced by ultraviolet, X rays, and neutrons. The neutron-induced mutants were obtained through collaboration with G. E. Stapleton.

To date, nineteen mutant genes have been tested for linkage with A and B, the two principal loci controlling mating reaction. On the basis of present results, two of these appear to be linked with A while none shows evidence of linkage with B. In twenty-three other combinations of genes studied, only one questionable case of linkage has been discovered. Thus far, attempts to introduce more than three separate biochemical deficiencies into a single stock have been unsuccessful. Preliminary studies have been made on the effect of ultraviolet irradiation upon gene changes at the B locus in stocks which carry the specific modifier M. Of 428 mutants induced by ultraviolet

TABLE 3

Hypothetical expectations of $T4r_{48}$ plaque type mutants in intercrosses

(The calculations are based on independence of all loci. For two factor crosses this will yield about 35 per cent recombinants and 65 per cent parentals.)

CROSS	PER CENT EXPECTED IN YIELD					TYPE NUMBER OF CROSS
		# 1	# 2	# 5	# 6	
#1 × #1		100	0	0	0	A
#1 × #2		50	50	0	0	B
#1 × #5		32	36	32	0	C
#1 × #6		23	27	27	23	D
#2 × #2		0	100	0	0	E
#2 × #2		18	64	18	0	F
#2 × #5		0	50	50	0	G
#2 × #5		9	41	41	9	G
#2 × #6		0	32	36	32	I
#5 × #5		0	0	100	0	J
#5 × #5		0	18	64	18	K
#5 × #6		0	0	50	50	L
#6 × #6		0	0	0	100	

Based on having equal multiplicity of parental types and no selective action against any locus or combination of loci.

TABLE 4

NO. OF EXPERIMENT	CROSS	TYPE	NUMBER OF PARTICLES INFECTING CELLS (Avg. per cell)					YIELD IN PER CENT*			
			#1	#2	#5	#6		#1	#2	#5+	#6+
4	#1 × #2a	B	4.8	3.8	0.0	0.0	Found	64	46	0	0
							Expected	50	50	0	0
6	#1 × #5a	C	8.6	0.0	5.5	0.0	Found	52	26	17	5
							Expected	32	36	32	0
15	#1 × #5a	C	-	-	-	-	Found	44	26	26	3
9	#1 × #5b	C	-	-	-	-	Found	37	40	24	0
16	#1 × #5b	C	3.6	0.0	3.2	0.0	Found	39	33	27	0
5	#1 × #6	D	9.5	0.0	0.0	5.7	Found	40	36	13	11
							Expected	23	27	27	23
7	#1 × #6	D	5.6	0.0	0.0	6.7	Found	29	40	7	24
20	#2a × #2b	E	-	-	-	-	Found	0	100	0	0
							Expected	0	100	0	0
1	#2a × #5a	H	0.0	6.3	11.9	0.0	Found	8	44	42	6
							Expected	9	41	41	9
17	#2a × #5a	H	-	-	-	-	Found	12	45	35	8
10	#2a × #5b	G	0.0	6.2	5.7	0.0	Found	0	52	48	0
							Expected	0	50	50	0
18	#2a × #5b	G	-	-	-	-	Found	0	48	47	5
11	#2b × #5b	G	0.0	6.6	7.6	0.0	Found	0	47	53	0
2	#2a × # 6	I	-	-	-	-	Found	0	73(?)	?	27 Poor differentiation
							Expected	0	32	36	32
3	#2a × #6	I	-	-	-	-	Found	0	73(?)	?	27 Poor differentiation
21	#2a × #6	I	0.0	12.5	0.0	6.5	Found	0	58	13	29

TABLE 4 (Cont'd)

NO. OF EXPERIMENT	CROSS	TYPE	NUMBER OF PARTICLES INFECTING CELLS (Avg. per cell)					YIELD IN PER CENT *			
			# 1	# 2	# 5	# 6		# 1	# 2	# 5+	# 6+
12	#5a × #5b	J	-	-	-	-	Found	0	17	83(?)	(?) Poor differentiation
							Expected	0	18	64	18
19	#5a × #5b	J	-	-	-	-	Found	0	15	69	16
8	#5 × #6	L	0.0	0.0	3.5	5.6	Found	0	0	48	52
							Expected	0	0	50	50
13	#5a × #6	L	-	-	-	-	Found	0	0	48	52
14	#5b × #6	L	-	-	-	-	Found	0	0	55	45

* = The expected percentages depend on equal multiplicity of the two infecting parental types. They also depend on infecting with a sufficiently high number of both types. The effect of either a low total or an unequal multiplicity of infection is to depress the yield of recombinants.

+ = Under some conditions differentiation of #'s 5 and 6 is difficult. No method has been found to improve this situation, and therefore caution must be exercised in interpretation of data which depend on such differentiation.

irradiation of such stocks, three resulted from gene changes at the B locus. This may be compared with previous studies of spontaneous mutation in the same stocks. Here 112 of 517 mutants resulted from gene changes at the B locus.

EFFECTS OF RADIATION ON *PARAMECIUM*

R. F. Kimball (Leader)
Nenita T. Gaither Mary K. King

Retardation of cell division by ultraviolet. (Kimball, Gaither, King) In previous quarterly reports, it has been shown that ultraviolet irradiation of *Paramecium aurelia* results in retardation of cell division which lasts for several divisions before the normal rate of division is restored. The earlier experiments were carried out with daily counts of the number of animals. To obtain a more exact picture of the way in which the effect was distributed to various division intervals, especially at lower doses, more frequent observations were needed. An experiment was carried out in which observations were made once an hour for three days, once every six hours for five more days, and once a day thereafter until all lines of descent had recovered the normal division rate. Five different doses of monochromatic 2650 A ultraviolet were given, and thirty animals were isolated from each dosage group and from the unirradiated controls. Precautions were taken during the first day to keep the exposure to visible light to a minimum to avoid photoreactivation. Daily transfers to fresh medium were made to keep the conditions as nearly like the daily isolation lines of other experiments as possible.

The results are shown graphically in Figs. 5, 6, 7, in which the arithmetic means of the times taken to go through a given division interval after irradiation are plotted against dose. Note the difference in scale between Fig. 6 and Figs. 5 and 7. In general, the results are similar to those obtained by the daily observation method and confirm the usefulness of that method. The first interval is longer than the second, the third and fourth intervals are on the average considerably longer than either the first or the second, the fifth and sixth are progressively shorter, and almost complete recovery of the normal rate has occurred by the completion of the sixth division. However, there are certain features which were not clearly brought out

Figure 5

Time from the division preceding irradiation to the first division after irradiation (first interval) and from the first to the second division. Standard errors are indicated.

FIG. 5

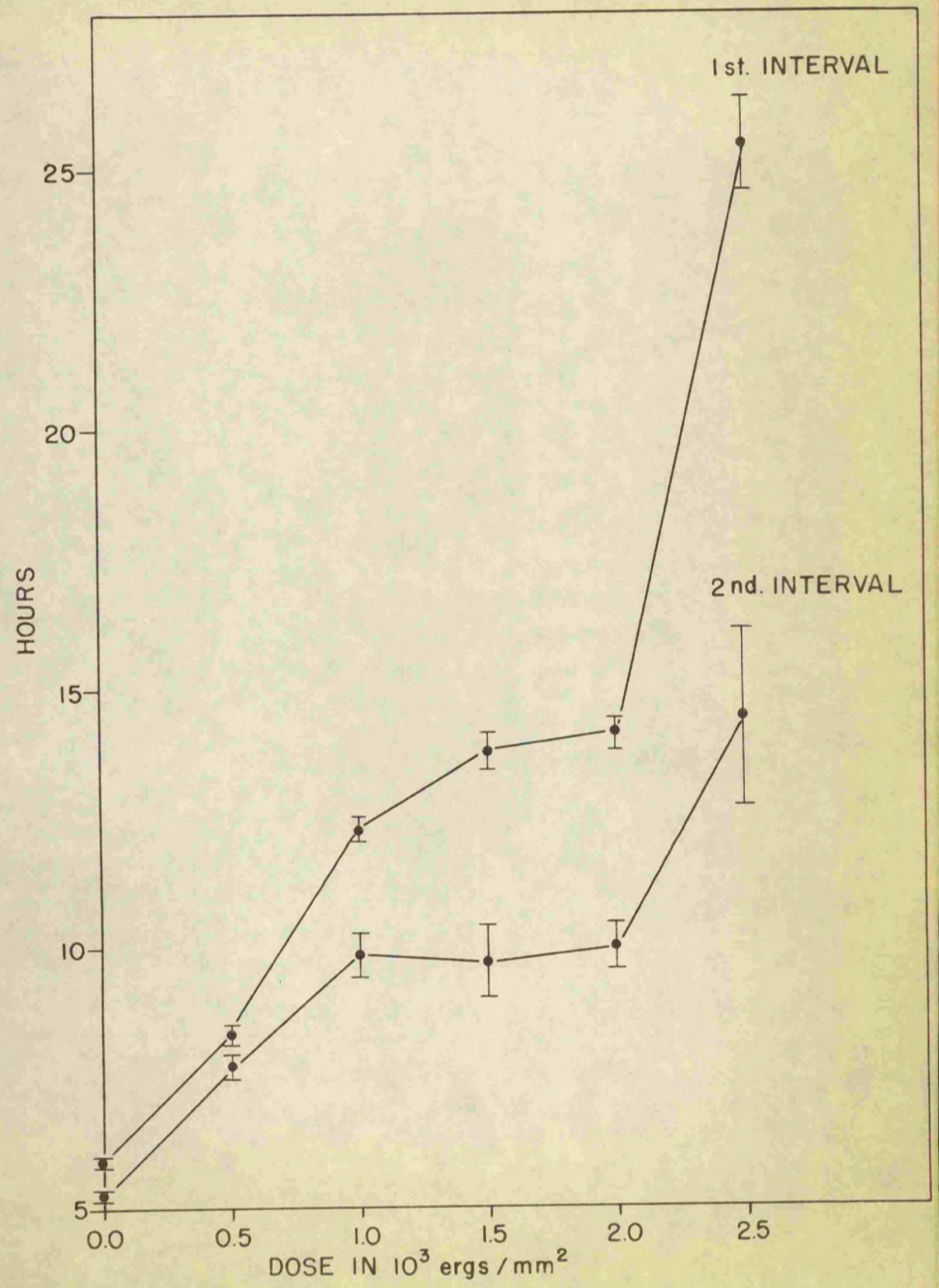

1st. INTERVAL
2nd. INTERVAL
HOURS
25
20
15
10
5
0.0
0.5
1.0
1.5
2.0
2.5
DOSE IN 10^3 ergs / mm^2

Figure 6

Time for the third interval, the fourth interval, and the sum of the two intervals. The time for the first interval is included for comparison.

DWG. #9001

FIG. 6

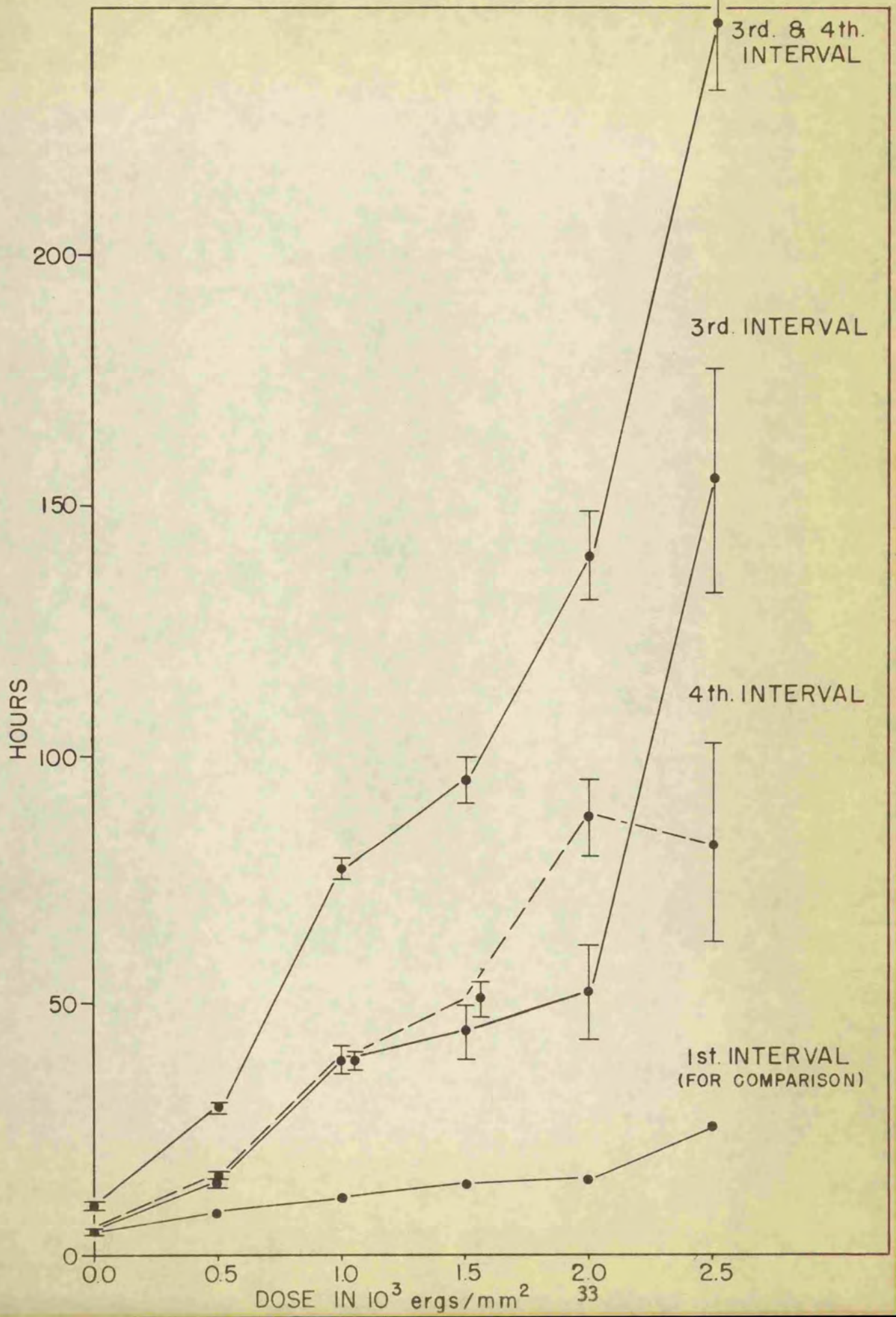
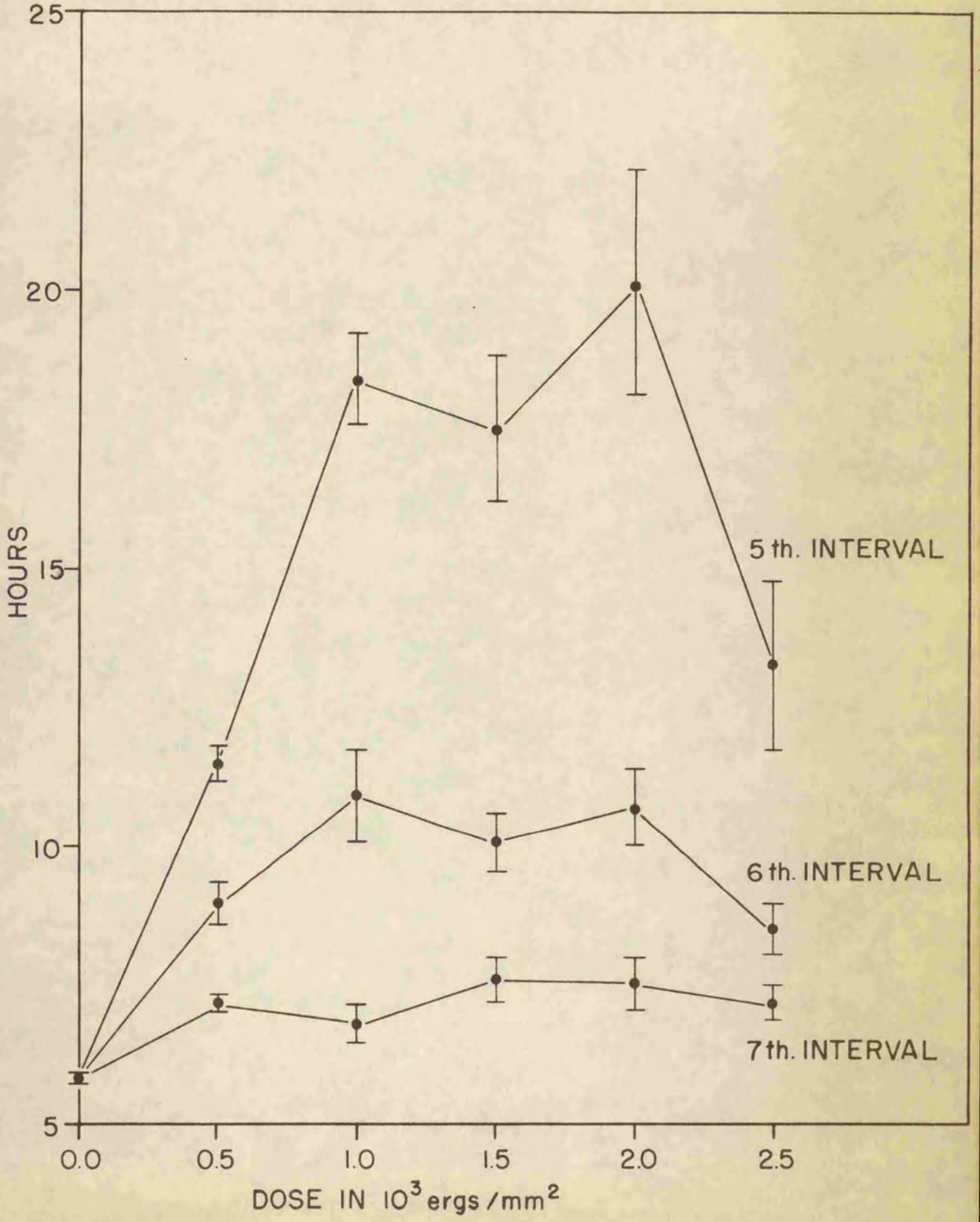


Figure 7

Times for the fifth, sixth, and seventh intervals.

FIG. 7


25
20
15
10
5
HOURS
5 th. INTERVAL
6 th. INTERVAL
7 th. INTERVAL
0.0
0.5
1.0
1.5
2.0
2.5
DOSE IN 10^3 ergs / mm^2

or were not discernible at all in the previous data. The relation between intervals is clearly not constant with dose but changes in such a way that the inequalities mentioned above become greater with increasing dose. Moreover the dosage relations for the various intervals do not appear to be the same as judged from the form of the curves. The great variability of the third and fourth intervals at higher doses makes the form of the curves for the two taken individually rather doubtful, but the sum of the two appears to increase fairly smoothly as some power of the dose. At higher doses, it is quite obvious that the third and fourth intervals account for most of the total retardation. Altogether the analysis in terms of the separate division intervals suggests that retardation may be a complex process with several component parts.

Within a group of lines given the same treatment there appears to be no correlation between the lengths of successive intervals with one important exception. The length of the third interval is inversely correlated with that of the fourth, at least at higher doses, as shown in Fig. 8. In this figure, the times for the third interval are plotted against those for the fourth for two different doses. At the higher dose, there is a distinct inverse correlation. The very long intervals on the two tails of the curve have been spoken of as the "cessation" period in previous reports but it is obvious that there is a whole spectrum of cases from those in which there is one long cessation period to those in which both the third and fourth intervals are longer than normal, but neither is extremely long. The correlation diagrams for intermediate doses are intermediate between the two extreme cases shown here. Through the kindness of Mr. Moshman of the Mathematics Panel, curves of the form $y-5 = a\,(x-5)^b$ have been fitted to the correlation data for the five doses where y is time in hours for the fourth interval and x, time in hours for the third interval. The two times were decreased by the approximate control time of five hours. The evidence shows that a increases with dose and suggests that b decreases with dose. All values of b were negative save that at the lowest dose.

Thus a complete description of the relations of various components of retardation to each other and to dose is a complex matter. Fortunately, the total number of divisions which are retarded by radiation does not seem to change appreciably with dose. Thus a quantity such as time to the sixth division furnishes a reasonably accurate way of measuring the total delay in division produced by a given treatment. To test how well this time can be

Figure 8

Correlation diagrams for time for the third and fourth intervals for two different doses of ultraviolet.

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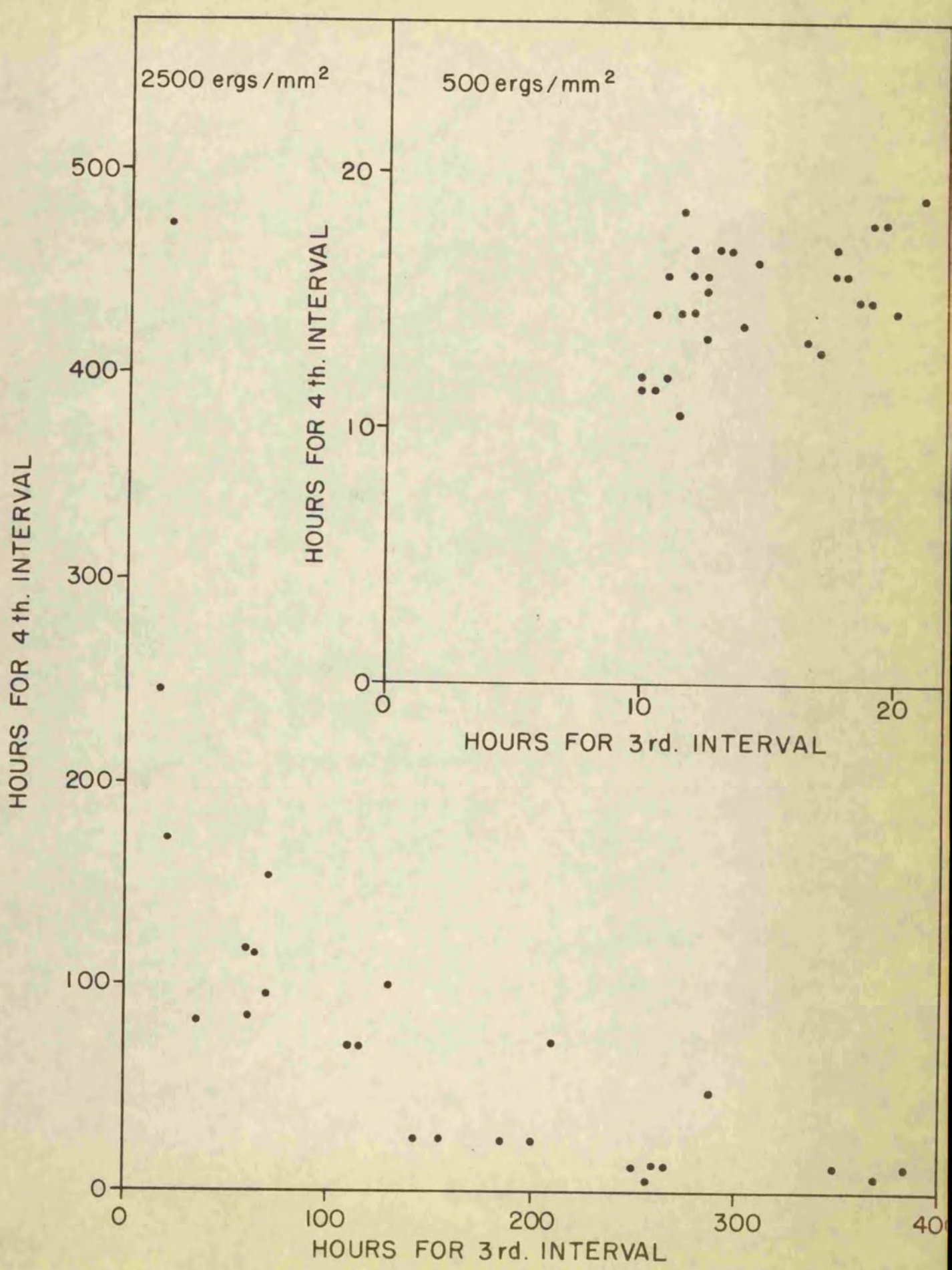
DWG. #9003

FIG. 8



ascertained by daily rather than more frequent observations, the data from the experiment with frequent observations were recalculated in the manner in which they would have been if only daily observations had been available. The times to the sixth division calculated in this way are compared with the times to the sixth division calculated from the complete set of observations. The comparisons are given in Table 5. Even at the lowest dose where a daily period is quite long in comparison with the total time involved, the errors are not great. Thus time to the sixth division as determined by experiments with daily observations is an adequate measure of the total effect on cell division.

TABLE 5

A comparison between times to the sixth division calculated on the same material on the basis of frequent observations and daily observations

DOSE OF 2650 A ULTRAVIOLET IN ERGS/MM^2	TIME TO THE SIXTH DIVISION IN HOURS		DIFFERENCE EXPRESSED AS A PER CENT OF THE FREQUENT OBSERVATIONS CALCULATION
	FREQUENT OBSERVATIONS	DAILY OBSERVATIONS	
500	65.9	70.9	7.6
1000	128.8	125.9	2.3
1500	146.7	145.5	0.8
2000	195.9	194.3	0.5
2500	299.7	296.7	1.0

Photoreactivation of the effects of ultraviolet irradiation. (Kimball, Gaither, King) Several experiments designed to increase the body of data available on photoreactivation in respect to retardation of cell division and mutation induction have been carried out using monochromatic ultraviolet of wave lengths 2650 A and 2804 A. This material together with that already obtained is now being analyzed statistically and a paper is being made ready for publication. Since the analysis is not complete, no detailed report will be given at this time.

The effect of oxygen upon ultraviolet induced radiation damage. (Kimball, Gaither, King) Several experiments have been performed to test the possibility that the amount of oxygen present might influence the outcome of ultraviolet irradiation. The results have been extremely variable, and there are distinct reasons for believing that the different exposure chambers which were used may not transmit the ultraviolet equally. Efforts are now being made to control such disturbing factors, but the data suggest that any effects of oxygen which exist are probably small.

EFFECTS OF RADIATION ON THE RATE OF MITOSIS

J. Gordon Carlson* (Leader)
Mary E. Gaulden
Nyra Harrington
Marjorie Hix

Effect of radiation on the osmotic pressure of neuroblast cells of chortophaga viridifasciata. (Carlson and Harrington) Studies on the effect of gamma rays given at a low intensity reveal that a change is produced in the osmotic pressure relationship in the neuroblast cells by irradiation. Using an intensity of 4 r/hr, embryos were treated for periods varying from one to six days, thus the doses given were 96, 192, 384, and 576 r. At various intervals after treatment, the embryos are made into hanging-drop culture preparations and then examined under the microscope at 38° C along with control embryos. A solution which was isotonic to the controls was hypotonic, with varying degrees, to the treated cells, thus an arbitrary means of designating osmotic pressure differences could be established.

The osmotic pressure of embryonic cells which have been treated with gamma rays for one day is the same as the control cells. There is a difference, however, in osmotic pressure of cells when the embryos were treated for a period of two days or more. The effect becomes more pronounced as the time of irradiation increases.

Experiments followed using X rays delivered at a high intensity. With the exception of 1000 r, all doses given were about the same as those given with the low intensity gamma rays. No change in osmotic pressure was observed

* Consultant

after 100 r, while 200 r showed variation within the first five hours after X radiation. Embryos receiving 1000 r in ten minutes show a change within two hours after treatment. There is some individual variation, since some of the cells show a difference in osmotic pressure immediately after receiving 1000 r, while cells in other embryos will not show any change for an hour or so.

This experiment shows that osmotic pressure variations produced by irradiation are not due to an intensity factor, but depend on the total dose received by the cells. Cells receiving 100 r or less do not show any osmotic pressure change, while cells treated with 200 r or more show a distinct osmotic pressure difference.

Effect of oxygen tension on radiation-induced mitotic inhibition. (Gaulden and Nix) Experiments have been completed which indicate that the amount of oxygen present during irradiation modifies the extent of mitotic inhibition produced by the radiation. Grasshopper eggs were placed in polystyrene chambers which were evacuated and flushed with the desired gas three times and then given 64 r of X rays while in an atmosphere of a particular gas. Immediately following irradiation the chambers were opened to the air of the room. The total time of treatment from beginning of evacuation through irradiation was about five to seven minutes. Embryos were dissected from the eggs and made into hanging-drop preparations for microscopic study. The number of cells in mid-mitosis (prometaphase, metaphase, and anaphase) was recorded every 22 minutes for six hours after treatment. Since 22 minutes is the average duration of mid-mitosis (at 38° C), and since the treatments used do not affect this duration, the number of cells completing mitosis in a given period of time can be determined.

The following gases or mixtures of gases were used: 100 per cent oxygen, air (21 per cent oxygen), 10 per cent oxygen and 90 per cent helium, 2 per cent oxygen and 98 per cent helium, nitrogen, carbon dioxide, and also vacuum. Irradiation of cells in the higher concentrations of oxygen results in greater mitotic inhibition, that is, the period after irradiation during which there are no cells in the mid-mitotic stages is prolonged, and recovery from this inhibition period is slower than in cells irradiated in lower concentrations or in absence of oxygen. The difference between the mitotic inhibition produced by irradiation in 100 per cent and that produced by irradiation in 21 per cent oxygen is not statistically significant (Fig. 9), whereas the decreased mitotic inhibition produced by irradiation, nitrogen, carbon dioxide,

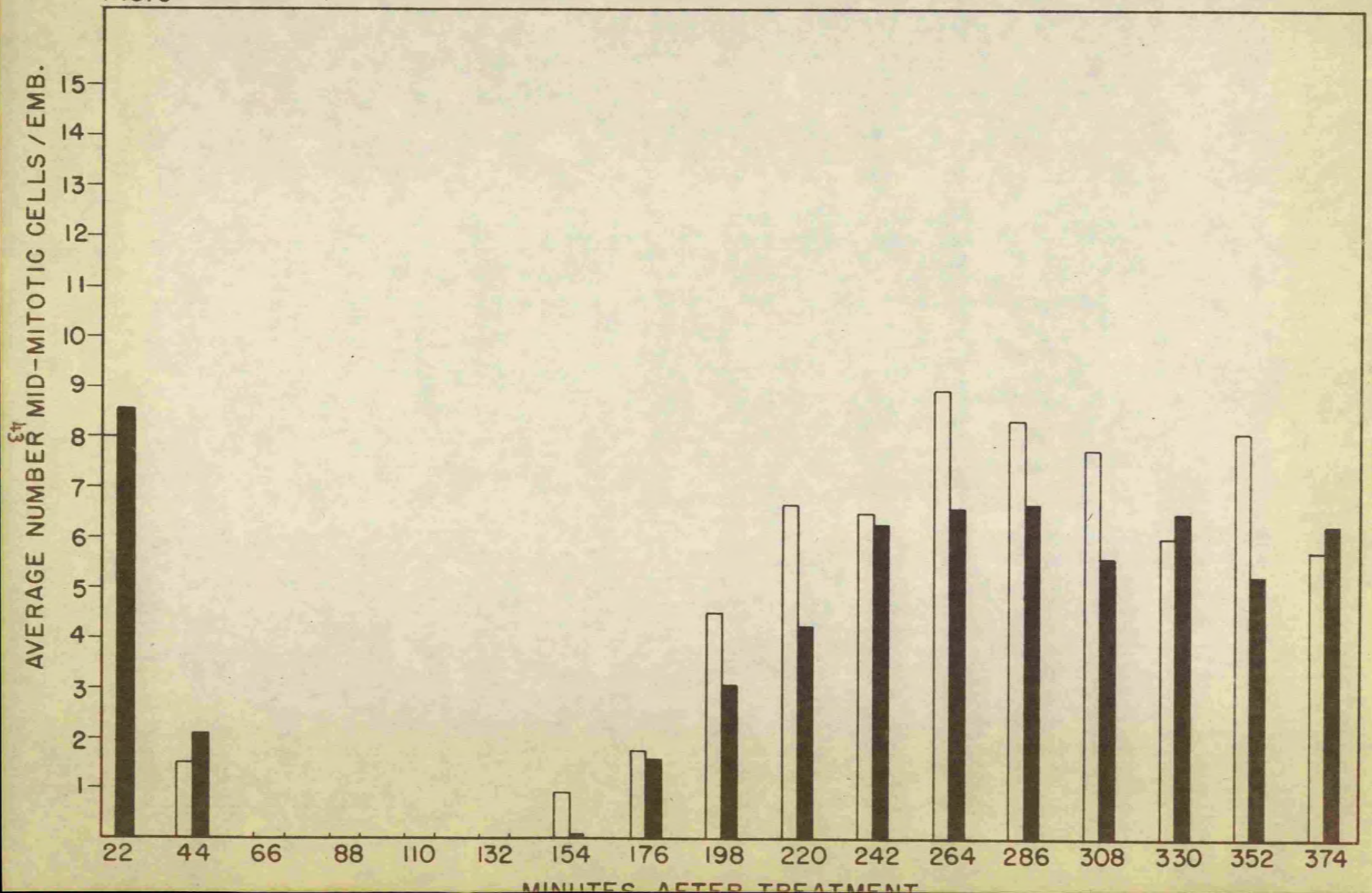
Figure 9

White bars: embryos irradiated with 64 r X rays in 21 per cent oxygen (air).
Black bars: embryos irradiated with 64 r X rays in 100 per cent oxygen.

UNCLASSIFIED

DWG. #9004

FIG. 9



or vacuum (Figs. 10, 11, 12) is quite significantly different from that produced in 21 and 100 per cent oxygen. The data obtained with irradiation in 2 and 10 per cent oxygen are now being treated statistically to determine their significance. Cells treated with the various gases and/or vacuum without radiation do not show any mitotic inhibition. It appears, therefore, that the degree of sensitivity of cells to X radiation with respect to mitosis can be correlated with the amount of oxygen present at the time of irradiation.

Mitotic rate of cells in the grasshopper egg. (Gaulden and Nix) Work has been continued on a study begun last quarter to determine whether the mitotic rate of cells removed from the egg immediately after irradiation and grown in artificial culture medium is the same as the mitotic rate of cells which are not removed from their natural environment until some hours after treatment. This can best be determined by establishing the time at which the maximum number of mid-mitotic cells occurs following radiation-induced mitotic inhibition.

One group of eggs was irradiated with 64 r of X rays and placed at 38° C along with a control group that had received no irradiation. Embryos were removed and fixed at 22-minute intervals over a period of six hours, the embryos being mounted in the same manner as when prepared for study in the living condition. The number of cells in mid-mitosis was determined for each fixation time which corresponded with each observation made on living preparations.

Preliminary experiments show that in embryos which are not removed from the egg immediately after treatment the greatest number of cells in mid-mitosis is found 190 to 220 minutes after treatment. In hanging-drop preparations the greatest number of mid-mitotic cells is found 240 to 265 minutes after treatment. More experiments are in progress.

Beta-ray exposures. (Gaulden) A study has been initiated to determine in some detail the effects of beta rays on cell structures and the mitotic process in the grasshopper embryo neuroblasts with a view to comparing these effects with those produced by other types of radiation. With the help of Dr. C. W. Sheppard and Mr. Herman Cember, Health Physics trainee, an apparatus has been devised for treating the neuroblasts which takes into consideration the physical and biological conditions which must be satisfied if accurate estimations are to be made of the amount of beta radiation reaching the cells.

Figure 10

White bars: embryos irradiated with 64 r X rays in 21 per cent oxygen (air).
Cross-lined bars: embryos irradiated with 64 r X rays in nitrogen.

UNCLASSIFIED

DWG. #9005

FIG. 10

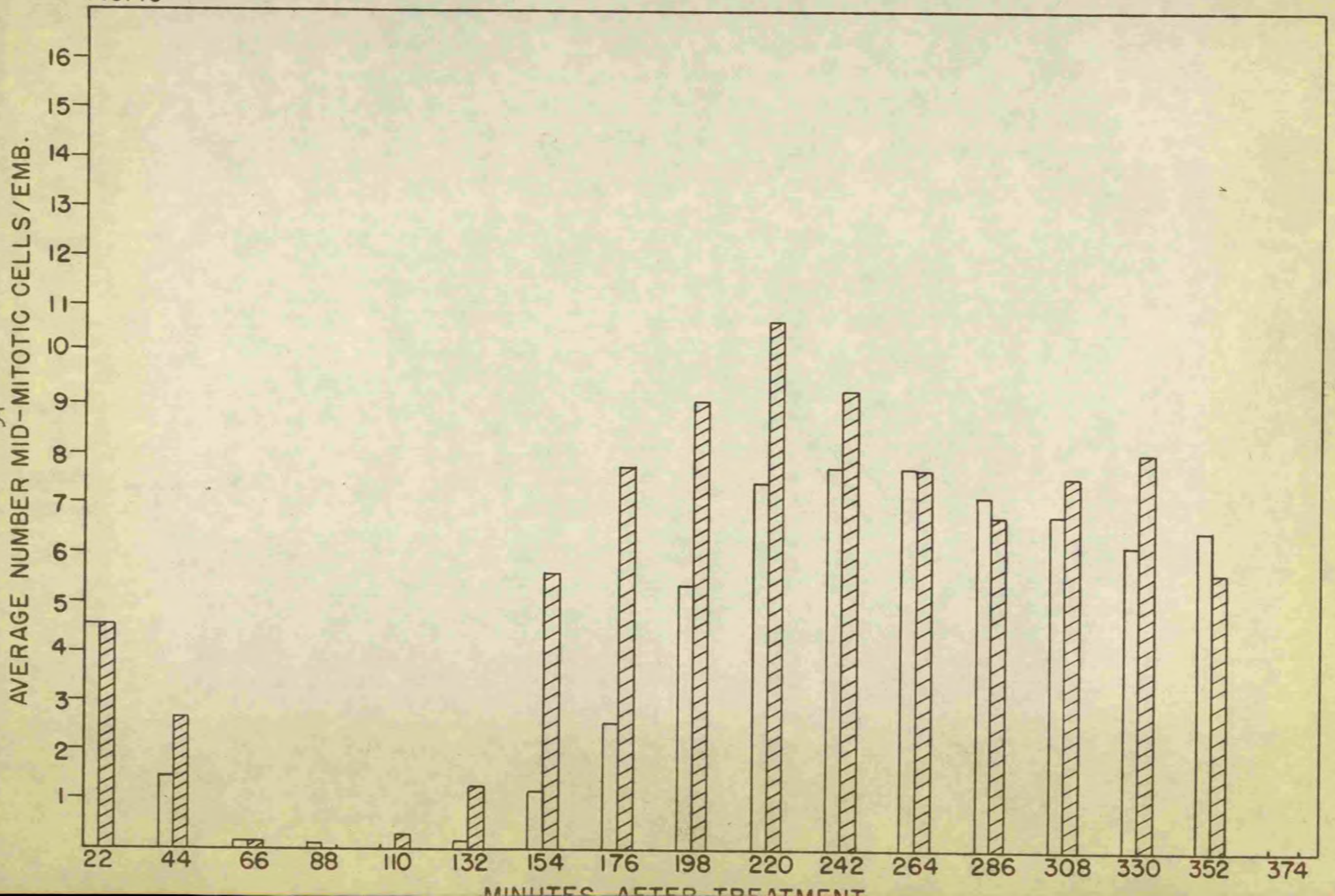


Figure 11

White bars: embryos irradiated with 64 r X rays in 21 per cent oxygen (air).
Cross-hatched bars: embryos irradiated with 64 r X rays in carbon dioxide.

UNCLASSIFIED

DWG. #9006

FIG. 11

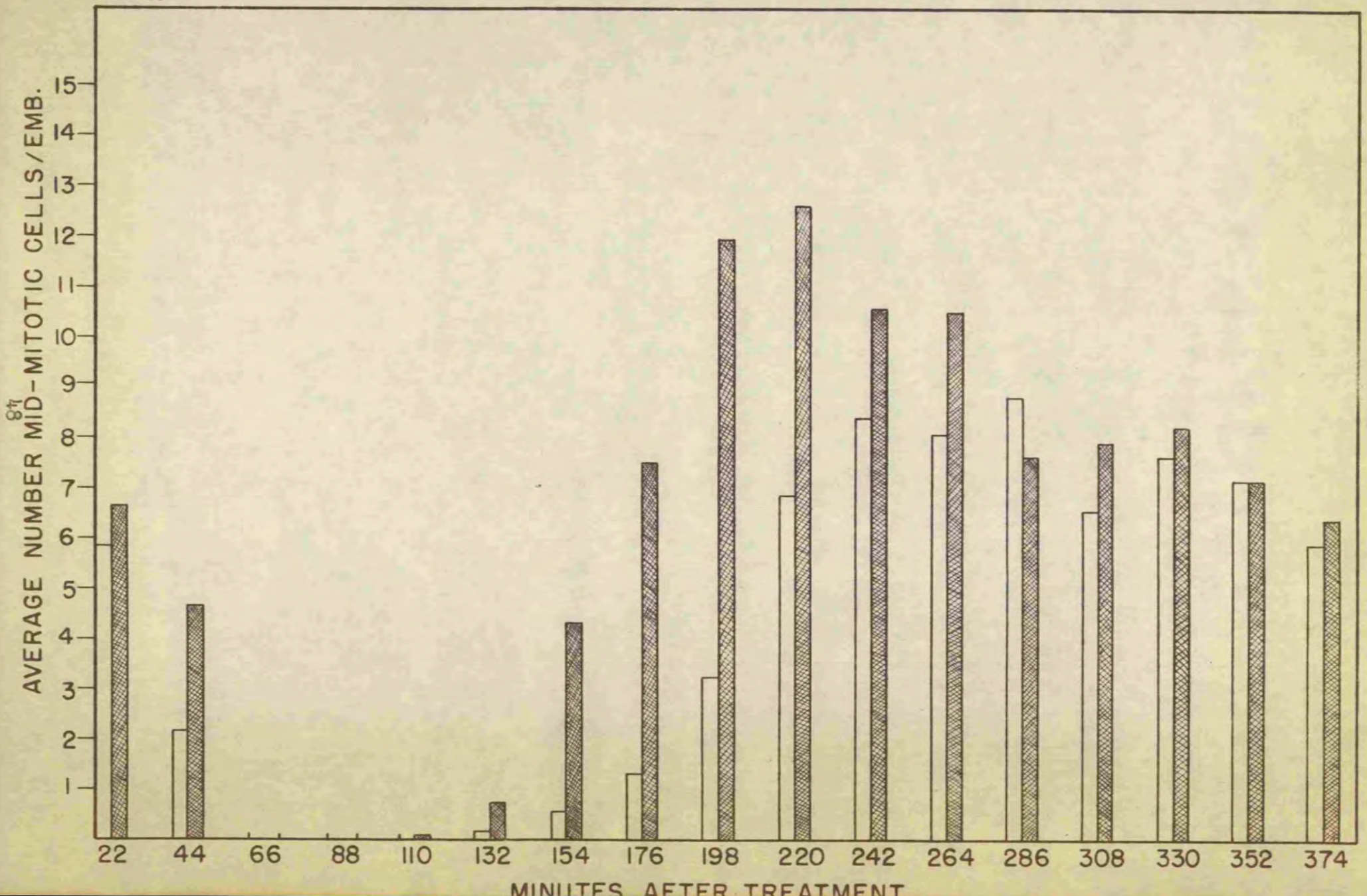


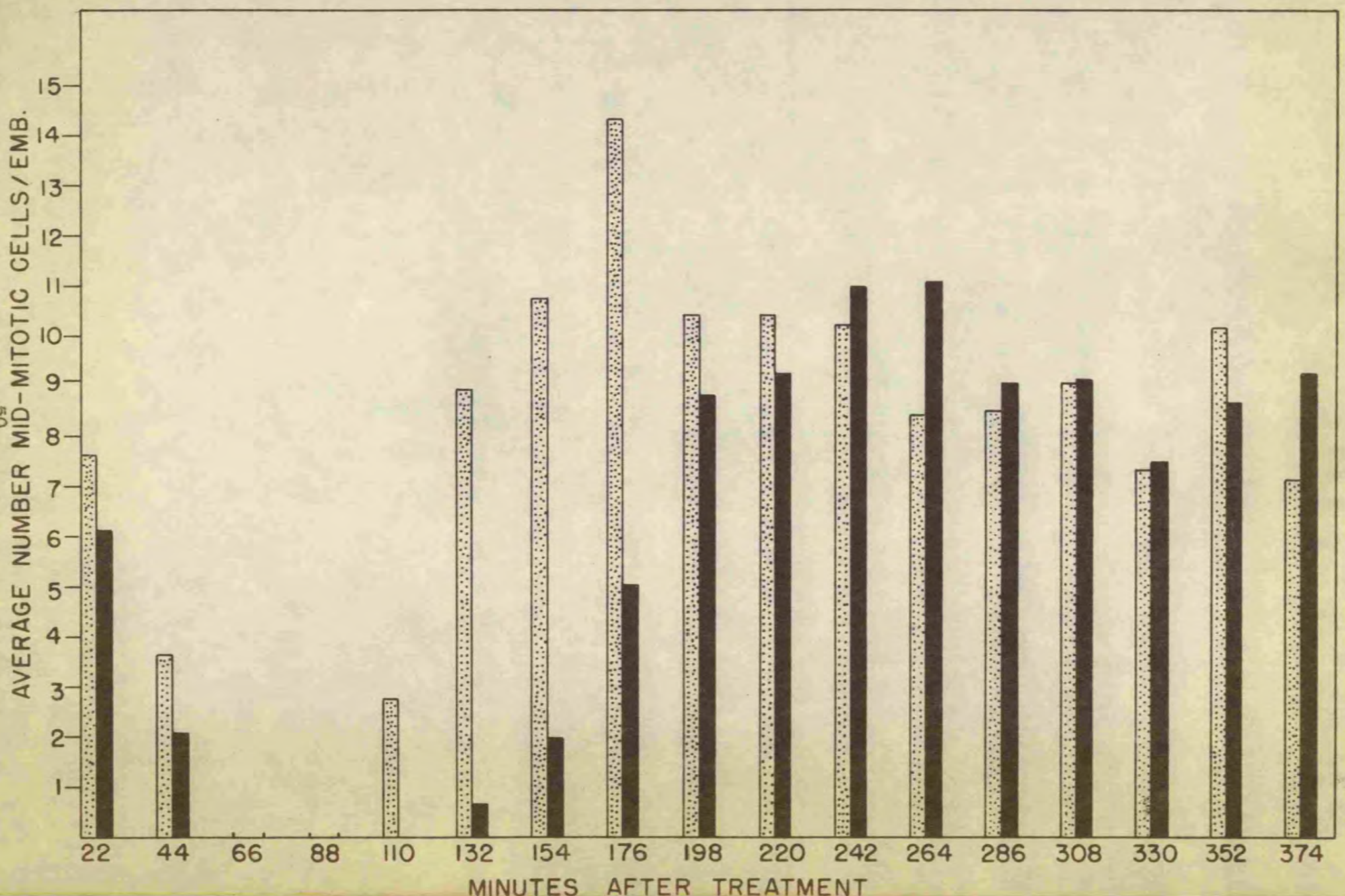
Figure 12

Stippled bars: embryos irradiated with 64 r X rays in vacuum.
Black bars: embryos irradiated with 64 r X rays in 100 per cent oxygen.

UNCLASSIFIED

DWG. #9007

FIG. 12



The exposure chamber consists of two close fitting rings between which can be stretched a sheet of rubber hydrochloride (0.0002 in. thick) to make a "dish." The rubber hydrochloride membrane absorbs a negligible amount of beta rays and at the same time holds a liquid (culture medium). The cells, surrounded by culture medium, are placed in the bottom of the dish and the source, a ^{32}P bakelite plaque, is placed in contact with the lower surface of the dish. Thus the cells can be placed almost in direct contact with the source. The culture medium provides a uniform material around and above the ce[illegible], a condition which eliminates discontinuities in the system and thereby allows for more accurate calculations of the intensity of the radiation at the level of the cells.

EFFECTS OF RADIATION ON *DROSOPHILA* GENETICS

William K. Baker* (Leader) Elizabeth Sgourakis

The effect of oxygen concentration on the induction of gene mutations. (Baker and Sgourakis) Previous studies indicate that the frequency of X-ray induced gene mutations in *Drosophila* is greatly reduced if the flies receiving the radiation are maintained in an atmosphere of low oxygen concentration during exposure. Experiments were performed which, it was hoped, would clarify various aspects of this oxygen effect, namely: (1) Is this effect one of indirect action of X rays on the genes? (2) Will cysteine, which protects the sulfhydryl enzymes from the action of oxidizing agents, lower the induced mutation rate in flies which spend the larval period growing on cysteine-containing media.

In order to test for an indirect effect, mature virgin females of the Muller-5 strain of *Drosophila melanogaster* were exposed to X rays while in an atmosphere of pure oxygen at a temperature of 2.0° C. Immediately after exposure these females were mated for a two-hour-period with untreated, mature wild-type males of the Oregon-R strain. By progeny testing it was possible to determine if any of the "activated substances" produced in the reproductive tracts of the irradiated females induced any mutations in the untreated sperm. Of the 1507 wild-type X chromosomes tested in this fashion, only 0.47 per cent bore recessive lethals. This figure is not significantly higher than the spontaneous mutation rate for the Oregon-R strain.

* Consultant

In the cysteine experiments, mature stock females of the Oregon-R strain were permitted to lay eggs on fresh standard culture medium to which cysteine hydrochloride had been added in the following concentrations of 0.1, 1.0, and 2.0 per cent. The larvae which hatched from these eggs fed on these media, and in all bottles, except for those containing 2.0 per cent cysteine hydrochloride, there was a good yield of adult flies. Adult Oregon-R males were selected from these bottles and irradiated in atmospheres of either pure oxygen or nitrogen at 2.0° C (3000 r, 250 kvp, 15 ma). After treatment, these males were mated to untreated, mature, virgin females of the Muller-5 strain. Using the Muller-5 method of detecting sex-linked recessive lethals, the following results, shown in Table 6, were obtained:

TABLE 6

GAS	PER CENT CONC. OF CYSTEINE HYDROCHLORIDE	NO. CHROMOSOMES TESTED	PER CENT LETHAL
Oxygen	Controls (no cysteine)	258	16.3
	0.1	310	15.8
	1.0	163	13.5
Nitrogen	Controls (no cysteine)	539	5.8
	0.1	544	5.8
	1.0	505	6.1

(These data are based on F_2 counts only. Confirmatory test crosses have not yet been examined.)

The differences in the X-ray sensitivity of the flies grown in the various concentrations of cysteine are not significant. It should be noted that a different method of administration of the cysteine might produce a protective effect.

Experiments were performed to determine if the mutations induced in the sperm are randomly distributed among the fertilized eggs laid during different time intervals after mating. Oregon-R males were subjected to X rays in

atmospheres of oxygen or nitrogen at two temperatures -- either 26.5 or 1.5° C. After irradiation these males were mated to Muller-5 virgin females. At the end of every 24 hours, over a period of nine days, these parents were transferred to fresh culture bottles. By progeny testing it was possible to determine the mutation rate of the sperm which fertilized the eggs laid during each 24-hour period. Preliminary results indicate that the distribution of these mutations is not random. For the first three days after mating, there is no significant difference in mutation rate induced in flies tested in oxygen and nitrogen at the warm temperature. But in oxygen at this temperature, the rate of mutation for the fourth 24-hour period differs significantly from the previous 24 hours by a threefold increase. On the eighth and ninth days after treatment, there is a significant increase in the number of mutations produced in nitrogen at the warm temperature over the previous four days.

At 1.5° C the percentage of mutations recovered in oxygen-treated flies during the third 24-hour period differs significantly from the previous 24-hour period. There is no apparent difference in the nitrogen-treated sperm throughout the duration of the experiment. These experiments raise the possibility that cells in different stages of spermatogenesis may differ in oxygen concentration within the cells under this method of treatment.

EFFECTS OF RADIATION ON MUTATION OF FUNGI

Alexander Hollaender (Leader) Frances L. Martin
G. E. Stapleton R. B. Grayson

Effect of oxygen tension on sensitivity of bacteria to X rays. (Hollaender, Stapleton and Martin) The last quarterly report (ORNL-644) described in detail various factors and conditions which modify, to a great extent, the X-ray sensitivity of bacteria and fungi. In the light of the evidence presented it is difficult to conclude that only the "direct action" of this radiation plays an important role in producing the lethal effect.

Of the factors and conditions studied, two seemed of sufficient interest and importance to warrant further investigation at this time: (1) effect of oxygen tension and (2) protective action of organic medium during irradiation.

Effect of oxygen tension. As previously shown, the X-ray sensitivity of strain B/r of *Escherichia coli* is dependent, to a considerable degree, on the oxygen tension during irradiation. In these studies the bacterial cells were grown aerobically in nutrient broth 8 g/liter for 24 hours at 37° C. Previous to irradiation the cells were removed from the broth by centrifugation, washed and suspended in phosphate buffer, filtered through cotton filters, then samples of the filtered suspensions were placed in small glass irradiation vessels in which they could be evacuated, after which they were bubbled with oxygen or nitrogen for 15 minutes previous to irradiation.

Evacuation of suspensions to be treated with oxygen was discontinued once it was established that this process had no detectably harmful effect on the suspended cells.

After irradiation the suspensions were diluted appropriately and plated on nutrient agar (Difco) then incubated aerobically at 37° C for 24 hours. As shown in Table 7 or Fig. 13, the ratio of survivors at 60,000 r under the two different gases is

$$\text{nitrogen/oxygen} = > 1000$$

whereas the relative sensitivity for oxygen-treated samples as compared with nitrogen-treated ones is approximately threefold. (The relative sensitivity of the bacteria is the ratio of doses of radiation necessary, under the two conditions, to produce the same quantitative response.)

The same modification of sensitivity has been demonstrated on *E. coli*, strain B, which has been reported to be more sensitive to radiation than B/r. These experiments have confirmed this finding. Since strain B is more sensitive to X rays, the rate of killing is greater under both conditions studied, nevertheless the ratio of sensitivity of oxygen-treated cells to nitrogen-treated cells is approximately equal to that found for B/r. (Fig. 14).

Since it is well established that *E. coli* is a faculative anaerobe, it was decided to compare the relative sensitivity of this organism grown anaerobically previous to irradiation, with the same strain grown under strictly aerobic conditions.

TABLE 7

Comparative sensitivity of aerobic and anaerobic E. coli strain B/r

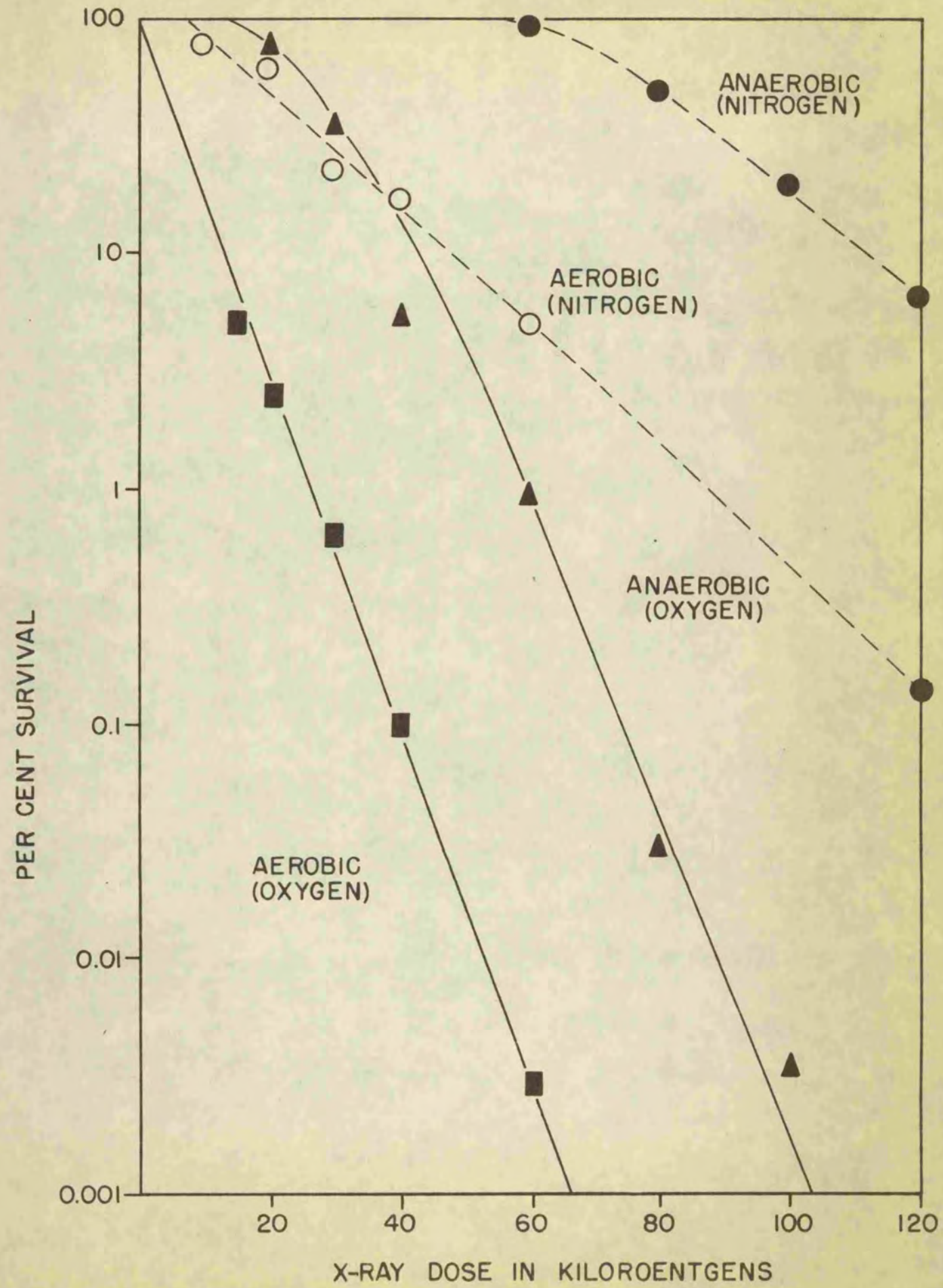
DOSE IN ROENTGENS	AEROBIC				ANAEROBIC			
	OXYGEN		NITROGEN		OXYGEN		NITROGEN	
	PER CENT SURVIVAL	NO. OF COLONIES COUNTED	PER CENT SURVIVAL	NO. OF COLONIES COUNTED	PER CENT SURVIVAL	NO. OF COLONIES COUNTED	PER CENT SURVIVAL	NO. OF COLONIES COUNTED
20,000	2.51	319	66.50	4383	77.5	269	106.5	548
30,000	0.626	5295	20.77	3048	35.0	2094	105.5	1826
40,000	0.113	1428	16.92	3004	5.09	1774	95.2	461
60,000	0.0038	4326	4.66	7909	1.97	3139	107.2	1769
80,000					0.0306	403	35.2	1416
100,000					0.0038	574	20.2	418
120,000			0.0143	541			4.75	374

All exposures in M/15 phosphate buffer pH = 6.8, Temperature = 2° C.

DWG. #9008

FIG. 13

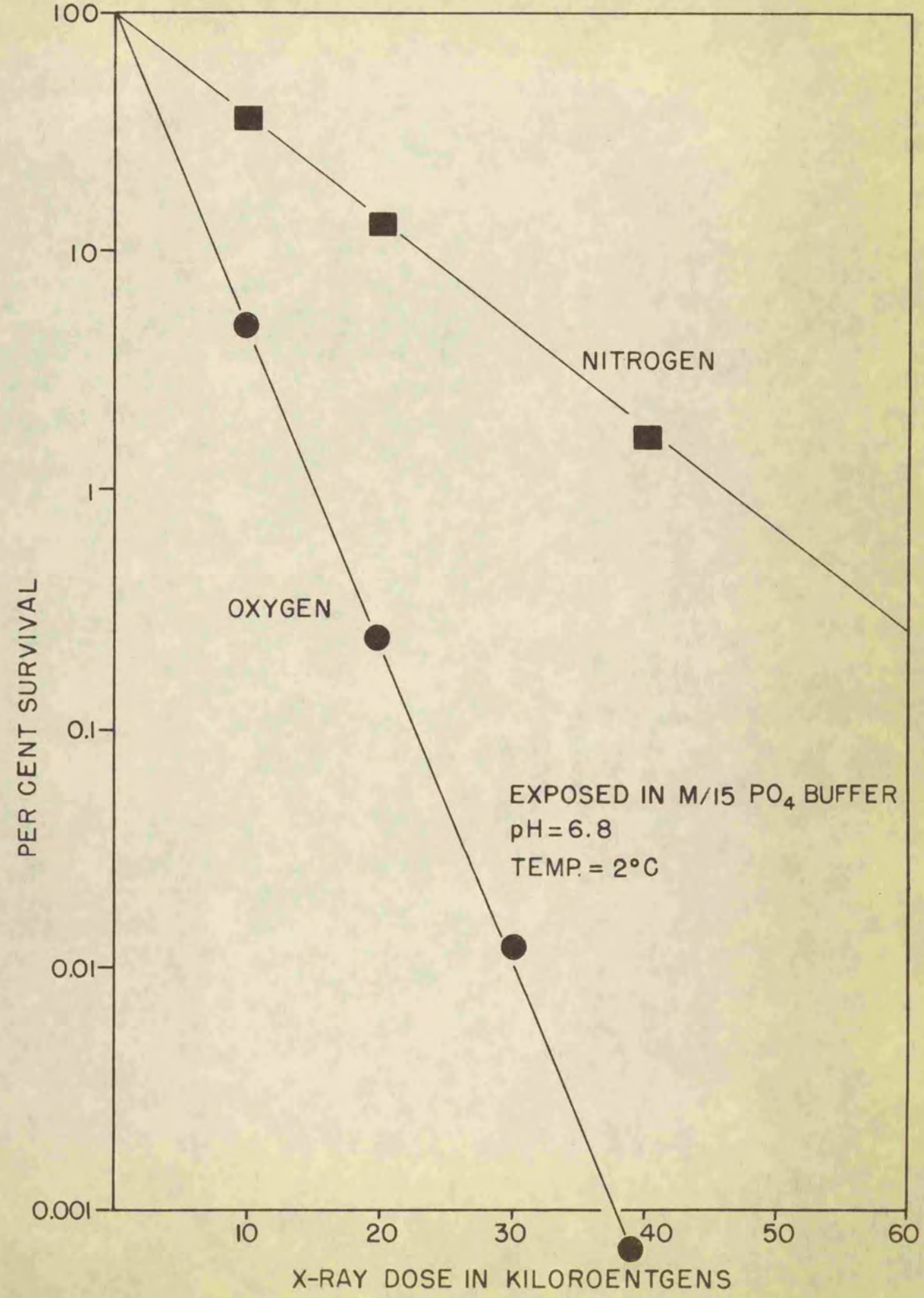
SURVIVAL OF E. COLI (STRAIN B/r)



DWG. #9009

FIG. 14

SENSITIVITY OF E. COLI (STRAIN B)



A small inoculum was transferred to nutrient broth to which buffer and 0.2 per cent glucose had been added; the inoculated culture was then bubbled with nitrogen continuously for the duration of the growth period of 24 hours at 37° C. The aerobic culture was bubbled with air continuously for the same length of time. Suspensions of both the anaerobically and aerobically grown bacteria were bubbled before irradiation with oxygen or nitrogen and then exposed to X rays. After irradiation the samples of both cultures were plated and incubated under aerobic conditions at 37° C for 24 hours. No attempt has been made to grow the organisms anaerobically after irradiation. The bacteria grown anaerobically before irradiation were found to be sufficiently radioresistant to warrant extending the dose to 120,000 r.

Table 7 and Fig. 14 show that, when the anaerobic cells are exposed in the presence of oxygen or nitrogen, a definite threshold dose is established. This is in the case of cells kept anaerobic before and during irradiation, a dose of 60,000 r or more being necessary to produce any lethal effect.

It is felt that perhaps the threshold dose in the case of those cells grown anaerobically prior to irradiation, then placed in the presence of oxygen during irradiation, was an artifact created by the return of these cells to consumption of oxygen during the exposure. To test this possibility anaerobic cells were held for an hour previous to irradiation in phosphate buffer bubbled with oxygen. The results showed no similarity in sensitivity to aerobic cells, treated with oxygen, but rather the survival curve checked point for point that previously determined for anaerobic cells.

The ratio of survival for the cells grown anaerobically prior to radiation is

$$\text{nitrogen/oxygen} \cong 10^6,$$

The ratio of sensitivity between oxygen and nitrogen treated anaerobic cells is again, perhaps fortuitously, very close to three.

If the sensitivity of the extreme cases is compared, that is, cells grown aerobically prior to irradiation and irradiated in the presence of oxygen with those grown anaerobically before irradiation and irradiated in the absence of oxygen, a factor of ten is found.

The possibility of oxygen still being present in the anaerobic cells is not ruled out, since some of the manipulations are not carried out under strictly anaerobic conditions; also, traces of oxygen are undoubtedly present in the nitrogen gas used.

This suggests an interesting question concerning radiosensitivity of strictly anaerobic microörganisms, or the sensitivity of microörganisms whose respiration has been minimized by poisons such as cyanide, azide, or other chemicals.

Both of these questions are being investigated at the present time.

Protective action of organic medium during irradiation. It was reported previously that bacteria (*E. coli* — B/r) were more sensitive to X rays, when exposed in phosphate buffer, than in nutrient broth (Difco) 8 g/liter. Since broth contains a wide variety of amino acids it was decided to test the protective action of amino acids, first in groups and then individually if the group tests appeared promising.

A group of representative amino acids were prepared in buffer solutions and deaerated by evacuation and then held under nitrogen until the bacterial cells were introduced. All of the amino acid solutions were so prepared, since it was found in the early phases of this study that, unless fresh solutions were used, the protective action of the amino acids was lost. All of the solutions, with the exception of cysteine, lost completely their protective capacity, if bubbled with oxygen before irradiation.

The effect of amino-acid solutions as protective agents was studied from two points of view; (a) the effect of amino acid concentration on bacterial survival, (b) the protective action of optimum concentration of amino acids as obtained from (a) on survival as a function of X-ray dose. Glycine, alanine, norleucine, aspartic acid, glutamic acid, lysine, tryptophane and cysteine were used, in concentrations up to 0.02 molar.

The results indicate (Fig. 15 and Table 8) that only glutamic acid and cysteine afforded appreciable protection over the range of concentrations used. Per-cent survival against amino-acid concentration, has been plotted. A constant dose of 60,000 r was used in all experiments, the ratio of survivors giving an indication of the protective action. Since all the amino acids except cysteine and glutamic acid showed approximately the same protection, the average range of values for the group is plotted.

FIG. 15

EFFECT OF AMINO ACIDS ON X-RAY SENSITIVITY

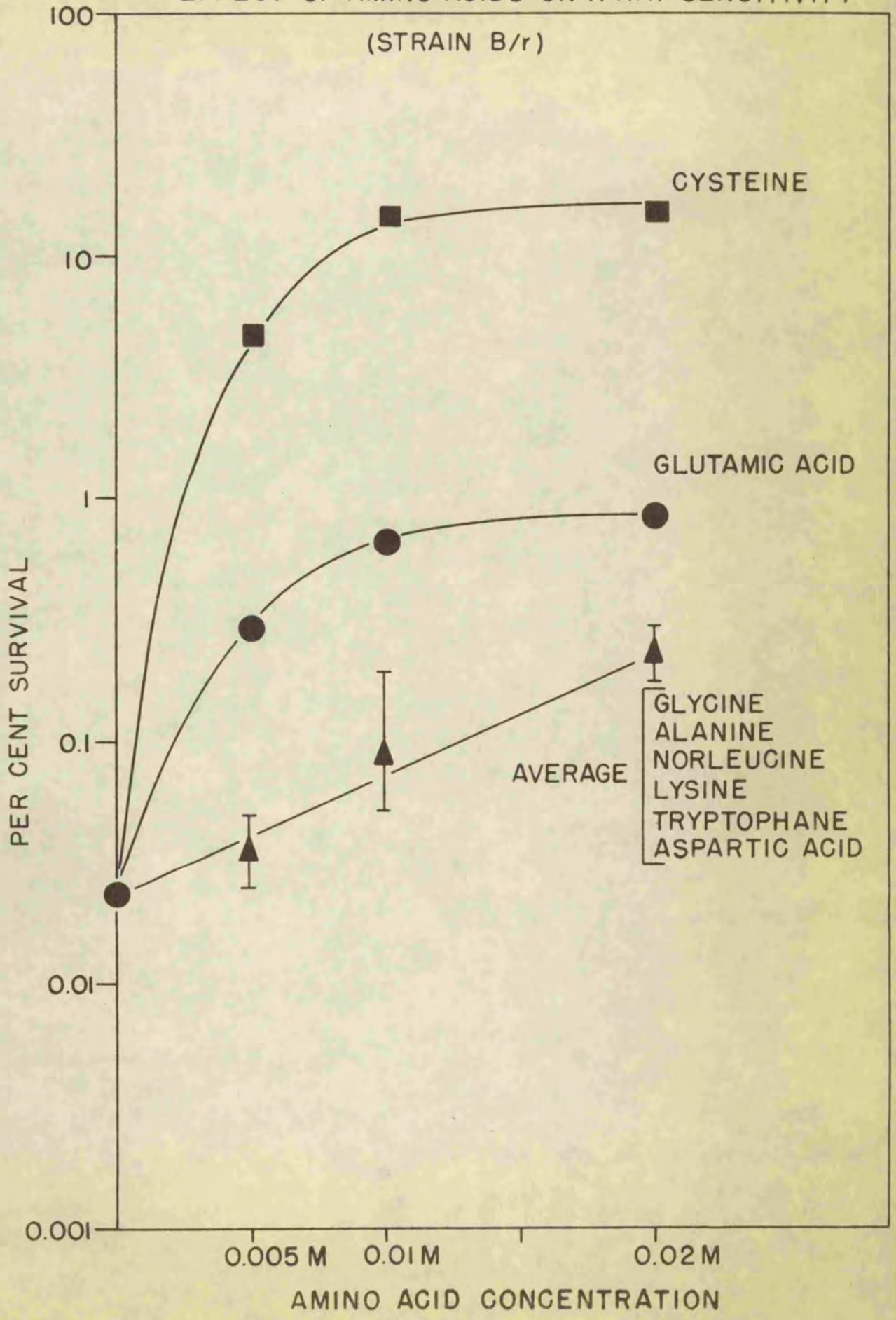


TABLE 8

Effect of various amino acids on X-ray sensitivity (X-ray dose = 60,000 r)

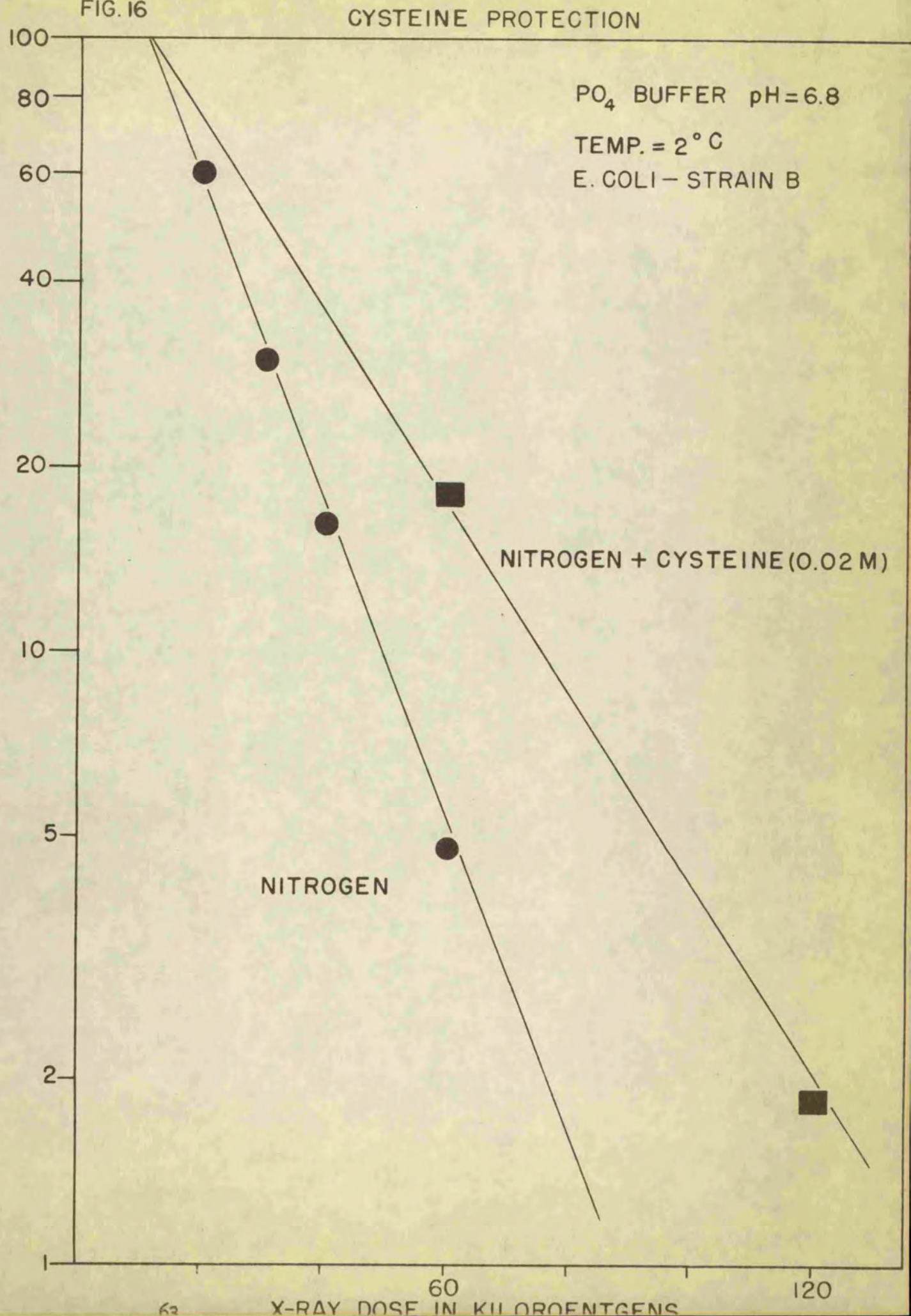
M CONC.	GLYCINE		LYSINE		TRYPTOPHANE		GLUTAMIC		CYSTEINE	
	PER CENT SURVIVAL	NO. OF COLONIES	PER CENT SURVIVAL	NO. OF COLONIES	PER CENT SURVIVAL	NO. OF COLONIES	PER CENT SURVIVAL	NO. OF COLONIES	PER CENT SURVIVAL	NO. OF COLONIES
0.000	0.0163	654	0 0232	1102	0 0256	2851	0.0216	1182	0.0334	1927
0.005	0.0243	1794	0.0337	2024	0.0175	721	0.345	775	4.785	646
0.010	0.0546	2541	0.0345	1909	0.207	397	0.685	1534	14.35	2500
0.020	0.1922	1476	0.3140	1306	0.2275	1173	0.822	830	14.50	2188

Finally, the sulfur-containing amino acid cysteine was tested in combination with the usual nitrogen treatment (as previously explained) to determine if the amino acid had a protective action over and above that afforded by oxygen replacement by nitrogen. Fig. 16 is a plot of survival as a function of X-ray dose for nitrogen and nitrogen plus cysteine (0.02 molar in phosphate buffer). As suspected from the data (Fig. 15 and Table 8) the sensitivity of the bacteria is reduced beyond the value obtained by nitrogen treatment alone. When, on the other hand, cysteine was added in the same concentration to *anaerobically* grown cells irradiated in the presence of nitrogen, no significant increase in survival was noted.

Discussion. It appears from the data presented here that there are two different effects; (1) radiation produces changes in the medium which can be reduced by lowering the oxygen tension of the medium; (2) the studies with anaerobic cells, on the other hand, indicate that essentially complete removal of oxygen from the cells also results in lowering of their X-ray sensitivity. The combination of the two protective systems, which is shown in Fig. 14, results in extreme resistance of the organisms to radiation. The possibility that respiration is intimately tied up with the radiation sensitivity seems to be well borne out by these tests. The use of respiratory inhibitors should give further information on this question.

FIG. 16

DWG. #9011



MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION ON MICE

W.L. Russell (Leader)
Liane B. Russell
Louis Wickham
J.C. Kile
Josephine S. Gower
Jane Crowell
Gloria Jones
Mary Henderson

The incidence of sterility and partial sterility in the offspring of X-irradiated mice. (W. L. Russell, Gower, Jones and Henderson) Data have already been presented (Biol. Quarterly Report, ORNL-644) on the incidence of sterility and partial sterility in the male offspring of irradiated male mice. Results obtained from a group of 15 female offspring of irradiated males are now adequate to establish which of these offspring are partially sterile.

Radiation factors were the same as those given in the earlier report. The irradiated males were 101 × C3H F_1 hybrids. These were mated to females of a noninbred test strain. The 15 female offspring used in this experiment were conceived within from two to six days after irradiation of the father. Each of these offspring was mated with a vigorous and fertile 101 × C3H F_1 hybrid male. The number of litters and mean litter size for each of these matings are given in Table 9.

None of the 15 females was completely sterile, but it is apparent from the table that five (Nos. 16, 23, 28, 29 and 39) can clearly be classified as partially sterile. A sixth (No. 13), which died after producing only two litters, was provisionally classed with the partially steriles. Her status has been confirmed by tests of two of her offspring. Both of these proved to be partially sterile. The results for all females are summarized in Table 10.

For similar dose ranges Snell (Genetics, 20:545, 1935) obtained reduced fertility in 33 per cent of 55 tested female offspring of irradiated males, and Hertwig (Z.i.A.V., 79:1, 1940) in 44 per cent of 96 tested females. The 40 per cent obtained in our small sample is thus in close agreement. Since the data for male and female offspring are similar, they have been combined in Table 11 for a comparison with the results of Snell and Hertwig.

TABLE 9

Number of litters and mean litter size for each test mating

SERIAL NO. OF ♀ AND DOSE GIVEN TO HER SIRE	NO. OF LITTERS	MEAN LITTER SIZE
5 (1000 r)	5	10.0
7 (500 r)	12	8.8
13 (750 r)	2	3.0
15 (750 r)	12	8.3
16 (750 r)	11	4.5
17 (750 r)	10	7.4
22 (500 r)	14	7.6
23 (500 r)	12	4.5
28 (750 r)	14	3.2
29 (1000 r)	14	3.4
30 (1000 r)	14	8.9
32 (500 r)	1	10.0
37 (500 r)	13	8.5
39 (750 r)	16	3.0
44 (750 r)	11	9.4

TABLE 10

Incidence of sterility and partial sterility in female offspring of irradiated males

DOSE TO ♂ PARENT (r)	OFFSPRING			
	STERILE	PARTIALLY STERILE	FERTILE	TOTAL
500	-	1	4	5
750	-	4	3	7
1000	-	1	2	3
Total (Mean dose 717 r)	-	6	9	15

TABLE 11

Incidence of sterility and partial sterility in offspring (male and female combined) of irradiated males

INVESTIGATOR	DOSE TO ♂ PARENT (r)	OFFSPRING			
		PERCENTAGE			TOTAL NUMBER
		STERILE	PARTIALLY STERILE	FERTILE	
Snell	400-1200 (mostly 600-800)	11.5	25.3	63.2	182
Hertwig	500-1000 (mean 764)	6.1	33.3	60.5	114
Russell	500-1000 (mean 669)	8.1	27.0	64.9	37

Recently the results of Lorenz, *et al.* (Radiology, 49:274, 1947), who detected no partial sterility in the offspring of mice exposed to 8.8 r per day, have been widely quoted with the apparent implication that they are at variance with the findings of Snell and Hertwig. Hertwig, however, had already shown that the incidence of partial sterility is high only in offspring conceived within a short period (about four weeks) following irradiation. If the experimental procedure of Lorenz, *et al.* is analyzed with this in mind, it is easy to explain why no partial sterility was detected by them. The results obtained here, 13 affected individuals in a sample of 37, represent only a small group accumulated incidentally to another project, but they have seemed worth presenting because, as far as they go, they do support the work of Snell and Hertwig.

If reciprocal chromosome translocation is regarded as the principal cause of the partial sterility, then it is apparent that the radiation induced incidence of this type of aberration is very much higher in mice than it is in *Drosophila*.

Artificial insemination of mice. (Kile) The development of a convenient and productive method for the large-scale artificial insemination of mice has been completed. Good results were obtained by inseminating cyclic females using the method previously described (Biol. Quarterly Report, ORNL-537). The advantage of using the cyclic estrus is appreciable, as the waiting periods of three to six weeks required by other methods are eliminated. The females were inseminated during the cyclic estrus from 1½ to 4½ hours following mating with a vasectomized male. This sterile mating is a convenient way to effect embryonal implantation.

Method. Females of the C57 brown strain were kept with vasectomized C57 black males in a room in which the daily light cycle was controlled by an electric timing switch. The lights were turned off at 7 A.M. and on at 3 P.M.; and mating, which accompanies the onset of estrus, usually occurred between 10 A.M. and 2 P.M. The sperm suspension was introduced through the wall of each uterine horn from 1½ to 4½ hours following mating. Semen was collected by stripping the vasa deferentia of a C57 brown male with watchmaker's forceps. The paternity of the offspring could be determined by the disposition of pigment in the eyes at birth, as well as by coat color a few days later.

The females were anesthetized with ether and a mid-ventral incision from 4 to 6 mm long was made 1 cm anterior to the clitoris. The uterine horns were alternately lifted from the abdominal cavity with a small glass hook and injected with from 0.22 cc to 0.10 cc of sperm suspension with a 28-gauge hypodermic needle. The laparotomy was closed with two layers of silk sutures.

Results. Of 103 inseminations performed, 74 (71.8 per cent) were fertile. The mean litter size of the delivered young was 5.97. The results are given in Table 12.

TABLE 12

Insemination of cyclic mice following sterile matings

SPERM MEDIA	NUMBER INSEMINATED	NUMBER FERTILE	PER CENT FERTILE	NO. LITTERS DELIVERED	MEAN SIZE OF LITTERS DELIVERED
NaCl	59	42	71.2	38	6.34 ± .44
Salt-Dex.	44	32	72.7	29	5.48 ± .46
Totals	103	74	71.8	67	5.97 ± .32

As is shown, there was no significant difference obtained with the two sperm media. Many of the females were inseminated two or more times. Three of them produced three litters each. Six of the females failed to deliver their full-term litters, possibly the result of surgical damage at the time of insemination. One other female ate her young prior to counting. The difference between columns 3 and 5 in Table 12 is due to these seven females.

Border cases, such as questionable mating times, were not excluded from insemination, so it is possible that the percentage of fertility can be increased somewhat if inseminations are made only under optimum conditions. However, the success obtained here may already approach that occurring from natural matings in the inbred strain used.

Effects of low doses of X rays on the development of mouse embryos. (Russell, Russell and Henderson) Following irradiation at developmental stages critical for certain characters a high incidence of characteristic abnormalities was obtained (see ORNL-595). This made it appear likely that

doses considerably lower than those used previously (200 r, 300 r, 400 r) — possibly low enough to fall within the usual human fluoroscope range — would produce abnormalities and thus constitute a human hazard.

In addition to looking for severe structural abnormalities, it was decided to use material in which quantitative changes could also be easily detected in a limited sample For this purpose, the strains of mice chosen were two which show natural variability with respect to certain characters (the positions of the thoracolumbar and lumbosacral borders respectively). The relative proportions of types are constant for each strain, and since we are dealing with individuals which are genetically alike, environment is thus proved to play a role in the development of these characters. It was thought that even a small dose of radiation, administered at the particular critical time (determined in previous experiments), might change the relative proportion of types from that characteristic for controls Since the development of a great many characters (in addition to the ones studied in this experiment) has been shown to be influenced by environmental factors, the present investigation has widespread application

The two strains were irradiated at three stages of pregnancy and with four doses (200 r, 100 r, 50 r, 25 r). A control littermate was used with each irradiated female. To date, up to 91 newborns are available per stage-dose group, with a total of 291 irradiated animals in one strain and 223 in the other, the control numbers being 378 and 252 respectively. Since the study of skeletons is not yet completed, only a progress report can be given, citing samples from two stage-dose groups of one of the strains in comparison with a control sample (see Table 13). Several other characters which are also being studied are not included in this table.

It may be seen that, even with 100 r, the incidence of severe structural abnormalities is high (one-third of all individuals) and that the shift in the quantitative character is complete Some effect may thus be expected from the 50-r and perhaps even 25-r irradiations It should be pointed out that these doses fall into the range used in human fluoroscopy.

TABLE 13

		NUMBER OF SIDES WITH INDICATED GRADE OF RIB DEVELOPMENT							PER CENT SEVERE STRUCTURAL ABNORMALITIES	
		IN RIB NO. 14					NO. 15			
TREATMENT	NO. OF ANIMALS	0	1	2	3	4[a]	1	PER CENT OF SIDES WITH FULLY FORMED No. 14	RIBS	STERNUM
Controls	26	5	10	4	16	17	0	32.7	0	0
100 r	27	0	0	0	0	52	2	100.0	33.3	29.6
200 r	5	0	0	0	0	10	0	100.0	20.0	60.0

[a] indicates fully formed fourteenth rib, as is also present when a low grade fifteenth rib has appeared

MICROBIOLOGY

TRACER STUDIES ON METABOLISM

S. F. Carson (Leader) E. F. Phares
D. S. Anthony W. J. Jefferson
Mary V. Long

Propionic acid fermentations. (Carson, Anthony, Phares) Pyruvate was carbon source with $\overset{*}{C}H_3COOH$ and $CH_3\overset{*}{C}OOH$ in tracer amounts (high specific activity). Following are tabulated the specific activities (c/s/mg $BaCO_3$) of individual carbon fragments of the resulting propionic acid.

	$\overset{*}{C}H_3COOH$	$CH_3\overset{*}{C}OOH$
CH_3	0.72	0.035
CH_2	0.82	0.027
$COOH$	0.04	1.20

These results give very strong support to the theory of formation of at least a substantial amount of C_4 precursor of propionate through C_2 condensation.

These results are mainly possible due to the elegant degradation of propionate.

Pigeon liver and breast muscle. (Carson, Anthony, Phares, Pomper) Feed material used was $CH_3\overset{*}{C}OCOOH$ with minced tissues suspended in Krebs-Ringer solution. When malate or succinate was to be isolated, carrier was added at the *beginning* of the experiment (with the tissue acting on pyruvate*). The gas phase contained 90 per cent oxygen and 10 per cent carbon dioxide; the latter contained 26 atom per cent C^{13}. Methods of degradation previously described are: (a) succinate $\longrightarrow$ fumarate + malate; (b) malate $\longrightarrow$ lactate + CO_2; (c) lactate $\longrightarrow$ acetate + CO_2; (d) acetate $\longrightarrow$ methylamine + CO_2.

Pyruvate* was separated by HSO_3^- extraction and subsequently tested by acid-steam distillation (pyruvic acid is "60 per cent steam volatile"; no activity appeared in the distillate). If any acetate* had been present, it also

should have appeared in the distillate, since acetic acid is "100 per cent steam volatile".

Table 14 shows the results. All counts reported are at least 10 × background; background is about 0.007 c/s/mg barium carbonate (this figure allows comparison since background equals 0.3 c/s, and disc usually contains about 40 mg barium carbonate).

TABLE 14

MALATE	LIVER		MUSCLE	
	C^{14}	C^{13}	C^{14}	C^{13}
COOH	0.05			
CHOH	0.84		0.61	
CH_2	0.16		0.001	
COOH	0.06	1.251	0.03	1.083

SUCCINATE	LIVER		MUSCLE	
	C^{14}	C^{13}	C^{14}	C^{13}
COOH	0.005		0.002	
CH_2	0.20		0.07	
CH_2	0.04		0	
COOH	0.005	1.093	0.002	1.084

Note: C^{14} = c/s/mg $BaCO_3$

C^{13} = atom per cent C^{13}

These experiments are being repeated under conditions in which there is a much greater conversion of pyruvate to succinate, in order (1) to be able to omit primary addition of succinate, and (2) to obtain much higher specific-activity succinate, so that the succinate can be given the most rigorous and complete purifications and tests.

The C^{13} values are an excellent check on the *nonparticipation* of a "CO_2 + C_3 reaction" for the formation of liver succinate, muscle malate and muscle succinate. Liver malate, on the other hand, shows C^{13} excess in carboxyl as would be expected.

Methods research. (Phares, Anthony) A method of partition chromatography has been used to separate the nonvolatile acids, which will eventually include lactic acid. Previously reported systems, chiefly chloroform-butanol, would not accomplish separation of lactic acid from succinic acid. An equal mixture of ethyl ether and amyl alcohol against an inside phase of 0.5 normal sulphuric acid on celite was successful.

Preliminary work on ion-exchange separation of these acids is being carried out.

Oxidation of methyl amine derived from acetic acid by the Schmidt reaction appears to be best accomplished with alkaline permanganate in a closed system. The usual persulfate and van Slyke oxidation yield only 70 - 75 per cent of the calculated carbon dioxide.

When persulfate oxidation occurred in the presence of Cl^-, ten times the usual silver nitrate in the presence of sodium chloride (this is an excess of Ag^- over Cl^-) permitted about 50 per cent oxidation of succinic acid.

BIOCHEMISTRY

BIOCHEMISTRY OF NUCLEOPROPERTIES, AMINO ACIDS AND ENZYMES

C. E. Carter (Leader)
W. E. Cohn
D. G. Doherty
F. Vaslow
E. Volkin
J. X. Khym
M. L. Morse
M. Helen Jones
Ann R. Webster
E. A. Lloyd
J. R. Totter (Res. Participant)

Heterogeneity in pyrimidine nucleotides. (Cohn) In earlier reports evidence has been presented for the existence of isomerism in the naturally occurring purine ribonucleotides, adenylic and guanylic acids, the exact nature of which still remains to be established. The first step in the establishment of this heterogeneity, namely, two peaks in the ion-exchange elution diagram, has now been duplicated in the pyrimidine nucleotides, cytidylic and uridylic acids, isolated and characterized by our usual accepted procedures, in which no trace of purine compounds could be detected. This, together with small but significant differences in spectral properties, is taken as evidence of heterogeneity, if not true isomerism, in the pyrimidine nucleotides, thus raising the number of isolatable mononucleotides from yeast nucleic acid to eight.

In addition, careful analysis of the cytidylic acid fraction isolated by our usual methods from an enzymatic hydrolysate of desoxyribonucleic acid, which yields the four desoxyribonucleotide fractions expected, has resulted in the isolation of a small amount of material possessing spectral properties similar to, but differing significantly from, desoxycytidylic acid (280/260 ca. 3.1; maximum, 287 mμ. at *p*H 2). The base liberated from this nucleotide has spectra identical with those reported for 5-methyl cytosine.

Samples of the five desoxynucleotides have been sent to other laboratories currently investigating vitamin B_{12} and folic acid requirements in bacteria.

Structure of desoxynucleotides. (Carter and Webster) The desoxypentose nucleotides prepared by ion-exchange methods following the enzymatic degradation of thymus nucleic acid have been studied as substrates for a specific 5′ nucleotidase isolated from bull semen. The results of this investigation

have shown that all the desoxynucleotides liberate inorganic phosphate in the presence of the specific enzyme. The kinetics of the enzymatic hydrolysis of phosphoester group of muscle adenylic acid and desoxyadenylic acid are identical. Desoxyguanylic acid is hydrolyzed at approximately the same rate whereas desoxycytidylic and thymidylic acids are degraded at a slower rate. None of the nucleotides isolated from ribose nucleic acids are hydrolyzed by the enzyme. The results of these investigations indicate that in all the desoxynucleotides phosphate is esterified on the 5 position of the deoxyribose. The studies of the acid hydrolysis of desoxyadenylic acid have shown that, although the phosphoester group is hydrolyzed by a specific 5′ nucleotidase, acid hydrolysis liberates the group at a rate similar to that for the acid hydrolysis of yeast adenylic acid, a 3′ phosphoester. It is concluded from these data that the purine desoxynucleotides are 5 phosphoesters but that this linkage in desoxyribose compounds is acid labile.

Chemical synthesis and identification of new nucleic acids. (Doherty) A new fractionation scheme was devised to separate the isomeric adenylic acids in sufficient quantities for chemical degradation studies. Investigation of the stability of the pure adenylic acids "A" and "B" in aqueous solution revealed that they were mutually interconvertible. This reaction was slow at the *p*H of the adenylic acids and was catalized by the presence of hydrogen ions. An equilibrium mixture of the two components was obtained from either pure isomer. Since the purine nucleotides are somewhat unstable under acid conditions some degradation to adenosine and adenine took place. This transformation was confirmed by W. E. Cohn with the acid-stable purine nucleotide cytidylic acid.

Several characters of the adenylic acids have been prepared and, on hydrolysis, have yielded identical compounds. A direct proof of the structure of adenosine-3′-phosphoric acid without first recourse to degradation to inosinic acid has been devised. An optically inactive ribitol phosphoric acid was obtained upon reduction, confirming Levine's early work on adenosine-3′-phosphoric acid. Application of this procedure to adenylic "A" has also yielded an optically inactive ribitol phosphoric acid. Thus the weight of present evidence indicates that the isomerism is not owing to the position of the phosphoric acid radical but rather, is an intramolecular structural isomerism. Further proof of this is being sought. This procedure is being extended to all the available nucleotides to further establish their structure.

Preparation and analysis of mammalian ribonucleic acid. (Volkin, Carter and Jones) Ribonucleic acids (RNA) have been isolated and purified from mammalian tissues by a procedure utilizing their insolubility in concentrated solutions of guanidine hydrochloride (Report ORNL-585). Nucleotide analyses of these preparations were carried out by degrading the RNA in mild alkali and resolving the resulting mononucleotide components by anion-exchange chromatography. The results of the analyses of eleven such preparations are reported here. The preparative scheme has been modified slightly and will be described in some detail.

METHOD 1

The fresh tissue is immediately homogenized for five minutes with three volumes per gram tissue of 0.15 *M* NaCl, 0.02 *M* phosphate *p*H 6.8. Desoxyribonucleoprotein is removed by centrifugation at 3000 g for 30 minutes. Both operations are carried out at 0 - 4° C. The supernatant solution is now made 2-molar with guanidine hydrochloride. After warming at 36° C for 30 minutes and chilling at 0°C for 30 minutes, ribonucleoprotein precipitates, while the bulk of the tissue protein remains in solution. The nucleic acid-protein complex is washed with cold 2-molar guanidine hydrochloride then twice extracted with chloroform, octyl alcohol (4:1). To insure complete separation of the nucleic acid from the protein by the latter process, the extraction is carried out at 45°C in the presence of 2-molar guanidine. Extractions in the cold, or in saline or water solutions, result in incomplete separation on nucleic acid. RNA is then precipitated in the cold with two volumes of ethanol, washed twice with 70 per cent ethanol and dissolved in water.

METHOD 2

The possibility of nuclease action on RNA during the preliminary steps of the preparation can be obviated by immediately homogenizing the tissue in concentrated guanidine hydrochloride. The latter reagent is an effective protein denaturant and the presence of any enzymatic activity in high concentrations of this reagent is quite unlikely. Duplicate liver RNA preparation made by the two methods yielded essentially identical analytical compositions, indicating that, in liver at any rate, little or no nuclease hydrolysis occurs in the first procedure.

In this method, the tissue is immediately homogenized with three volumes per gram tissue of cold 2.5-molar guanidine hydrochloride solution. The rest of the method follows that of Method 1, except that the RNA protein complex is washed at least three times with cold 2-molar guanidine hydrochloride to insure complete removal of any contaminating desoxyribonucleic acid.

The method of mononucleotide analysis of RNA, described in detail in ORNL-585, will be briefly summarized here. RNA was quantitatively hydrolyzed to mononucleotides by 0.5 *N* NaOH at 37°C for 17 hours. The hydrolysate was diluted to 0.02 *N* NaOH and run through the resin column (Dowex-1). After washing free of excess alkali, the elution of the mononucleotides was carried out with increasing concentration of hydrogen chloride. Hydrogen chloride concentrations 0.001, 0.003, 0.005 and 0.007 *N* eluted in succession cytidylic, adenylic, uridylic and guanylic acids, respectively. Quantitative determination of mononucleotides in the effluent fractions was carried out by ultraviolet spectrophotometry, based on the following molar extinction coefficients at 260 mμ: cytidylic, 21,800; adenylic, 39,000; uridylic, 31,000; and guanylic 32,700. Recovery of nucleic acid in terms of mononucleotides was based on the total absorption units at 260 mμ in the eluates compared with that contained in the original hydrolysate.

The analytical compositions of eleven ribonucleic acid preparations from various sources are shown in Table 15. For comparative purposes, nucleotide compositions were expressed as molar ratios, with that nucleotide of lowest molar quantity assigned unity. In all cases this latter nucleotide was uridylic acid. In summary it may be stated that there are differences in RNA composition for the same tissue of different species and likewise, the RNA of various tissues from one species differ in composition. Of the pyrimidine nucleotides cytidylic acid predominates over uridylic, while of the purines, guanylic acid is always in excess of adenylic. In this respect, the extremely high guanylic acid content of calf thymus and pancreas are noteworthy. The purine/pyrimidine ratios are close to one in some cases, but never less than one in any preparation. Rabbit liver 2 was ribonucleic acid prepared by Method 2 from a different animal from that of rabbit liver 1. The similarity in composition between the two preparations is evident. On the other hand, the two calf liver ribonucleic acid preparations were derived from the same liver by Methods 1 and 2 respectively, and here also the analytical composition compares favorably.

TABLE 15

Analytical composition of mammalian ribonucleic acids.

RNA SOURCE	PER CENT UNITS RECOVERED	MOLAR RATIOS				PURINE/PYRIMIDINE RATIO
		CYTIDYLIC	ADENYLIC	URIDYLIC	GUANYLIC	
Rabbit liver 1	94	1.70	1.01	1.00	2.03	1.13
Rabbit liver 2	92	1.69	1.05	1.00	2.02	1.12
Rat liver	95	2.21	1.17	1.00	2.14	1.03
Regenerating rat liver	97	1.97	1.08	1.00	2.05	1.05
Mouse liver	96	1.49	1.16	1.00	1.88	1.22
Mouse hepatoma	98	1.87	1.21	1.00	1.87	1.07
Calf liver 1	96	1.77	1.19	1.00	2.13	1.20
Calf liver 2	93	1.84	1.15	1.00	2.15	1.16
Calf spleen	99	2.05	1.16	1.00	2.28	1.13
Calf thymus	96	2.13	1.53	1.00	3.64	1.65
Calf pancreas	100	1.77	1.05	1.00	3.62	1.69

Enzyme-substrate equilibria. (Vaslow and Doherty) Work has continued on the measurement of chymotrypsin-substrate equilibria, with considerable time being spent in refining the measurement techniques. To date measurements of the assumed uniunimolecular dissociation constant, $K = \frac{[E][S]}{[ES]}$ as a function of *p*H and temperature, are given in Table 16. The experiments were run with 1 per cent chymotrypsin solution, and 0.1 *M* sodium chloride and 0.01 *M* phosphate salt buffer. The substrate, bromine-labeled acetyl dibromotyrosine varied in concentration from 5×10^{-7} to 1×10^{-5} without affecting the value of K. The molecular weight of chymotrysin was taken as 40,000.

TABLE 16

Variations of K with pH and temperature

*p*H	5°C	20°C	*p*H	5°C	20°C
5.3		4.8×10^{-3}	7.1	1.1×10^{-2}	
5.8		6.1×10^{-3}	7.4	1.6×10^{-2}	
7.6		2.3×10^{-2}	7.5	1.7×10^{-2}	
			8.0	0.1	

Taking the value of K at 5°C and *p*H 7.6 as 1.6×10^{-2}, the values of $\Delta F°$ for this *p*H at 5 and 20°C respectively are -2300 and -2200 cal/mol. The value of $\Delta S°$ is +7 cal/mol and of $\Delta H°$ is -250. These tentative figures refer to the apparent equilibrium

$$\text{enzyme} + \text{substrate} \rightleftharpoons \text{enzyme - substrate}$$

and do not take into account the strong *p*H variation shown experimentally. More complete measurements of the *p*H variation and of the reaction kinetics must be made before the nature of the primary enzymatic process can be determined.

Infrared spectroscopy. (Vaslow and Doherty) The infrared spectra of acids, and of several other derivatives of adenine have been obtained. In the range of 5.8 to 6.3 μ, adenine and its derivatives have two strong bands characteristic of double-bond vibrations. Without specifically identifying their origin, the wave lengths for the compounds are given in Table 17.

TABLE 17

Absorption wave lengths of adenine derivatives in microns.

COMPOUND	WAVE LENGTH	
Adenine	6.00	6.27
Adenosine	6.00	6.25
Sodium salt adenosine-3′-phosphoric acid	5.98	6.25
Adenine HCl	5.88	6.18
Adenine sulphate	5.89	6.18
Adenosine-3′-phosphoric acid	5.88	6.18
Adenosine-5′-phosphoric acid	5.88	6.18
Adenylic acid A	5.93	6.18
Sodium salt adenylic acid A	5.94	6.22

It is seen that the adenylic acid bands are those corresponding to the salts of adenine rather than to the free base. Furthermore, the formation of the sodium salt, tying up the phosphoric acid residue, brings the spectrum to the adenine free base type. It is, therefore, believed that the adenylic acids in the crystalline state exist as either inter- or intramolecular salts. Chemical evidence indicates that adenylic "A" may have an intramolecular salt linkage.

Nucleic acid metabolism of microorganisms. (Morse and Carter) Investigation of *Escherichia coli,* strains B and B/r, has continued with emphasis on the cytological appearance of the two strains. Cytological evidence which supports the differences in desoxyribonucleic acid content previously reported has been obtained. The B/r strain contains increased (two to four) numbers of Feulgen-positive bodies when examined in 24-hour nutrient broth cultures. The B strain contains one or, at most, two bodies per cell at this time. The cytological appearance of the B/r strain, however, is influenced by nutrient conditions, the nature of which is not completely known at present but which is believed to be associated with sugar to phosphate ratio (Duguid, J. Path. Bact., 60:265, 1948). After growth in fresh medium, B and B/r become cytologically indistinguishable. Somewhat unsuccessful attempts have been made to secure a photographic record of the above cytological differences. It is hoped that experiments now in progress will show whether the extra nuclear material in the B/r strain is genic material.

RADIOBIOCHEMISTRY

C. W. Sheppard (Leader)

W. T. Burnett, Jr. E. B. Darden, Jr.
Leon Wish Gertrude Beyl

Radiological physics. (Darden) The principal activity has been in preparing a somewhat comprehensive report on the use of thimble chambers for gamma-ray measurement. This report includes a thorough review of the early literature and a complete discussion of the theory of thimble chamber measurements.

Erythrocytes. (Beyl) Work continues on the nature of the disturbance of erythrocyte potassium accumulation. Preliminary studies on the effect of radiation on glycolysis, on cholinesterase activity of erythrocytes, and on the effect of oxygen are in progress. A rather complete study of the sodium transport processes in irradiated cells has been made and will be reported in the near future.

Colloids. (Wish, with the assistance of the Pathology and Physiology group.) Experiments on the fate of colloidal gold containing ^{198}Au after intravenous injection in normal rabbits and mice were continued. However, the sols were prepared with trisodium citrate instead of ascorbic acid as the re-

ducing agent. By varying the *p*H of the gold chloride solution before the addition of the citrate, small, medium, and large-sized particle sols were obtained. Several of their physical properties have served as a qualitative measure of particle size, namely: color, transparency, Tyndall beam intensity, and its extinction by polarization. The electron microscope is now being used for more quantitative measurement of particle size.

Since the very small particles, 5 mμ or less, approach the region of ionic gold, 0.1 to 0.2 mμ, it is extremely essential to ascertain whether any ionic gold escapes reduction. The interpretation of physiological data will depend on the physical nature of the injected gold. By the measurement of the redox potentials of the trisodium citrate and gold chloride solutions and mixtures of the two in the cold, it was possible to evaluate the per cent of the total gold remaining in solution. The results are shown in Table 18.

TABLE 18

SAMPLE NO.	FINAL *p*H	COLOR	PARTICLE SIZE	ESTIMATED Au (PER CENT OF TOTAL)
1	7.00	light pink	very small	2
2	7.05	pink	small	1
3	6.72	dark pink	small	1
4	6.57	dark pink	small	1
5	5.40	red	medium	0
6	4.32	deep red	large	0

The ionic gold remaining in the medium and large particle sols was found to be practically zero but, as the *p*H was raised, approximately 1 to 2 per cent of the total gold was ionic. It is believed that this is due to the slower rate of formation of the higher *p*H sols.

The disappearance from the circulation of the small, medium and large particle sols was investigated in rabbits and mice. The mice were of an inbred strain and of uniform age and sex. These were sacrificed serially and the amount of radioactivity in the blood and various organs measured. The excreta of three mice, each injected with one of the sols, was collected and analyzed for the radioisotope. All activities were measured with an automatically recording gamma-ray ionization chamber. By including determinations of

the initial activity of the total animal and of the carcass, excellent material balances were obtained. Only the disappearance from the blood was followed in rabbits. Radioautographs of various tissues were made.

During the first few minutes following injection most of the activity was found in the circulation and thereafter the rate of disappearance depended on the size of the colloidal particles injected. Within a few hours approximately 90 per cent of the activity of the large particles appeared in the liver and spleen, whereas most of the small sol remained in the circulation in this time. Similar disappearance curves were obtained for both the rabbits and mice, although the disappearance was slower in the mouse. Large amounts of the activity were found in the urine and feces of animals injected with the small particle sol, but only a trace appeared in the excreta of those injected with the large colloid.

The amount of activity injected is sufficient to accumulate a radiation dose of about 2,000 r in the liver and spleen when a large particle sol is used. This produced a definite over-all weight loss after about three days. Of the organs studied, the greatest decrease in weight occurred in liver and spleen.

Radioautographs showed a progressive relative increase of activity in the macrophages of the liver and spleen.

Blood volumes. A paper on the direct plasma and cell volume determinations in normal and hypervolemic mice using the classical T-1824 dye and radioisotope techniques has been prepared and is now being reviewed.

High specific activity ^{59}Fe has been obtained and electrolytic cells are being made for the electroplating of iron. Both ^{59}Fe and ^{55}Fe can be determined by this method. Special counters are being constructed by the Instrument Department for this work. These isotopes are to be used to measure cell volumes directly and to estimate the life span of erythrocytes in irradiated animals.

Mineral metabolism. (Burnett) The mode of action of manganese in biological systems is not known although the element is an essential micronutrient. Since its functions in mammalian metabolism may be related to certain enzyme systems, as suspected from *in vitro* activation, it has been elected to begin a study of the biological behavior of manganese with the pancreas, the cells of which elaborate a variety of enzymes.

By injecting rats and mice with radiomanganese, confirmation was obtained of the scattered data in the literature indicating that the pancreas contains a relatively high concentration of manganese. A definite time lag was observed between the time of injection of the isotope and its appearance in the pancreas, a fairly constant level then being maintained. Uptake by the liver proceeded at a much faster rate as did the subsequent excretion by the liver into the intestinal tract.

The next step was to establish the appearance of manganese in pancreatic juice. For this, dogs were provided with artificial pancreatic fistulas by Dr. R. R. Bigelow (consultant-Oak Ridge Hospital) and given intravenous injections of radiochemically pure manganese chloride solutions containing either the 2.59-hour or the 310-day isotope of manganese. The radioactivity appeared in the essentially pure juice in amounts sufficient to be measured directly in a gamma-ray ionization chamber so that the changes in concentration of manganese could be followed, under different physiological conditions, without resorting to laborious and unreliable chemical methods. Since the manganese content of pancreatic juice does not appear to have been previously reported, the data are being reinforced and extended by spectrographic analyses. It is of interest that in one experiment carried out over a period of several days the concentration of manganese as well as the total nitrogen concentration increased during the peak periods of flow which occurred immediately after feeding the dog. There was also a concurrent increase in the manganese-total nitrogen ratio. This appears to be one of the first pieces of evidence obtained by an *in vivo* experiment suggesting a possible relationship between manganese and the pancreatic enzyme systems.

The rapid disappearance of radioactivity from the circulation of the dog within several minutes after being introduced into the blood stream, an event demonstrated with an external counting arrangement, makes it seem reasonable to assume that the manganese was converted into the colloidal form before being taken up by the liver. The blood of the dog two hours after injection contained only 0.028 per cent of the injected dose per ml, whereas the pancreatic juice contained 0.145 per cent. Seventy hours after, the values for the blood and pancreatic juice were 0.026 and 0.155 per cent, respectively.

The evidence thus far indicates that the liver is a distribution center for manganese that is utilized by other tissues as well as being the principal organ for ridding the body of an excess of the element. In this connection it

should be noted that Hahn and associates (Sheppard, Wells, Hahn, and Goodell, J. Lab. & Clin. Med., 32:274-286, 1947) have shown that when radioactive manganese colloidal particles are injected intravenously more radioactivity can be found in the pancreas than can be accounted for on the basis of the phagocytic activity of the cells of this organ. The suggestion has been made that some of the particles are broken down into the ionic form for redistribution to other tissues.

The manganese content of the kidneys was comparable to that of the pancreas; nevertheless, as also found by other investigators, very little activity was excreted in the urine of dogs injected with radiomanganese. A finding which will be given further consideration is the considerable activity found in the stomach walls, small amounts also being found in the stomach contents.

PLANT PHYSIOLOGY

G. R. Noggle (Leader) Eleanor Schumacher

Production, isolation and purification of C^{14}-labeled carbohydrates. (Noggle and Schumacher) A method for the chromatographic separation of the monosaccharides, disaccharides and trisaccharides from plant extracts has been perfected. The details will appear in a paper now in preparation.

Using the above method in conjunction with the method for separating glucose and fructose (Noggle and Bolomey) several batches of radiosugars have been prepared. To date 2 g of radioglucose, 1.5 g of radiosucrose and 0.5 g of radiofructose have been purified and crystallized. Some of the sugars are to be turned over to the Isotope Division for sale while the remainder will be used in experiments. Ten millicuries of C^{14} per week are now being put into plants for biosynthesis purposes.

Studies on carbohydrate metabolism is detached sugar beet leaves. (Noggle and Schumacher) Some studies have been made on the effect of infiltrating sugar solutions into detached sugar beet leaves while they are in a photosynthesis apparatus. The leaves were pulled from the plants and the petioles placed in water. The leaves were then held overnight in a refrigerator. The next morning the ends of the petioles were cut and placed in different sugar solutions. The material was then placed in a vacuum desiccator and evacuated for one hour. Atmospheric pressure was restored and the leaves,

with their petioles still in the solutions, were placed in the photosynthesis chamber (for full description of apparatus, see paper by Noggle and Bolomey, "The Biosynthesis of Carbon-14-labeled Compounds. I. The Separation of Glucose and Fructose)." Carbon dioxide containing C^{14} was generated and admitted to the chamber. The final concentration of carbon dioxide was 10 per cent. The apparatus was placed in a water bath at 18°C and illuminated for 50 hours. At the conclusion of the period of photosynthesis the leaf chamber was swept with carbon dioxide-free air and the leaves were then removed and weighed. The volume of the sugar solutions remaining in the flasks was also measured. The petioles were immersed in 20 ml of the various solutions. After 50 hours, the volumes of the solutions were as shown in Table 19.

TABLE 19

	SOLUTION REMAINING (ml)	SOLUTION TAKEN UP (ml)	WET WEIGHT (g)
Control (water)	5.4	14.6	19.0
Glucose 0.1 *M*	4.8	15.2	19.0
Fructose 0.1 *M*	9.0	9.0	16.0
Sucrose 0.1 *M*	5.0	15.0	19.5
Raffinose 0.1 *M*	14.6	5.4	14.0
Glucose + Fructose 0.1 *M*	9.6	10.4	18.0

The leaves were extracted in boiling 80-per-cent ethanol and the extracts analyzed for sugars, results of which are shown in Table 20.

The sucrose was analyzed by the invertase method. The amount of raffinose could not be found by this method but a paper chromatogram showed raffinose to be present. The above results would indicate the presence of an active enzyme in sugar beet leaves which is able to split raffinose.

The effect of the nutritional level of the leaves on the ability of the plant to utilize infiltrated sugars is also being studied.

Radiosugars have also been used for infiltrating the leaves but this part of the project has just been initiated.

TABLE 20

Concentration of sugar in plant extracts (per 25 g fresh wt)

	TOTAL SUGAR (mg)	SUCROSE (mg)	FRUCTOSE (mg)	GLUCOSE (mg)
Control	135	35.5	12.5	87.0
Glucose	324	113.5	57.5	153.0
Fructose	232	94.0	53.0	84.0
Sucrose	530	192.0	150.0	188.0
Raffinose	440	192.0	65.0	185.0
Glucose + Fructose	370	113.0	87.0	170.0

Yeast biosynthesis. (Noggle) Three strains of yeast, *Torulopsis utilis* var. *Major*, *Candida pseudotropicalis* and *Saccharomyces cerevisiae* var. *ellipsoideus* are being grown on different carbon sources. From this work it is hoped that a satisfactory method of introducing C^{14} into yeast can be developed. Various extraction and isolation techniques for analyzing the yeast cells are also being studied.

BIOPHYSICS

PHOTOSYNTHESIS

W. A. Arnold (Leader)

R. W. Koza Jane Taylor

Photosynthesis. (Arnold and Taylor) Testing during the past month of an automatic Warburg apparatus, built by the Instrument Department, has shown satisfactory operation for the measurement of photosynthesis. The readings are recorded on a Brown recorder with such sensitivity that the width of the paper corresponds to about 130 mm on the ordinary manometer.

Dr. Hutner has shown that streptomycin acts on the green plant *Euglena gracilis* in such a manner that it produces colorless cells. This experiment is being repeated here with *Chlorella* to see if this action of streptomycin is a general one.

PATHOLOGY AND PHYSIOLOGY

Jacob Furth (Leader)
J. B. Kahn
R. H. Storey
Mary Knoohuizen
Jane Beale
W. D. Gude
J. J. Lane
Germaine Click
Arthur Burke
Eschol Ledford

Exposure of mice to slow neutrons. (Hematology in association with Dr. G. Andrews, Opthalmology by Dr. K. W. Christenberry - Assisted by Lane and Gude) This project, outlined in earlier reports, is making satisfactory progress. Animals of one set are being killed at various intervals following exposure to graded quantities of neutrons; others are kept until natural death. Table 21 shows the number of animals in the major groups of the latter (aging) series.

TABLE 21

Status of slow-neutron (aging) series (both sexes) on May 2, 1950

DOSE	NEUTRON		EQUIVALENT X RAY		CONTROLS
	EXPOSED	PER CENT SURVIVAL	EXPOSED	PER CENT SURVIVAL	
80 min.	T 93	95	61	95	48
(512 r)	B 87	64	-	-	
20 min.	T 72	100	64	100	57
(128 r)	B 69	100	-	-	57
5 min.	T 76	100	77	100	62
(32 r)	B 74	100	-	-	
TOTAL	471		202		167

T = top and B = bottom of exposure chamber.

The building up of this series began about nine months ago and is being continued at an increasing pace. Loss from any conditions other than fighting

was negligible. Thus far ten cases of leukemia occurred among the 273 mice of the neutron 20- to 80-minute series and only two among 123 siblings receiving an equivalent dose of X rays. No leukemia has yet been noted among the 167 control normal siblings. These data are most preliminary but are given because they show an interesting trend. Thus far only one case of carcinoma was noted and it occurred in the anogenital region in an X-ray mouse.

The inspection of the colony for cataract incidence has just been started by Dr. Christenberry. The results are shown in Table 22.

TABLE 22

Status of cataract incidence of neutron-exposed animals on May 10, 1950

80-MINUTE NEUTRON EXPOSURE	TIME AFTER IRRADIATION			TOTAL
	8-6 MOS.	6-4 MOS.	4-2 MOS.	
Right eye	18+, 1-	14+, 2-	4+, 2-	36+, 5-
Left eye	19+	14+, 2-	3+, 3-	36+, 5-
Both eyes	37+, 1-	28+, 4-	7+, 4-	72+, 10-
Total no. of eyes with cataracts	37	28	7	72
Total no. of eyes examined	38	32	12	82
Total no. of mice examined	19	16	6	41
Per cent with cataracts	97	88	58	87.7
Control siblings	4-	2-		6-

+ = cataract

- = no cataract

Most mice of the neutron 80-minute series, exposed two to four months ago, have slight postsubcapular opacities absent in normal mice, while most of those exposed for a longer period of time have definite cataracts as indicated in Table 22.

Following initial loss of weight the animals of all neutron and X-ray series gain weight which, after some time, is about the same in all groups. No gross late effects are noted after such single massive exposures.

The hematological changes are being studied in association with Dr. Andrews. The drop of white-cell count and recovery following the exposure to slow neutrons and to equivalent doses of X rays show a close parallelism as indicated in Fig. 17. (It is noteworthy in this connection that our slow neutron flux is practically free from fast neutrons but may contain as much as 3 or 4 r of contaminating gamma rays.)

The reticulocyte counts show an immediate and rapid drop followed by recovery after ten days with levels several times that of normal (Fig. 18). After 15 to 20 days there is a gradual return to normal. There is no difference in the rate of depression and recovery of reticulocyte levels between the neutron and equivalent X-ray series, indicating a similarity of action on erythropoietic systems.

Changes in cell and plasma volumes produced by total body X radiation. (Wish - Radiobiochemistry - Storey and Beale) Following irradiation of rabbits by 1000 r of X rays there is a large drop in erythrocyte volume as measured by ^{32}P-tagged homologous cells beginning with the third day and reaching a minimum at about the seventeenth day after irradiation, followed by a slow recovery (Fig. 19). There is at first a slight concomitant plasma-volume drop followed by a rise on about the tenth day elevating the lowered total volume but not bringing it up to entirely normal within 23 days. Similar changes occur in mice that have been exposed to 575 r (Fig. 20).

Direct plasma and cell volume determinations with isotope technics; organ-blood and hematocrit values in normal and hypervolemic mice. (Wish - Radiobiochemistry - Kahn, Storey, Knoohuizen and Beale) Direct single and combined cell and plasma volume determinations were also made on normal and polycythemic mice with ^{32}P-tagged red cells and ^{131}I-tagged plasma. The data indicate that the Evans-blue plasma volumes in mice are above the probably correct ^{131}I-plasma volumes by a factor of 1.2 and in rabbits by a factor of 1.08. The so-called mixing time of Evans blue is a withdrawal time of this dye by Evans blue-affin cells, most of which are probably macrophages. The cell volume calculations based on Evans-blue values are higher than those obtained by direct determinations with the ^{32}P technic by a factor of 1.58 in normal and 1.55 in tumor-bearing mice examined. The direct plasma and cell

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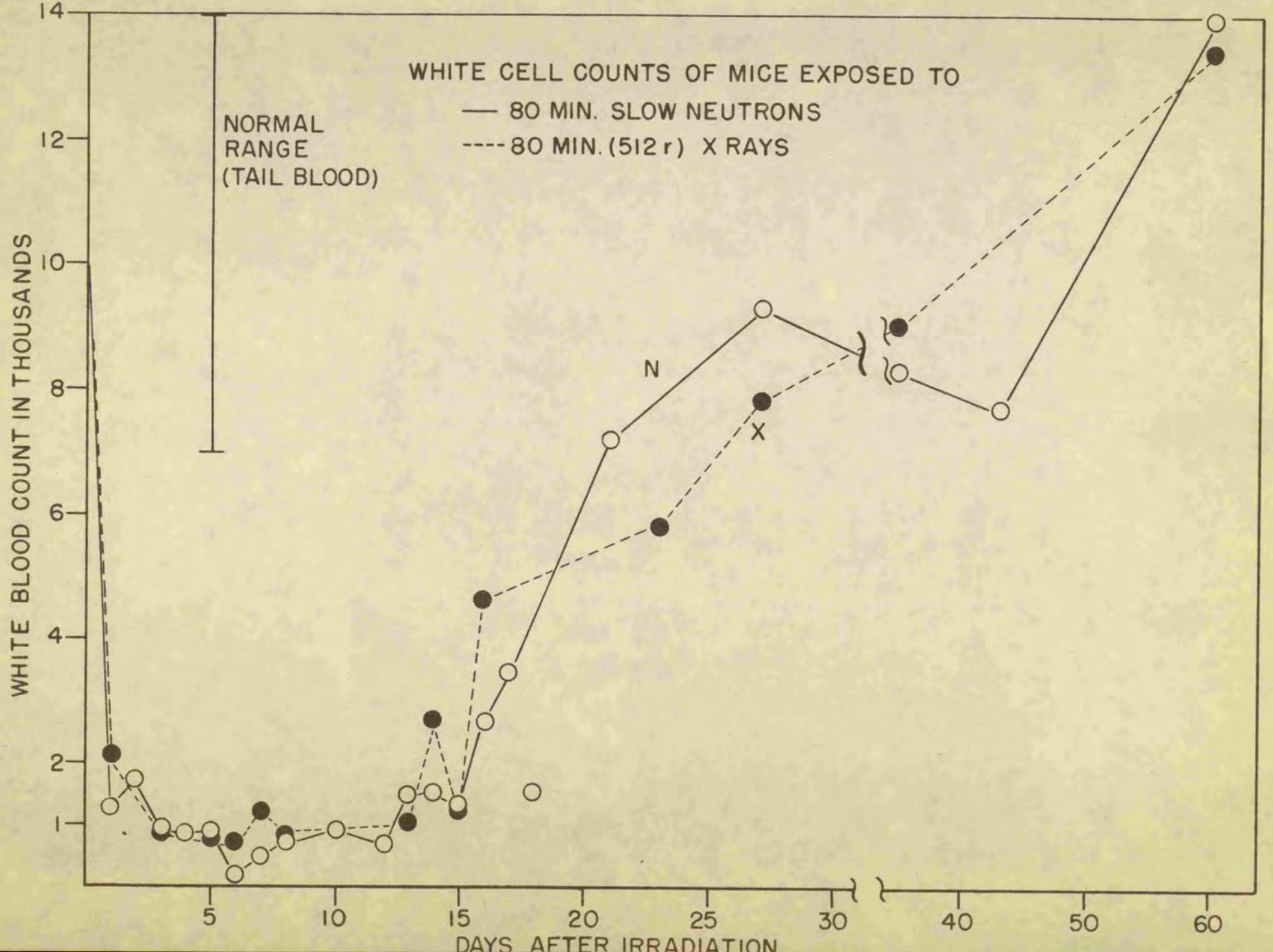
FIG. 17



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DWG. #9013

FIG. 18

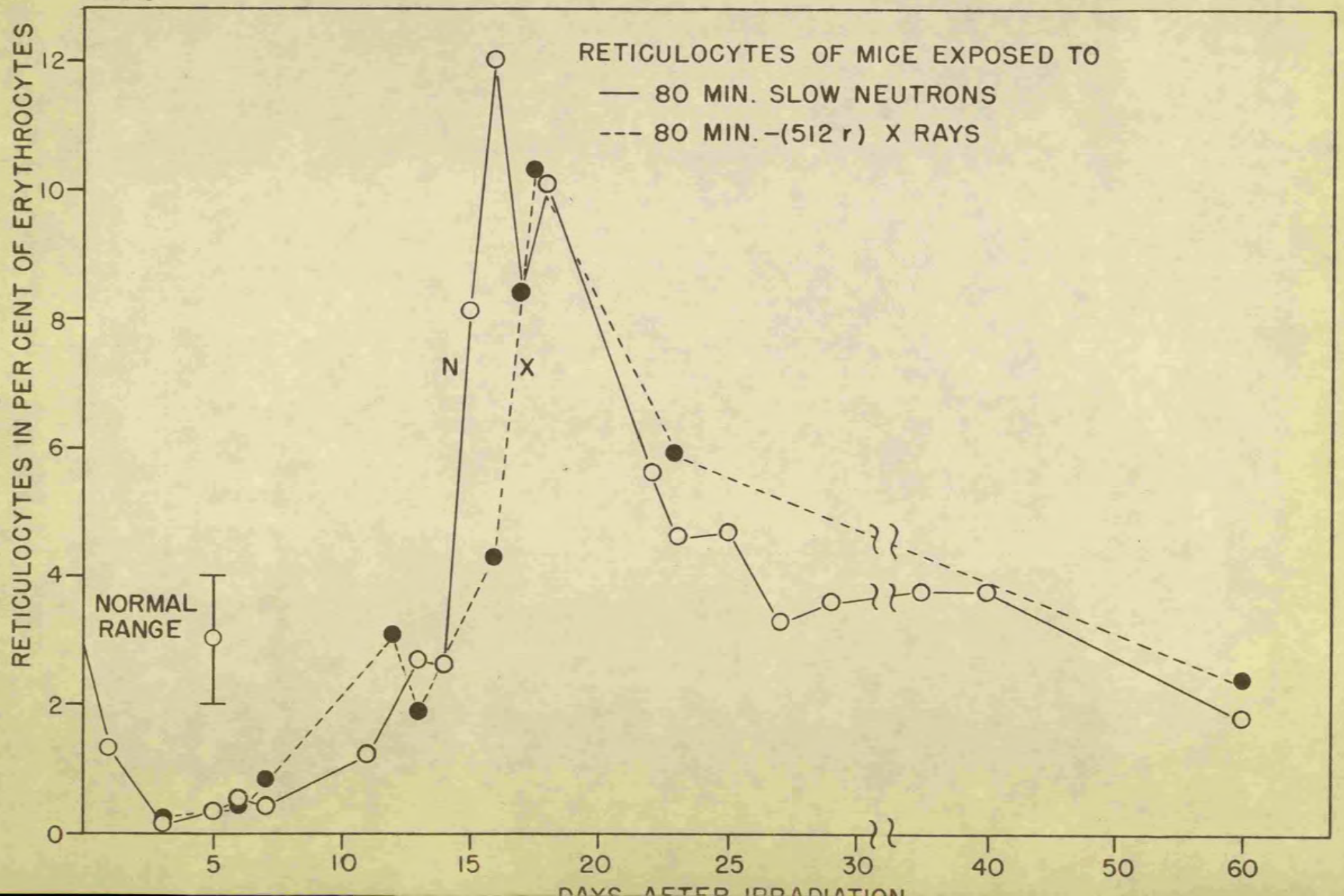

FIG. 19

DWG 9014
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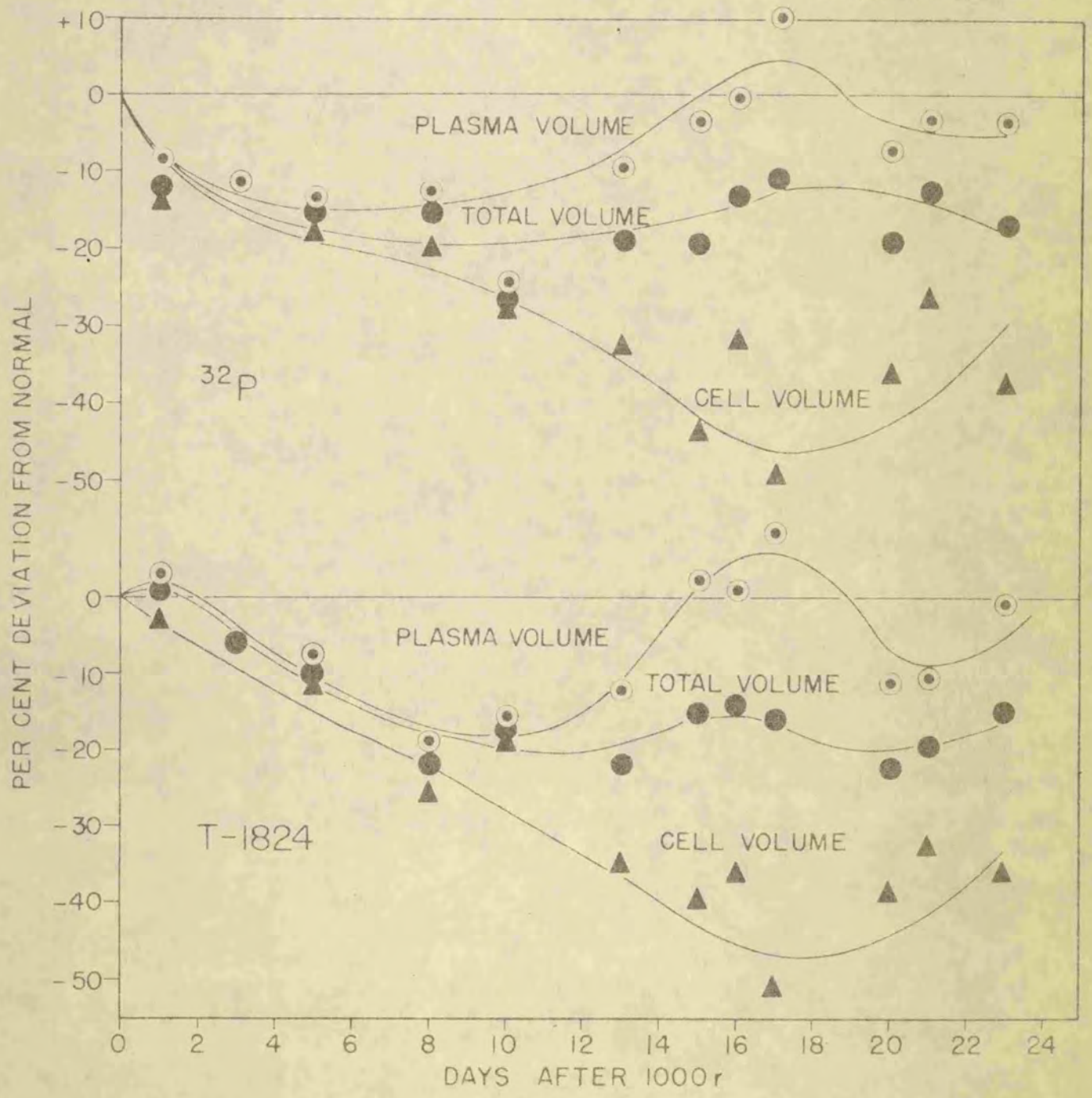
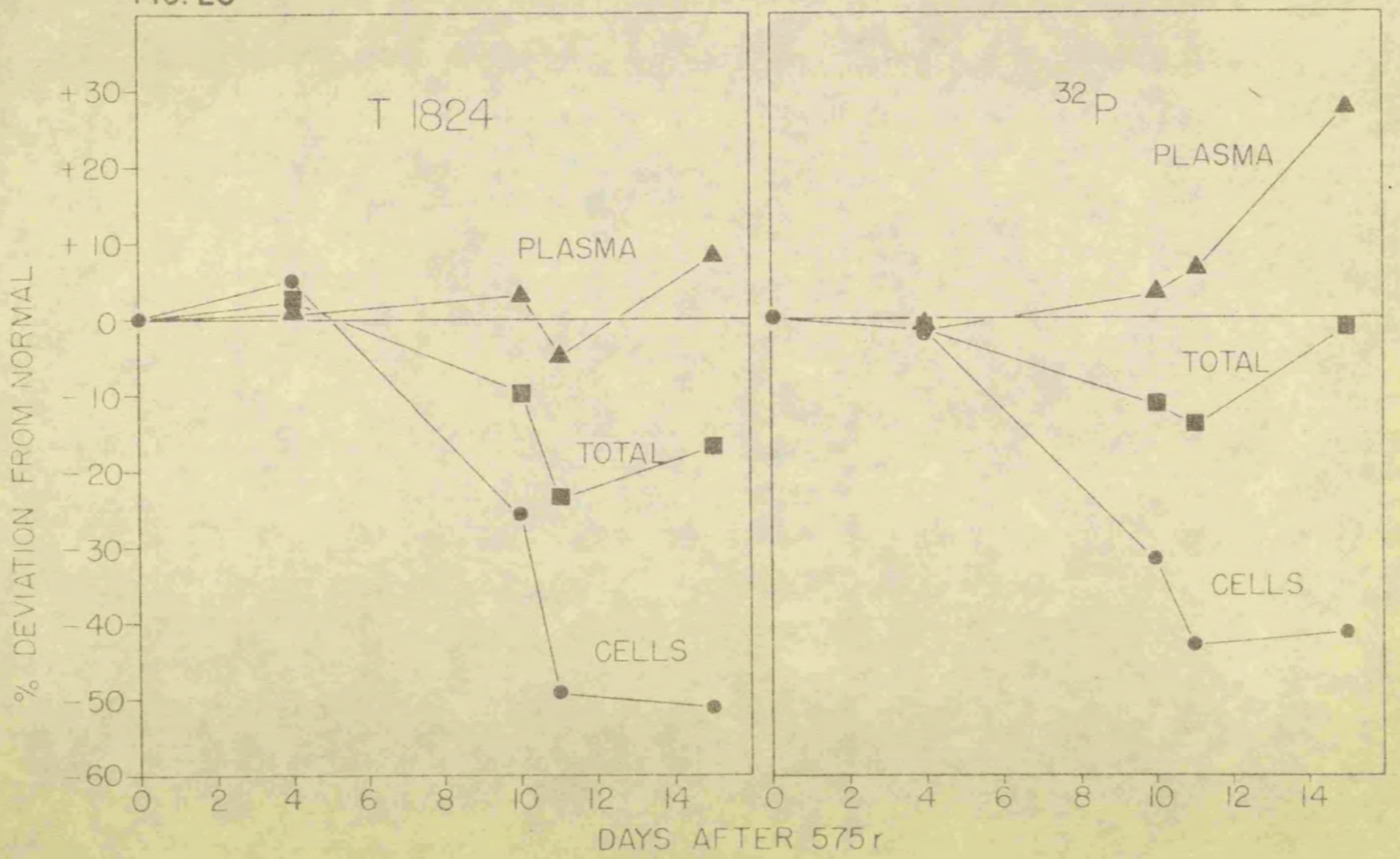


DWG 9015
UNCLASSIFIED

FIG. 20



volume determinations indicate that the average body hematocrit in mice approximates that of the venous hematocrit times 0.87. On the basis of these conversion values a single determination of either cell or plasma volume enables a calculation of the approximately correct total blood volume as shown by Fig. 21.

Hypervolemia caused by estrogen-secreting X-ray-induced tumors is due to an almost selective plasma-volume rise, while that with luteoma (also X-ray induced) is due primarily to polycythemia and to a lesser extent to a plasma volume rise. Correspondingly, normal male mice have a slightly higher red cell volume and females a higher plasma volume. These transplantable neoplasms are therefore suitable for the study of erythro- and plasmagenesis.

Earlier observations on the increased rate of disappearance in X-rayed animals on T-1824 dye, of iodinated protein, and of ^{32}P-tagged erythrocytes have been confirmed and extended. For illustration of these changes see Fig. 22 and Table 23. Future research is aimed to determine how much of the difference in greater disappearance rate is due to an increased capillary permeability and how much to alteration in macrophage function.

TABLE 23

Disappearance rate of ^{131}I-labeled plasma in X-rayed rabbits, expressed as per cent deviation from normal

SEX	DAYS AFTER 1000 r											
	1	2	3	4	6	7	8	9	11	13	15	21
♂	92†			200†					0	49†	206†	
♀	3	39†	93†	23†	68†	7†	123†	39†				6

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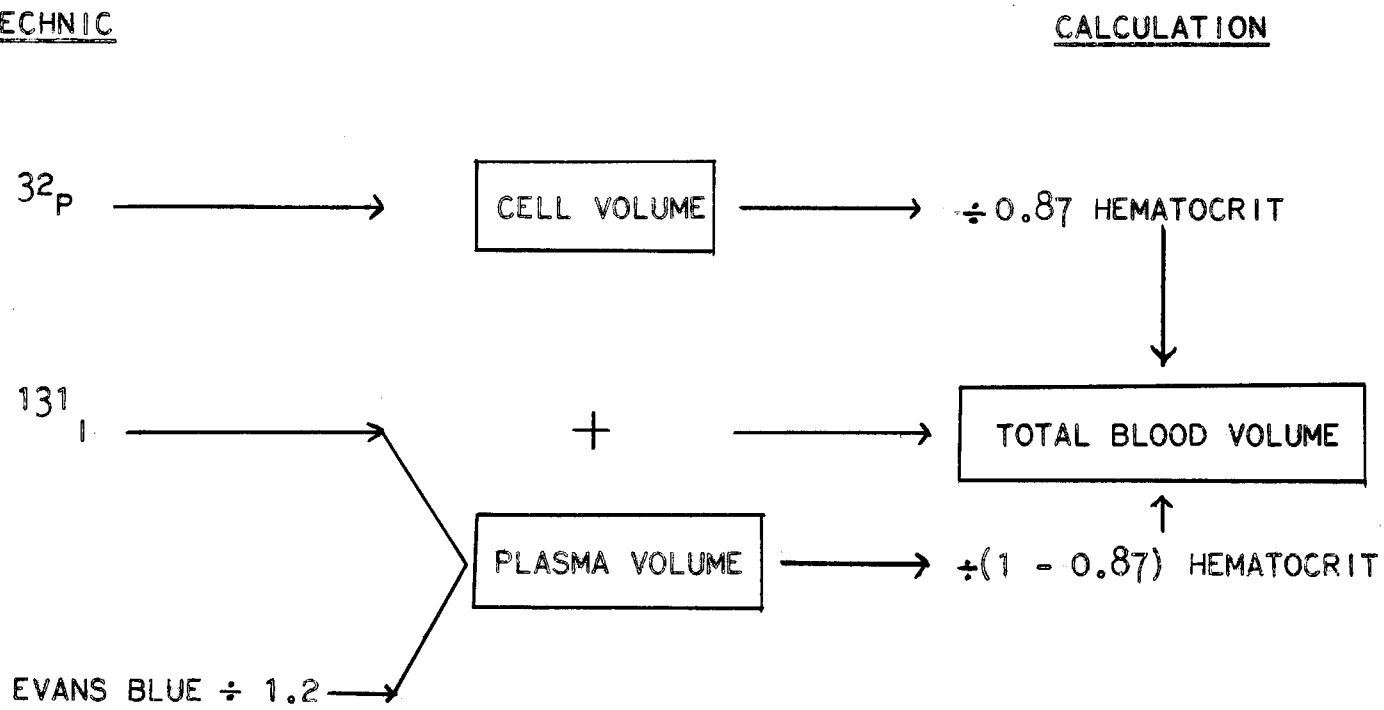
FIG. 21

DWG. #9016

CONVERSION VALUES FOR CELL OR PLASMA VOLUMES

USING ONE OF THREE DIFFERENT PROCEDURES



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DWG. #9017
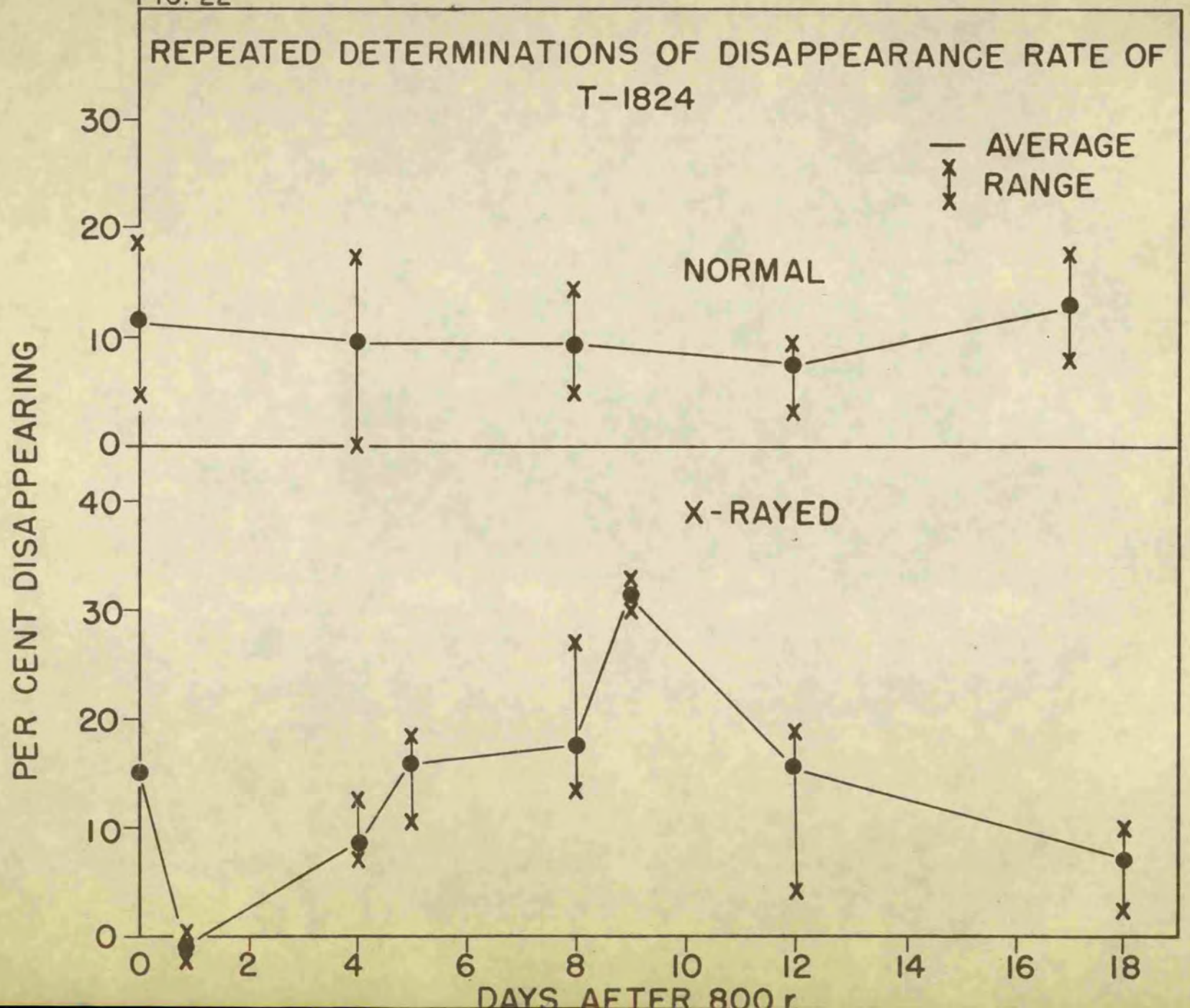
FIG. 22