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Progress Report

BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT

FOR PERIOD ENDING FEBRUARY 15, 1950

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ORNL 644
Progress Report

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BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT
for Period Ending February 15, 1950

Alexander Hollaender, Director

DATE ISSUED: APR 5 1950

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INTRODUCTION

Review of Research Progress. The study of the effect of oxygen tension on the sensitivity of organisms to X rays has occupied a number of groups in our Laboratory. Several interesting points have been brought out in these investigations: (1) The effect of oxygen on X-ray induced chromosomal rearrangement in *Tradescantia* has shown strong dependence on the concentration of oxygen. Apparently, there is a sharp increase in sensitivity up to 20 per cent oxygen in the atmosphere; further increase of the oxygen concentration increases the effect only slightly. Helium affords practically the same protection against radiation as nitrogen. A number of experiments were initiated for studying the combined effects of near infrared and X rays. The X-ray induced percentage of breakage as modified by infrared is influenced by the oxygen concentration during X radiation. (2) The sensitivity of *Paramecium* for X rays is influenced by oxygen tension. (3) The rate of mitosis of the grasshopper neuroblast shows modification depending on the concentration of the oxygen. (4) Bacteria were found to be excellent material for tracing the oxygen effect. Apparently, the sensitivity of bacteria is not only modified significantly by the oxygen tension but also by the (a) concentration of the bacteria in the suspension in which the bacteria are irradiated and (b) the concentration of the broth in which the bacteria are irradiated. Further investigation will probably reveal a number of other influencing factors. (5) It has been found that the sensitivity of bacteriophage to X rays is not influenced, under present experimental conditions, by the oxygen tension.

It appears that the modification of sensitivity of organisms to X rays by changing the oxygen tension promises to become a very important tool for radiation studies. A method is being developed to handle pollen grain of *Tradescantia* for the purpose of using it as a tool for studying radiation on low penetrating power. The effect of radiation on uracil has been the subject of a cooperative study between the yeast genetics investigators and the Biochemistry group.

The Mammalian Genetics group reports that the acute irradiation of female mice with 200 r during the first five days after copulation significantly decreases the number of living young born. This decrease is the result of at least two mechanisms: (1) Failure of implantation and (2) death between the

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time of implantation and day 10½ of pregnancy. Survivors at day 10½ are normal and continue thus to term. It is further stated that approximately one-third of the male offspring obtained shortly after irradiation of the male parent with X rays in the neighborhood of 700 r will be either partially or completely sterile.

The Microbiology group announces that biosynthesis of fumaric acid by a $2C_2$ condensation in the mold, *Rhizopus nigricans* has been confirmed and extended. It has been demonstrated that the carbon dioxide can undergo metabolic exchange with carboxyls of preformed fumaric acid. According to these experiments demonstration, in normal tissues, of the Wood-Werkman formation of C_4 dicarboxylic acids by locating the labeled carbon dioxide in carboxyls of these acids would not constitute unequivocal proof of net synthesis involving carbon dioxide. Evidence has been obtained for the probable operation of C_4 dicarboxylic acid respiratory cycle in this mold. This work has shown significant growth during the last few months, especially through the cooperation of the visiting investigator.

The study of the effect of heat on ultraviolet-irradiated bacteria has continued to give interesting results. A significant heat reactivation was noticed on *Escherichia coli*, strain B, after irradiation with X rays. No light reactivation was found in bacteria after X irradiation.

Important advances in the study of the metabolism of nucleic acids have been realized by the Biochemistry group. This group will sponsor a conference in Oak Ridge on April 13 and 14, to which some of the most outstanding nucleic acid chemists have been invited. The Radiobiochemistry group has been responsible for the training course in radiation physics. This is an extension of the radiochemistry course initiated earlier in the year. They have also taken over the responsibility of standardizing the X-ray source, and the designing of new facilities for X-ray apparatus. A number of reports are being written by members of this group, some in cooperation with Dr. A. S. Householder, of the Mathematics Panel.

The Plant Physiology group is making important strides in the work in production, isolation, and purification of C^{14}-labeled carbohydrates.

In the Biophysics group, Dr. Arnold and Dr. Holt are preparing a number of articles on the effects of ultraviolet irradiation and photosynthesis.

The study of effects of slow neutrons on mice is now moving along at a more rapid pace. This phase of the work of the Pathology and Physiology group

is necessarily slow, since only a few animals can be exposed at one time in the slow neutron facilities of the reactor. The blood volume and clearance study, an auxiliary in the study of X-ray effect, is continued in cooperation with the Radiobiochemistry group and has shown adequate development. Dr. Furth has also extended his work on X-ray induced ovarian tumors.

Traveling Seminars. Division members have been active in the Oak Ridge Institute-Oak Ridge National Laboratory cooperative program of traveling seminars. Lectures and papers given by biologists during the quarter are as follows:

Stanley F. Carson	University of Tennessee, Knoxville	"Hydrogen Transfer and Other Problems in Fermentation Chemistry"
Alan D. Conger	Univ. of Mississippi, Oxford	"Biological Effects of Slow Neutrons from an Atomic Reactor"
Jacob Furth	Univ. of Tennessee, Knoxville	"The Elements of the Ovary: Their Interrelationship, Controlled and Uncontrolled Growths"
Norman H. Giles, Jr.	Indiana Univ., Bloomington	"Reverse Mutations in *Tradescantia*"
Norman H. Giles, Jr.	Indiana Univ., Bloomington	"Effects of X Radiation in Oxygen Atmosphere"
Glenn R. Noggle	Assoc. Sou. Agri. Workers, and Assoc. Plant Physiologists, Biloxi	"A Chromatographic Method for the Separation of Photosynthesized Radiofructose and Radioglucose"
Glenn R. Noggle	Louisiana State Univ., Baton Rouge	"Isotopes in Plant Physiology Research"
Glenn R. Noggle	Louisiana State Univ., Baton Rouge	"Use of Isotopes in Agricultural Research"
Glenn R. Noggle	Southern Regional Research Laboratory, New Orleans	"Tracer Techniques in Plant Physiology"

Publications. Writings by Division members include papers and abstracts on which publication has been completed or which are in final prepublication stage. Many papers not listed are now being written, are in the reviewing committee, or have been submitted for open publication.

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
E. H. Anderson	Reactivation of Ultraviolet Irradiated Bacteria (Abstract)	Am. J. of Botany, 36: 807, 1949	
W. K. Baker and Elizabeth Sgourakis	Alteration of the X-ray Sensitivity of *Drosophila* by Means of Respiratory Inhibitors (Abstract)	Genetics, 35: 96, 1950	
W. K. Baker and E. Sgourakis	The Effect of Oxygen Concentration on the Rate of X-ray Induced Mutations in *Drosophila*	Accepted by Proc. Nat. Acad. Sci.	ORNL-575
Alfred Brauer	Localization of Prospective Areas in the Blastoderm of the Pea Beetle *Callosobruchus maculatus* Fabr. as Determined by Ultraviolet (2537 A) Irradiation Injury	J. Exper. Zool., 112:165, 1949	ORNL-364
Alfred Brauer	Localization of Prospective Areas in the Blastoderm of the Pea Beetle *Callosobruchus maculatus* Fabr. as Determined by Ultraviolet (2537 A) Irradiation Injury (Abstract)	Anat. Rec., 105:113, 1949	
J. G. Carlson	Some Conclusions Reached from Microdissection Studies of the Dividing Neuroblast of the Grasshopper Embryo (Abstract)	Anat. Rec., 105:19, 1949	
J. G. Carlson M. L. Snyder and A. Hollaender	Relation of Gamma-Ray Dosage Rate to Mitotic Effect in the Grasshopper Neuroblast	J. Cell. & Comp. Physiol., 33:365, 1949	ORNL-348

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
C. E. Carter	The Effects of X rays on the Metabolism of Nucleic Acids in Hematopoietic Tissues (Abstract)	Federation Proc., 8:351, 1949	
C. E. Carter and W. E. Cohn	The Separation of Three Naturally Occurring Adenine Ribonucleotides by Paper Chromatography and Ion-Exchange (Abstract)	Federation Proc., 8:100, 1949	
Alan D. Conger	The Effect of Boron Enrichment on Slow Neutron Irradiated Tissues (Abstract)	Genetics, 35: 102, 1950	
J. W. Foster S. F. Carson D. S. Anthony J. B. Davis W. J. Jefferson and Mary V. Long	Aerobic Formation of Fumaric Acid in the Mold *Rhizopus nigricans*	Proc. Nat. Acad. Sci., 35:663, 1949	ORNL-492
Mary E. Gaulden J. G. Carlson and S. R. Tipton	Effects of Carbon Dioxide and Nitrogen Gas on Mitosis as Observed in Living Neuroblasts of Grasshopper Embryo (Abstract)	Anat. Rec., 105:16, 1949	
N. H. Giles, Jr.	The Occurrence of Suppressors in a Methionineless Mutant of *Neurospora* (Abstract)	Genetics, 35:108, 1950	
R. F. Kimball	The Effect of Ultraviolet Light upon the Structure of the Macronucleus of *Paramecium aurelia* (Abstract)	Anat. Rec., 105:63, 1949	
R. F. Kimball	Inheritance of Mutational Changes Induced by Radiation in *Paramecium aurelia*	Genetics, 34: 412, 1949	ORNL-175
R. F. Kimball and Nenita Gaither	Photorecovery of the Effects of Ultraviolet Radiation on *Paramecium aurelia* (Abstract)	Genetics, 35: 118, 1950	
S. Pomper	Genetic Studies on Yeast (Abstract)	Genetics, 35: 130, 1950	

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
N. H. Giles, Jr. and H. P. Riley	The Effect of Oxygen on the Frequency of X-ray Induced Chromosomal Rearrangements in *Tradescantia* Microspores	Proc. Nat. Acad. Sci., 35: 640, 1949	ORNL-376
N. H. Giles, Jr. and H. P. Riley	The Effect of Oxygen on the Frequency of X-ray Induced Chromosomal Aberrations in *Tradescantia* Microspores (Abstract)	Genetics, 35: 131, 1950	
Liane B. Russell	X-ray Induced Developmental Abnormalities in the Mouse (Abstract)	Federation Proc., 8:367, 1949	
Liane B. Russell	X-ray Induced Developmental Abnormalities in the Mouse and Their Use in the Analysis of Embryological Patterns. I. External and Gross Visceral Changes	Submitted to journal	ORNL-595
Liane B. Russell	X-ray Induced Developmental Abnormalities in the Mouse and Their Use in the Analysis of Embryological Patterns (Abstract)	Anat. Rec., 105:13, 1949	
W. L. Russell and Josephine Gower	Offspring from Transplanted Ovaries of Fetal Mice Homozygous for a Lethal Gene (Sp) that Kills Before Birth (Abstract)	Genetics, 35: 133, 1950	
C. W. Sheppard and W. R. Martin	Isotopic Studies of Potassium Exchange Rates Between Cellular Elements and Plasma of Human and Canine Blood *in vitro* (Abstract)	Federation Proc., 8:145, 1949	
C. W. Sheppard and W. R. Martin	Sodium & Potassium Exchange Between Cells and Plasma of Mammalian Blood (Abstract)	Am. J. Physiol., 150:590, 1949	
G. E. Stapleton, and F. L. Martin	Comparative Lethal and Mutagenetic Effects of Ionizing Radiations on *Aspergillus terreus* (Abstract)	Am. J. of Botany, 36:816, 1949	

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
S. R. Suskind	Resuscitation of Heat-Inactivated Seeds by X Radiation	Submitted to journal	ORNL-579
H. J. Teas	Mutants of *Bacillus subtilis* that Require Threonine or Threonine Plus Methionine	J. Bact., 59: 93, 1950	ORNL-369
Maurice Whittinghill	Some Effects of Gamma Rays Upon Recombinations and on Crossing Over in *Drosophila melanogaster* (Abstract)	Genetics, 35: 140, 1950	

Visiting Speakers. An impressive array of speakers has been heard on the Biology Division seminar program this quarter. All are outstanding scientists in their chosen fields.

Dr. William H. Pearlman - Jefferson Medical School, Philadelphia-
"Steroid Hormone Metabolism"

Dr. Seymour Hutner - Haskins Laboratory, New York City -
"Various Aspects of Vitamin B_{12}"

Dr. F. W. Went - California Institute of Technology, Pasadena -
"Climatic Factors as Affecting Plant Growth"

Dr. Arthur Kornberg - National Institutes of Health, Bethesda -
"Enzymatic Degradation and Synthesis of Dinucleotides"

Dr. E. S. G. Barron - University of Chicago, Chicago -
"Recent Research on the Mechanism of Action of Ionizing Radiation"

Dr. H. E. Carter - University of Illinois, Urbana
"Lipid Studies"

Dr. Charles Hooker - University of North Carolina, Chapel Hill -
"Blood Progesterone"

Dr. Hans Neurath - Duke University, Durham -
"Kinetics of Proteolytic Enzymes"

Dr. B. M. Tolbert - Radiation Laboratory, University of California, Berkeley -
"Review of the C^{14} Work at the Berkeley Bio-organic Group"

Mr. G. A. Boyd - School of Medicine & Dentistry, University of Rochester - "Autoradiography"

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

Studies of Reverse Mutability in Inositolless Mutants of *Neurospora*. (Giles and Giles) The comprehensive analysis of a large number of ultraviolet and X-ray induced reversions in strains 37401 and 89601 is continuing. Crosses to wild-type, followed by random ascospore isolations and testings have been largely completed for approximately 82 ultraviolet-induced reversions. The results to date indicate that suppressor mutations account for less than 4 per cent of the total reversions.

A preliminary experiment on the effect of photoreactivation on reverse-mutation frequency in strain 37401 has been performed. Although reversions were obtained both in conidia exposed to ultraviolet alone, as well as in those receiving subsequent exposure to an H5 lamp, the frequency in the photoreactivated series (whose survival was approximately twice that of the ultraviolet alone series) was reduced by almost two-thirds. These experiments are being continued.

The Effect of Oxygen on X-ray Induced Chromosomal Rearrangements in *Tradescantia*. (Giles, Beatty and Fairchild) Additional experiments have been performed in an investigation of the mechanism of the oxygen effect. It has been shown that the removal of this gas during the irradiation period results in a decreased frequency of aberrations. The magnitude of this decrease in aberration frequency is less than that of the increase upon addition of oxygen during irradiation. This difference appears quite reasonable, since a considerably longer period is required for the evacuation of the exposure chamber than for the introduction of oxygen. A summary of the comparative data is presented in Table 1. From these experiments the conclusion may be drawn that the effect of oxygen is to increase the frequency of chromosome breakage rather than to modify the recovery process.

In previous experiments comparative exposures were made at only three oxygen levels, (a) in nitrogen or helium (no oxygen), (b) in air (*ca* 21 per cent oxygen), (c) and in pure oxygen. It seemed of considerable interest to obtain further data in order to determine the quantitative relation between aberration frequency and percentage of oxygen during irradiation. Consequently, two separate experiments were carried out (in one, clone 5 was used; in the other, clone 3 of Sax) in which exposures to a single X-ray dose — 400 r at 50 r/min — were made with seven different percentages of oxygen in the lucite chamber. The oxygen percentages were as follows: 0 per cent (irradiation in pure — 99.8 per cent — helium); 2 per cent (+ 98 per cent helium); 10 per cent (+ 90 per cent helium); 21 per cent (air); 60 per cent (+ 40 per cent helium); 99.5 per cent (pure oxygen from a commercial cylinder); and pure oxygen at an absolute pressure of 1500 mm of mercury (approximately 760 mm above normal atmospheric pressure at Oak Ridge. Before each exposure, inflorescences were placed in the chamber, which was then evacuated to approximately 1-2 mm Hg, and the particular gas or gas mixture admitted. This procedure was repeated five times. Following irradiation, the inflorescences remained in the chamber for approximately ten minutes in the same gas and were then removed to air. The results of these experiments are presented in Table 2 and Fig. 1. There is good agreement between the two experiments except for the two points in air, where, for some unexplained reason, the values obtained for clone 3 are considerably higher than expected. The data indicate that there is a very rapid rise in aberration frequency between 2 and 20 per cent oxygen, after which a gradual increase apparently occurs. The significance of the fact that essentially the same aberration frequencies were obtained in exposures made in pure (99.8 per cent) helium and 2 per cent oxygen (+ 98 per cent helium) is not yet clear. This may mean that not all of the dissolved air is removed from the tissues by the evacuation procedure. It is also desirable to check further the accuracy of the reported percentage of oxygen in the gas mixture used (obtained from a commercial source). The general problem of the effect of oxygen during irradiation at low oxygen tensions is being investigated further.

Experiments on the effect of infrared pretreatment in modifying the frequency of X-ray induced aberrations have been initiated and will be continued in cooperation with Dr. Beatty. The experimental exposure to infrared has

TABLE 1

Experiments demonstrating that the effect of oxygen in increasing the radiosensitivity of *Tradescantia* chromosomes is on the initial breakage mechanism rather than on the reunion process. All exposures: 300 r at 300 r/min.

SERIES NO.	PRETREATMENT CONDITIONS	EXPOSURE CONDITIONS	POSTTREATMENT CONDITIONS	NO. CELLS	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS PER CELL
1	Buds in vacuum	Vacuum	Vacuum — 10 min.	880	0.12 ± 0.01	0.11 ± 0.01
2	Buds in vacuum	Vacuum	Oxygen introduced (within 3 sec) to 1500 mm Hg — 10 min.	700	0.09 ± 0.01	0.10 ± 0.01
3	Buds in oxygen	Oxygen at 1500 mm Hg	Oxygen at 1500 mm Hg — 10 min.	150	0.70 ± 0.07	0.83 ± 0.07
4	Buds in oxygen	Oxygen at 1500 mm Hg	Evacuation (within 25 sec); vacuum — 10 min.	200	0.72 ± 0.06	0.85 ± 0.07
5	Buds in vacuum	1st 30 sec: vacuum 2nd 30 sec: oxygen introduced (within 3 sec) to 1500 mm Hg	Evacuation (within 25 sec); vacuum — 10 min.	350	0.39 ± 0.03	0.50 ± 0.04
6	Buds in oxygen	1st 30 sec: oxygen at 1500 mm Hg 2nd 30 sec: evac. (within 25 sec) to 1-2 mm Hg.	Oxygen introduced (within 3 sec) to 1500 mm Hg — 10 min.	518	0.61 ± 0.03	0.59 ± 0.3

TABLE 2

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.

OXYGEN PERCENTAGE	CLONE USED	NO. CELLS	NO. INTERCHANGES	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS	I.D. PER CELL
0	Clone 3	450	120	0.27 ± 0.02	104	0.23 ± 0.02
	Clone 5	400	113	0.28 ± 0.03	88	0.22 ± 0.02
2	Clone 3	284	73	0.26 ± 0.03	95	0.33 ± 0.03
	Clone 5	315	82	0.26 ± 0.03	77	0.24 ± 0.03
10	Clone 3	425	304	0.72 ± 0.04	305	0.72 ± 0.04
	Clone 5	200	152	0.76 ± 0.06	162	0.81 ± 0.06
21 (air)	Clone 3	350	322	0.92 ± 0.05	392	1.12 ± 0.06
	Clone 5	150	118	0.79 ± 0.07	116	0.77 ± 0.07
60	Clone 3	303	283	0.93 ± 0.06	364	1.20 ± 0.06
	Clone 5	200	181	0.91 ± 0.07	196	0.98 ± 0.07
100	Clone 3	250	249	1.00 ± 0.06	318	1.27 ± 0.07
	Clone 5	150	148	0.99 ± 0.08	159	1.06 ± 0.08
100 (at an absolute pressure of 1500 mm Hg)	Clone 3	225	245	1.09 ± 0.07	279	1.24 ± 0.07

Figure 1

Relation between percentage of oxygen in exposure chamber and frequency of chromosomal interchanges per cell in *Tradescantia* microspores. All exposures to one X-ray dosage — 400 r at 50 r/min. Two separate experiments, one with clone 3 and one with clone 5, as indicated.

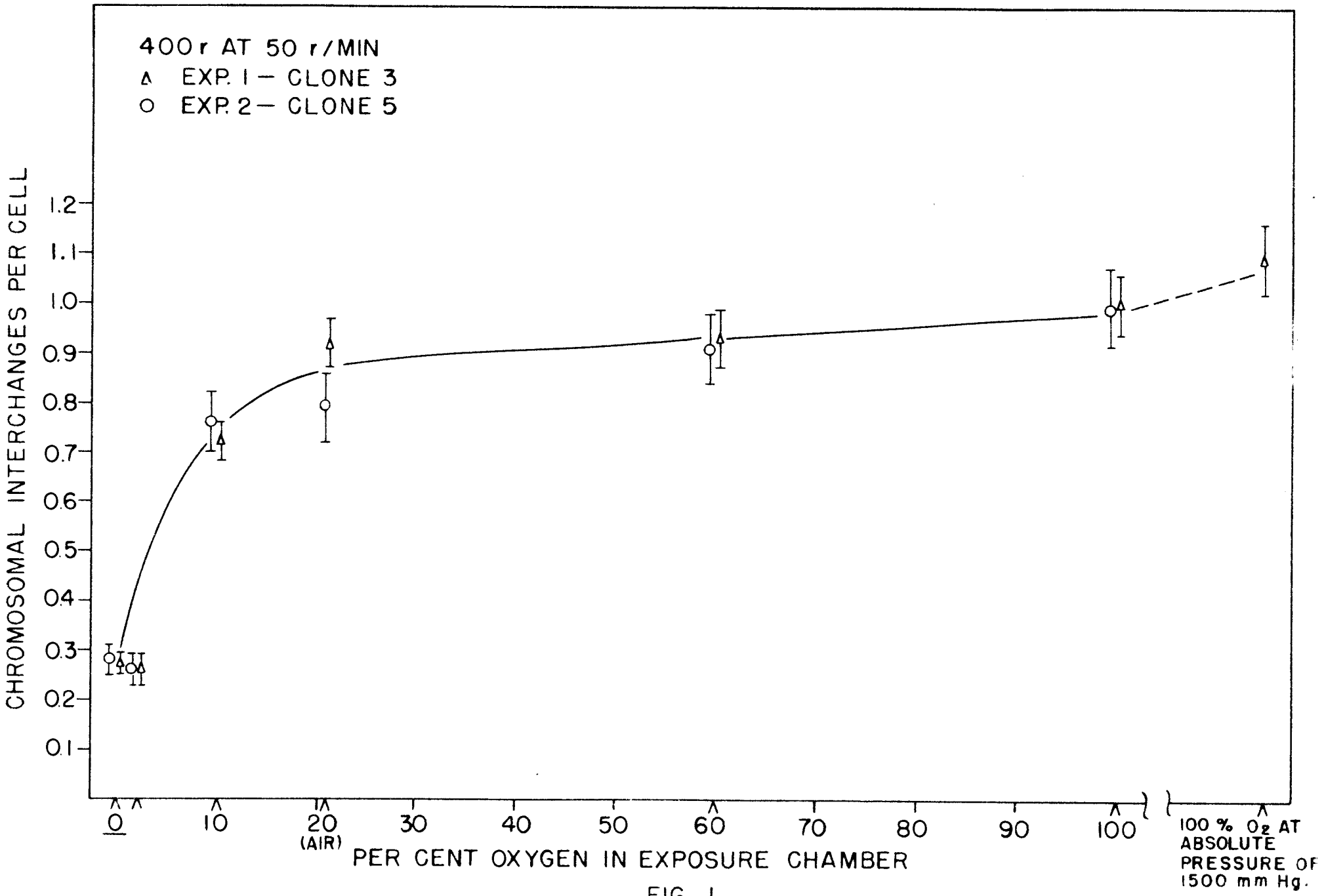

400 r AT 50 r/MIN
EXP. 1 — CLONE 3
EXP. 2 — CLONE 5
CHROMOSOMAL INTERCHANGES PER CELL
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
(AIR)
30
40
50
60
70
80
90
100
100 % O2 AT ABSOLUTE PRESSURE OF 1500 mm Hg.
PER CENT OXYGEN IN EXPOSURE CHAMBER

FIG 1

been performed in the same manner as in the original studies of Swanson and Hollaender (Proc. Nat. Acad. Sci., 32:295, 1946). In one series of exposures inflorescences were placed in a rotating test tube in air and pretreated with infrared. Then the infrared pretreated inflorescences were divided into two groups and exposed to X rays either in air or in a vacuum to test whether the oxygen effect is operative on infrared-sensitized breaks. Control series were included for simultaneous exposures with the infrared pretreated buds. The data are presented in Table 3. It appears that there is an appreciable effect of infrared in increasing the frequencies of aberrations in exposures made in air. Further, a marked reduction of aberration frequency results when oxygen is removed by evacuation. Since the percentage reduction in aberration frequencies is about equal in the control and infrared pretreated series, it appears that oxygen also affects breakage resulting from infrared sensitization. Additional exposures at high intensities are being planned to increase the magnitude of the infrared effect.

Irradiation of *Tradescantia* Pollen Grains. (Conger and Suskind) A proposed series of experiments to investigate the effect of X rays, protons, and alpha rays on *Tradescantia* chromosomes has been started. For these experiments, dry *Tradescantia* pollen grains will be used instead of the microspore cells of the anthers which are conventionally studied. The dry pollen grains offer numerous advantages for radiation experiments, since they are air-borne cells which can be handled with ease and subjected to various treatments either in the dry state or while they are growing on culture medium, and they are small enough to permit experiments with weakly penetrating rays; the cells are football shaped and about 43 μ long by 18 μ high, so that even alpha rays can penetrate them. Only a few of these treatments are possible with the microspore cells, growing as they do in the anther enclosed within the bud. The disadvantages are the labor and difficulty of culturing the pollen grains and the smaller numbers of cells available for analysis.

Dry pollen grains are irradiated, then sown on an agar culture medium (Bishop, Stain Tech., 24:9-12, 1949) on a slide and grown for about 20 hours in a moist glass chamber. The radiation-induced aberrations are analyzed in the pollen tubes at the pollen tube mitosis. The results of some of the first experiments, on the production of aberrations in these dry pollen grains by X irradiation are shown in the graph, Fig. 2. The chromatid aberrations analyzed are of the same type as those found in microspores. These results are

TABLE 3

Comparison of the oxygen effect on normal and infrared pretreated buds. All exposures -- 300 r at 50 r/min

PRETREATMENT	EXP. 50-II IRRADIATION IN AIR			EXP. 50-III IRRADIATION IN VACUUM			PER CENT OF ORIGINAL ABERR. FREQ. IN VACUUM	
	NO. CELLS	INTERCHANGE PER CELL	INT. DELE. PER CELL	NO. CELLS	INTERCHANGE PER CELL	INT. DELE. PER CELL	INTERCHANGES	INTERSTITIAL DELETIONS
Control (none)	385	.48 ± .035	.46 ± .034	450	.15 ± .018	.13 ± .017	31	28.2
Infrared (*ca* 4 hrs)	694	.58 ± .029	.68 ± .031	350	.18 ± 023	.18 ± 023	31	26.5

not final, but with further data it will be possible to construct a base line of X-ray effect on these cells for comparison with neutron and alpha-ray effects.

One experiment was made to test the effect of keeping the cells in an atmosphere of nitrogen during and after irradiation. The results, shown on the graph, agree with those reported by Giles and Riley (see Biology Division quarterly report, ORNL-537); chromosome aberrations are reduced by about one-sixth if the irradiation is done in nitrogen instead of in air.

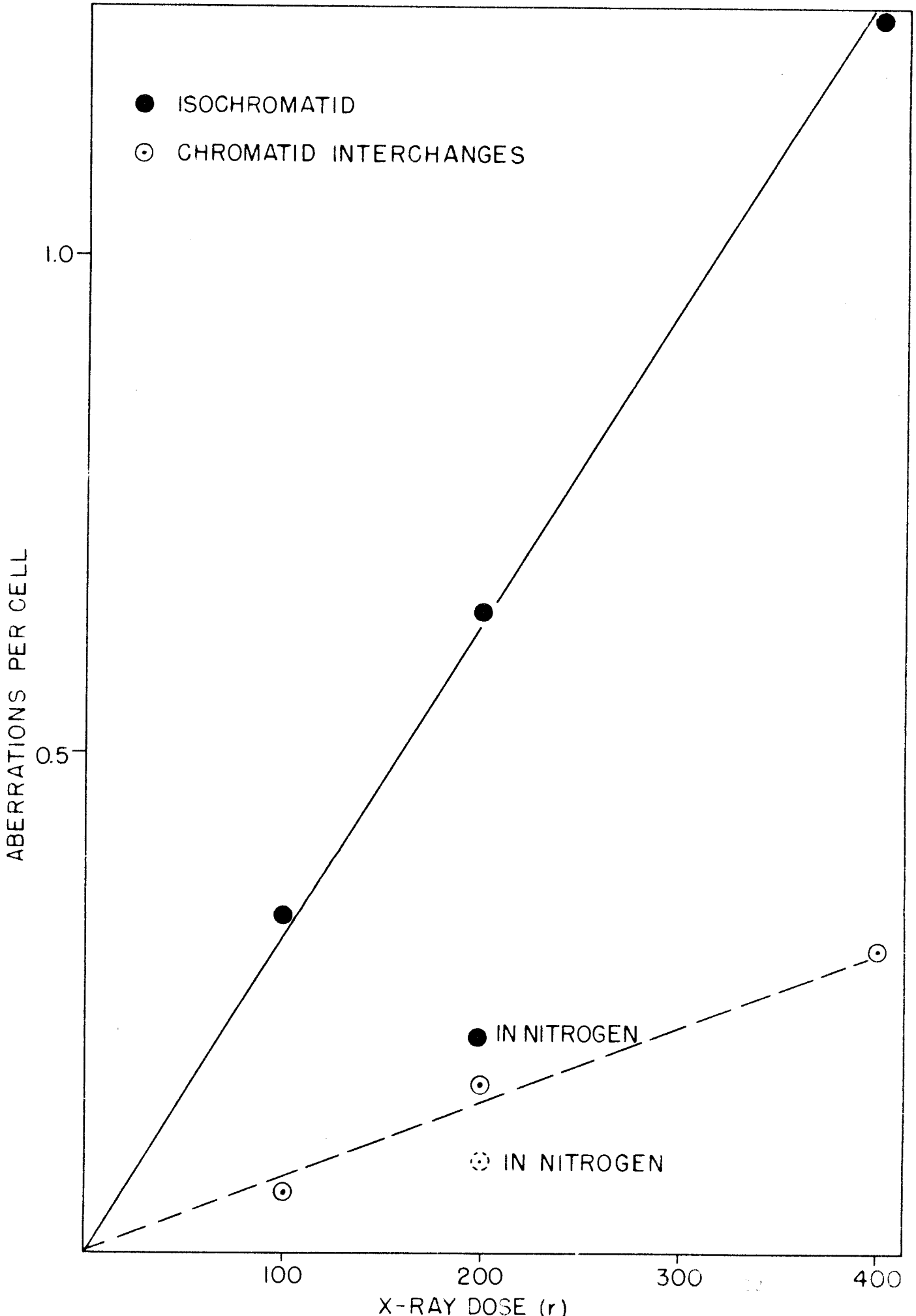
Yeast Genetics. (Pomper and Meyer) Three groups of mutants have been studied thus far. They require, respectively, purines, pyrimidines, and sulfur-containing amino acids. A partial account of the biochemical and genetical investigations of the purine-requiring mutants was given in the last quarterly report. In addition to the compounds tested for growth-promoting activity at that time, uric acid, alloxan, and 2,8-dioxyadenine have been tested, and found not to serve as metabolites for these mutants. Uric acid was observed to be inhibitory at concentration of 20-30 μg per ml. This inhibition could not be reversed by as much as 160 μg per ml of adenine or hypoxanthine. This compound was then tested on wild-type yeast, and found to be inhibitory. It is of interest to note that guanine, which inhibited the purineless mutants competitively, was without effect on the wild-type yeast tested. Various compounds were tested in an effort to reverse the inhibitory action of uric acid, but without significant success.

In collaboration with Dr. Giles, cross-feeding experiments have been carried out between certain purineless *Neurospora* mutants and some of the yeast mutants. There is, in *Neurospora*, a purple, adenineless mutant, which spontaneously throws off white, adenineless mutants. There is, in yeast, a pink, adenineless mutant (Ad_5), which also throws off white, adenineless cultures. It was felt that these might represent homologous situations in the two organisms, and that the white mutants might represent multiple blocks in the adenine biosynthesis. If this were so, then one might anticipate the accumulation of a precursor by the white mutant, of let us say *Neurospora*, which might be used by the pink yeast, and that the yeast mutant might in its turn produce a substance that the *Neurospora* mutant could use for growth. The experiments thus far have been negative, but are being continued with different combinations of mutants, in the hope of obtaining further information on the order of biosynthesis.

Figure 2

Chromatid aberrations induced in *Tradescantia* dry pollen grains by X irradiation in air (and in nitrogen at 200 r).

FIG. 2


ISOCHROMATID
CHROMATID INTERCHANGES
1.0
0.5
ABERRATIONS PER CELL
IN NITROGEN
IN NITROGEN
100
200
300
400
X-RAY DOSE (r)

With regard to the latter question, another approach is being employed. Crosses have been made between the above-mentioned pink, adenineless yeast, and various, nonallelic, white, adenineless yeasts. The analysis depends on the recovery of the four products of meiosis, and the phenotypic segregation of the pink pigment among the ascospores. If the pink gene (Ad_5) comes before the white gene (Ad_1) in a biosynthetic chain, then one should expect all of the asci to segregate 2:2 for color. If the situation is reversed, then one may expect 2:2, 4:0, and 3:1 depending on the segregation of the adenineless genes. The analysis is schematically presented in the following table.

TABLE 4

	$\dashv\!\rightarrow \longrightarrow \dashv\!\rightarrow \longrightarrow$ AD_5 (PINK) AD_1 (WHITE)	$\dashv\!\rightarrow \longrightarrow \dashv\!\rightarrow \longrightarrow$ AD_1 (WHITE) AD_5 (PINK)
ASCOSPORE GENOTYPE	COLOR PHENOTYPE	COLOR PHENOTYPE
Ad_1^+ Ad_5^+	White	White
Ad_1^+ Ad_5^-	Pink	Pink
Ad_1^- Ad_5^+	White	White
Ad_1^- Ad_5^-	Pink	White
ASCUS SEGREGATION	COLOR SEGREGATION	COLOR SEGREGATION
$Ad^+:Ad^-$	White:Pink	White:Pink
2:2	2:2	4:0
1:3	2:2	3:1
0:4	2:2	2:2

These experiments are not sufficiently advanced to report on in detail at this time, although there seems reason to believe that Ad_2 (a double mutant arising from irradiation of the tryptophanless mutant) precedes Ad_5. This over-all

rationale should be applicable whenever a mutant accumulates an assayable inert precursor.

A cross has been made of a Me_1^- Ad_4^- × a $Th^-Tr^-Ur_1^-$. Out of seven complete asci, four gave unexpected segregations of the Ad_4 gene (all other loci segregating regularly). Instead of the 2:2 segregation anticipated for a single factor cross, ratios of 3:1 and 4:0 (Ad^+:Ad^-) were obtained. The asci in which the aberrant ratios were obtained were subjected to population analyses in an attempt to establish whether the segregations were being obscured by mutational phenomena. In all cases, it was possible to demonstrate that 2:2 segregations had actually taken place at meiosis, but that mutation from adenine requirement to independence had occurred some time after germination. The finding was rather surprising, since the Ad_4^- culture had shown no marked instability in vegetative (haploid) reproduction. Study on this locus is being extended to determine quantitatively the reversion rate under a variety of vegetative conditions, and in crosses with other mutant stocks.

The pyrimidine mutants have been tested further on various possible metabolites, in addition to those listed in the last quarterly report. These include: 5-nitrouracil, 5-bromouracil, dialuric acid, formylurea, isobarbituric acid, isodialuric acid, formyloxaluric acid, uramil, pseudouric acid, allantoin, 5-acetic acid-hydantoin, adenine, adenosine, and adenosine-3-phosphate. Of these, 5-nitrouracil was the only one utilized. Utilization of uracil, cytosine, and uridine has been previously established. None of the compounds appeared to be inhibitory. The rate of utilization of uridine is markedly affected by *p*H for one of the pyrimidine mutants (Ur_2). At *p*H 6.5 to 7.0, Ur_2 grows well on uracil but not on uridine; at *p*H 4.5 to 6.0, it grows well on uracil and fairly well on uridine. This *p*H sensitivity is not observed with Ur_1. A cross was carried out between Ur_1 and Ur_2 and double mutants (Ur_1^- Ur_2^-) recovered. Two such double mutants were studied for their quantitative response to uracil, and for their *p*H sensitivity on uridine. The double mutants showed the same quantitative requirements as the single mutants. This may be regarded as inferential supporting evidence that the two genes lie in the same chain of reactions; otherwise, the requirements might be expected to increase. The double mutants seemed to resemble Ur_2 in their response to uridine.

Another study with the pyrimidine mutants, involving light regeneration of ultraviolet-inactivated uracil, has been done in collaboration with Dr.

Carter. Destruction of uracil by ultraviolet irradiation may be inferred from the fact that it loses both its characteristic absorption spectrum and its activity for the yeast mutants. Exposure to light, heat, or acid restores this activity. Further characterization of this reaction is being done by Dr. Carter.

A group of mutants has been found, after ultraviolet irradiation, which requires for growth the amino acid, methionine. Preliminary genetic and biochemical knowledge is now available on these mutants, which are divisible into at least three genetic groups and as many biochemical groups. One group grows on methionine, cystine, cysteine, homocystine, and homocysteine; another only on methionine; and the third is rather variable and hard to score with precision. It cannot yet be said with certainty how well the results with these mutants will fit in with the evidence from *Neurospora* on this biosynthesis.

Bacteriophage Investigations. (Doermann and Hill) Several investigations have been initiated with the bacteriophages of the T-series, active on the B strain of *Escherichia coli*. One of these has yielded conclusive results. In cooperation with the principal program of the Cytogenetics group, T2 bacteriophage has been subjected to X radiation both in the presence of air and with the air replaced by nitrogen. We are indebted to Mrs. F. L. Martin and Mr. George Stapleton for carrying out the air replacement with the same procedure that they have used in preparation of fungi and bacteria for similar experiments. The experiment was performed in both nutrient broth and in a buffered salt mixture. The results are shown in Table 5. No effect of oxygen removal can be detected here, either in the broth or in the buffer experiments. The great difference in survival in broth as compared to buffer is in agreement with the prior experiments of J. D. Watson of Indiana University (private communication).

The second program, which is still in its preliminary stage , is designed to yield a group of markers in the phage T4 for future genetic experiments with that virus. Crosses (*cf.* Hershey and Rotman, Genetics, 34: 44, 1949) have been made to determine differences in genetic composition of various T4 strains. The most thoroughly understood cross is one which may be designated by the symbols $T4r_{48} \times T4m_{41}$. Hershey and Rotman have described *r* and *m* factors in T2, but these are genetically different from those discussed here, as shown by a series of intercrosses with T2. The $r \times m$ cross described above

TABLE 5

Survival of bacteriophage T2 after X radiation

DOSE (kr)	SURVIVAL IN NUTRIENT BROTH	
	AIR	NITROGEN
0	1.00	0.93
75	0.28	- --
80	0.22	0.23
123	0.079	0.082
160	0.040	0.035
160	- - -	0.037
165	0.033	

DOSE (kr)	SURVIVAL IN BUFFERED SALT MIXTURE	
	AIR	NITROGEN
11	0.45	0.50
17	0.29	0.26
27	0.039	0.030
53	0.0000034	- ---

TABLE 6

Segregation in single bacterial bursts in the cross $T4r_{48}m^{+} \times T4r^{+}m_{41}$ and comparison with results obtained by Hershey and Rotman in the cross $T2Hr_{1}h^{+} \times T2Hr^{+}h$

CROSS	NO. BURSTS	K*	4K*	RECOMBINATION I PER CENT OF YIELD	RECOMBINATION II PER CENT OF YIELD
	25	$\leq$ 0.20	0.65 uncor.	8.9 ± 3.8	12.1 ± 6.2
			corr.	13.9 ± 5.3	19.0 ± 9.2
$T2Hr_{1} \times T2Hh$	52	$\geq$ 0.20	0.94 uncor.	15.5 ± 6.9	17.1 ± 7.1
			corr.	16.6 ± 6.3	18.1 ± 7.2
	19	$\leq$ 0.20	0.64 uncor.	12.1 ± 3.6	12.9 ± 4.6
			corr.	18.7 ± 7.1	19.3 ± 5.9
$T4r_{48} \times T4m_{41}$	58	$\geq$ 0.20	0.96 uncor.	17.0 ± 4.9	16.9 ± 5.6
			corr.	17.9 ± 5.1	17.8 ± 5.6

* *4k* is a factor devised by Hershey and Rotman to correct for inequality in yields of parental types. For discussion of the correction and its calculation, see Hershey and Rotman, Genetics 33:34, 1949.

TABLE 7

Preliminary results from crosses involving $T4_{r_{48}}$ plaque type mutants

PLAQUE TYPE	ORIGIN	PLAQUE TYPES YIELDED IN CROSSES WITH No. 2A	YIELDS IN OTHER CROSSES	REMARKS
#2a	Original parent strain	only #2		This is the strain used in cross shown in Table 6
#2b	From cross #1 × #5a	only #2		
#1	From first #6 stock	#1, #2		#1 apparently differs from #2a in a single locus only
#5a*	From first #6 stock	#1, #2, #5, #6	#5a × #5b yields #5, #2 and possibly #6 #5a × #6 yields only #5 and #6	#5a and #5b appear to be separate factors at nonhomologous loci
#5b*	From cross #1 × #6	----------	#5b × #6 yields only #5 and #6	
#6*	In expt. using #2 with multiple infection	#2, #6	#1 × #6 yields #1, #2, #5, #6, but ratios are peculiar.	#6 appears to contain both the #5a and #5b locus, but appears to lack the #1 factor.
#7	From first #6 stock	----------	----------	Contains an m factor, but as yet untested with other m factors.

* The differentiation of No. 5 and No. 6 is variable and difficult. For this reason some caution must be exercised in interpreting data depending on such differentiation.

was made in several different experiments, and the results are in all details comparable to the results of Hershey and Rotman's cross T2Hr_1 × T2Hm. The comparison of results is summarized in Table 6.

More genetic material for this type of investigation is available among a series of plaque types described earlier (Doermann and Dissosway, Carnegie Institution of Washington Yearbook No. 48, in press). Here five plaque types of T4r_{48} were described, and they are referred to as types #1, #2, #5, #6, and #7. Intercrosses among these types have given some preliminary information as to their genetic compositions. These results are summarized in Table 7. It is clear that the genetic make-up of these types is complex, and that they should yield material which can be used in further genetic experiments. To draw more specific conclusions than those mentioned in the table would be premature.

EFFECTS OF RADIATION ON PARAMECIUM

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

Photorecovery from the Effects of Radiation. (Kimball, Gaither, and King) The work of the quarter has been concerned primarily with building up a greater body of data on the effect of light upon mutation induction by 2650 A ultraviolet, measured by the amount of reduced vigor after autogamy (see Biol. Div. Quarterly Reports, ORNL-457 and ORNL-537, for details). The same treated material yielded data on the effect of light upon delay in cell division produced by 2650 A ultraviolet. Surprisingly, these latter results were not entirely in agreement with results for delay in cell division following 2804 A ultraviolet reported in the last quarterly report.

The methods of treatment were these already discussed in detail in the previous report (ORNL-537). Following exposure to monochromatic 2650 A ultraviolet, some animals were exposed to light from the H5 lamp while others were kept, as far as possible, in the dark. Daily division rate records were kept, and, after complete recovery from the delay in cell division, autogamy was obtained. The time to the sixth division after irradiation was used as a measure of the delay in cell division while the per cent of autogamous animals which produced less than the normal number of progeny in four days was used as a measure of mutation.

The results are plotted in Figs. 3 and 4. As previous results had indicated, light from the H5 lamp distinctly reduced the effect that would otherwise have been expected from the shorter ultraviolet. The two sets of curves in this report and the curve for death before the first division following exposure to 2804 A ultraviolet which was given in the last report are all in fairly good agreement with Novick and Szilard's (Proc. Nat. Acad. Sci., 35: 591, 1949) conclusion based on work with *Escherichia coli*, that the effect of light can be described as a decrease in the effective dose of ultraviolet by a factor between 0.3 and 0.4. The curves given in the last report for time to the sixth division following exposure to 2804 A ultraviolet appear to show a very much greater reduction. It is not clear at the moment how the discrepancy between this one set of curves and the corresponding curves for time to the sixth division in material exposed to 2650 A ultraviolet are to be explained.

In the previous report evidence was given that light from the H5 lamp might have a very slight effect in reducing the amount of mutation produced by X rays. Another experiment along this same line has failed to give any confirmation for such an effect. It can be concluded that either the light has no effect at all on X-ray induced mutations or an effect so slight as to be essentially undetectable by the present methods.

Effect of Lack of Oxygen upon the Action of Radiation. Two groups of animals were exposed to the same dose of X rays. One group was in air while the other was in an atmosphere of helium. Autogamous animals were obtained from both groups and the per cent of autogamous animals which produced less than the normal number of progeny in a four-day period was used as a measure of the amount of mutation. The results are shown in Table 8.

TABLE 8

TREATMENT	NUMBER OF TREATED ANIMALS	NUMBER OF AUTOGAMOUS ANIMALS	PER CENT NOT NORMAL
X rays, air	20	500	59.8
X rays, helium	20	500	16.2
No X rays, air	20	500	1.6
No X rays, helium	20	500	1.0

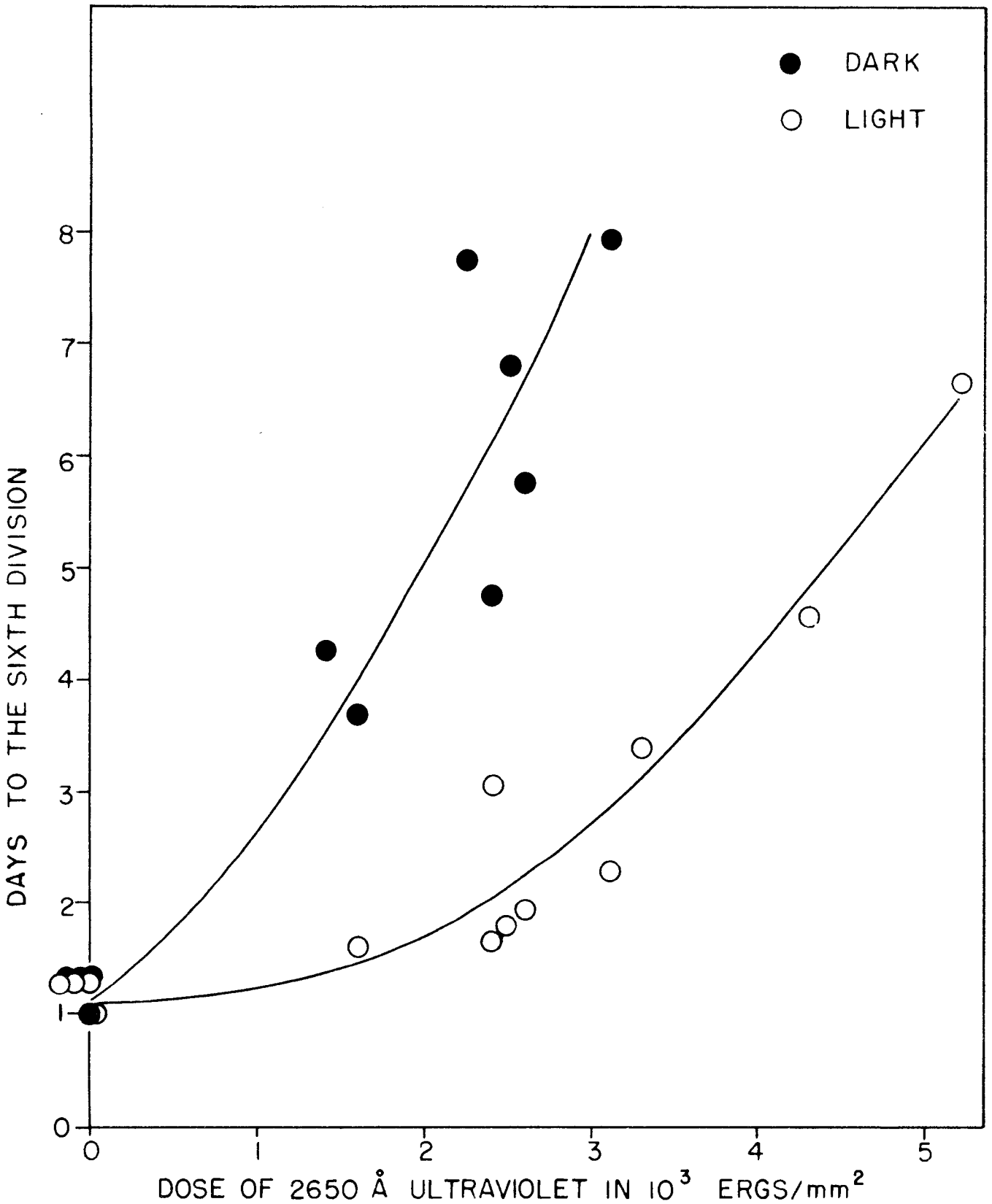

DARK
LIGHT
DAYS TO THE SIXTH DIVISION
0
1
2
3
4
5
6
7
8
0
1
2
3
4
5
DOSE OF 2650 Å ULTRAVIOLET IN 10^3 ERGS/mm^2

FIG. 3

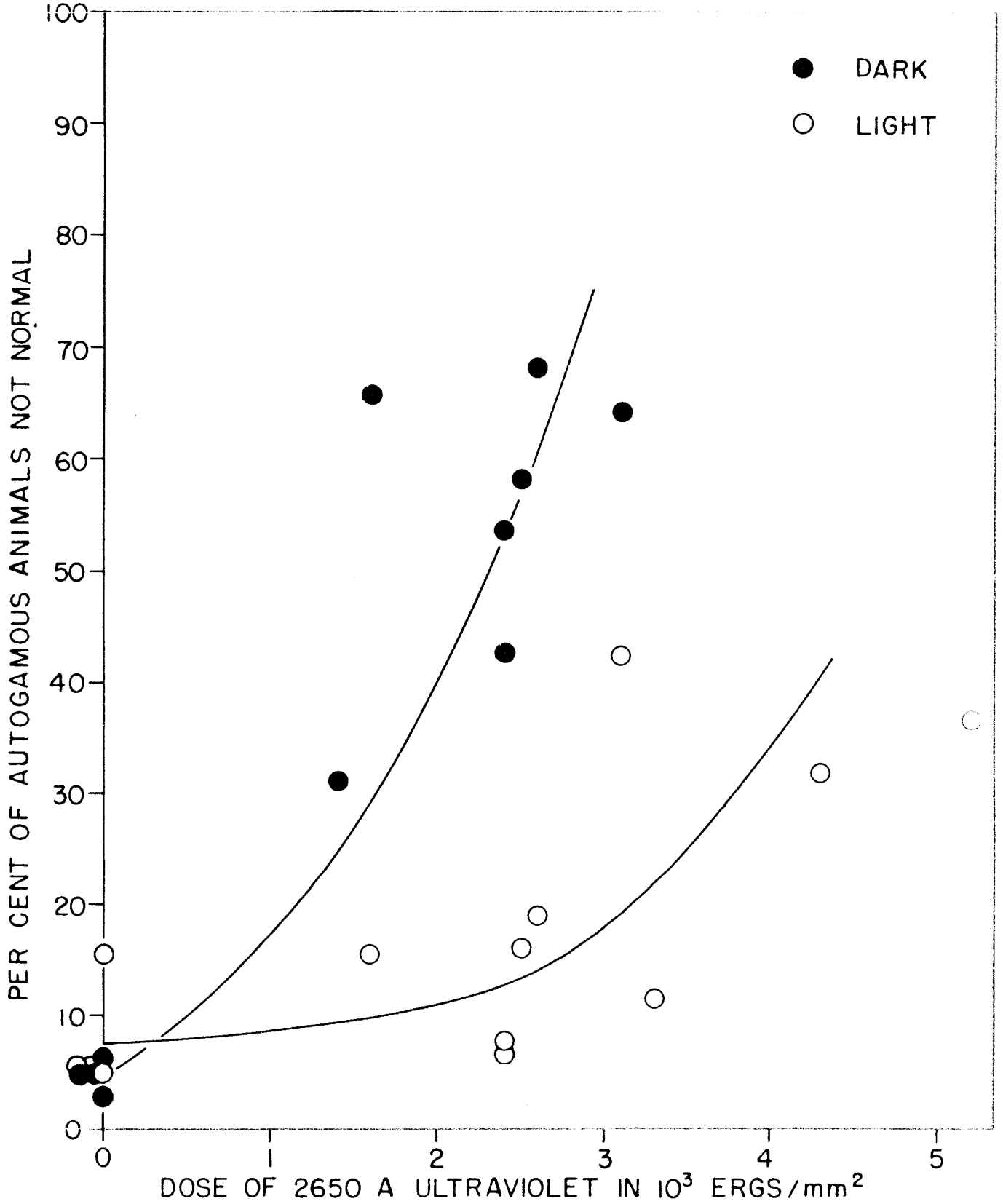

DARK
LIGHT
PER CENT OF AUTOGAMOUS ANIMALS NOT NORMAL
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
DOSE OF 2650 A ULTRAVIOLET IN 10^3 ERGS/mm^2

FIG. 4

It can be concluded that mutation induced by X rays in *Paramecium* is less in the absence of oxygen than in its presence. This is in line with results previously reported from this laboratory by Riley and Giles (Proc. Nat. Acad. Sci., 35:640, 1949) for chromosome aberrations in *Tradescantia* and by Baker (Genetics, 35:96, 1950) for lethal mutations in *Drosophila*. Further investigations of this matter are planned.

EFFECTS OF RADIATION ON THE RATE OF MITOSIS

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

Effects of Gamma Rays at Low Intensity upon the Rate of Mitosis in Neuroblast Cells of *Chortophaga viridifasciata*. (Carlson and Harrington) Studies on the effect of low intensity gamma-ray treatment on mitotic rate are being continued, using 4 r/hour intensity. Experiments are repeated for the dose of 576 r given during a six-day period. At the end of treatment the embryos are made into hanging-drop culture preparations and examined under the microscope at 38°C along with control embryos.

It was previously observed that there was much individual variation in response to the dose of 576 r. The number of mitoses in some embryos remained at zero during the entire day, while other embryos had fairly high mitotic counts. Further work showed that the isotonic point of the irradiated embryos was different from the control embryos. Some variation in the counts has been eliminated by finding the isotonic point for each embryo before the count is made. Other factors which may decrease the individual mitotic differences are being investigated.

Additional work is being done to see whether radiation of high intensity produces the difference in osmotic pressure that low intensity has. Embryos receiving 580 r in five minutes do not show any variation immediately after treatment. Experiments are now in progress to determine whether the effect on osmotic pressure is a delayed reaction.

Effect of Oxygen on Radiation-induced Mitotic Inhibition. (Gaulden and Nix) Due to unavoidable delay in calibration of the gamma-ray source, X rays were substituted in this study. Grasshopper eggs were placed in polystyrene chambers which were evacuated and flushed with the desired gas three times.

* Consultant

Along with the gas-treated embryos, another group of eggs was irradiated in air administered in the same manner as the other gases. The eggs were then irradiated at room temperature with 64 r of X rays while in an atmosphere of a particular gas and, immediately following, 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.

The results indicate that mitosis in the grasshopper neuroblast is less sensitive to radiation when the cells are irradiated in an atmosphere of nitrogen or carbon dioxide than when irradiated in air. Although the period of complete mitotic inhibition (period after irradiation during which there are no cells in the mid-mitotic stages) did not disappear, it was of shorter duration in those cells irradiated in nitrogen or carbon dioxide than in those irradiated in air, that is, cells given the former treatment begin to show recovery about thirty minutes earlier than those given the latter treatment. Not only did cells irradiated in nitrogen and carbon dioxide recover sooner than those irradiated in air, but more of them reached the mid-mitotic stages during the six hours of observation. The total number of cells passing through these stages in the six-hour period following radiation in air was only about 75 per cent of the total number of cells passing through these stages after irradiation in nitrogen or carbon dioxide. There appears to be little, if any, difference between the effects caused by irradiation in nitrogen as compared to those caused by irradiation in carbon dioxide. Preliminary experiments indicate that cells irradiated *in vacuo* exhibit the same reduced radiosensitivity as those irradiated in nitrogen or carbon dioxide.

In contrast to the above cited results oxygen increases the sensitivity of cells to radiation. Cells irradiated in oxygen have a slightly longer period of mitotic inhibition than those irradiated in air; also, fewer cells reach the mid-mitotic stages during the six-hour period of observation after irradiation. It appears, therefore, that the degree of radiosensitivity of cells with respect to mitotic inhibition can be correlated with the amount of oxygen present at the time of irradiation.

Experiments are now in progress to determine the effects of the evacuation plus exposure to each specific gas without irradiation. The data of all experiments are being treated statistically to determine significant points.

Chromosome Fragmentation. Before a study can be made of the effect of oxygen, or lack of it, on frequency of X-ray induced chromosome fragmentation

in the grasshopper neuroblast, some preliminary studies are necessary. For comparison of effects of two treatments on the frequency of chromosome fragments, determination must be made of the duration of the interval between the stage at which fragments are produced (using interphase or very early prophase) and the stage at which the fragments are observed (late anaphase) after different treatments which may affect differently the mitotic rate.

Seven eggs were irradiated in air and seven in nitrogen and then made into hanging-drop preparations. Cells treated in interphase were observed closely until they reached late anaphase, the time for each cell being recorded. Observations were made over a six-hour period after irradiation. The interphase-late anaphase duration was found to average about 278 minutes (at 38°C) when the irradiation had taken place in air, whereas the average duration was 240 minutes after irradiation in nitrogen. Approximately half the cells irradiated at interphase in air (total: 81) failed to reach late anaphase in the six-hour period of observation, while nearly all of the cells irradiated at interphase in nitrogen reached late anaphase in the same period of time. This is further evidence that the presence of oxygen is a critical factor in the sensitivity of cells to radiation.

Since fragment studies are made on embryos which are not dissected from the egg until several hours after irradiation, *i.e.*, they remain in the egg during the interphase-late anaphase interval, it is necessary to know whether the conclusions reached with cells in hanging-drop preparations concerning the duration of this interval after different treatments can be applied to embryos which are undissected. Experiments are now in progress to determine whether the mitotic rate of grasshopper neuroblasts is the same when the embryo is in the egg as when it is in a hanging drop of artificial culture medium. This study has been delayed considerably by a seasonal low supply of grasshopper eggs.

Mitotic Rate of Cells Having Low Metabolic Rate. (Gaulden and Harrington) Availability of large numbers of cells in the same stage of mitosis is of advantage for experimental use of various chemical and physical agents. Mitoses in the several hundred neuroblasts of the grasshopper embryo are not synchronous.

It has been found that if 14-day-old eggs are dried and placed at 17°C, the development of the embryos will come almost to a standstill. They show no morphological change over a period of seven days, while control embryos kept

moist at this temperature for the same period of time develop at a rate characteristic for this temperature. Dried eggs returned to moist dishes at the end of this time will resume, without showing any deleterious effects, the characteristic growth rate. Growth can be retarded considerably by drying eggs at room temperature, but the per cent of eggs recovering is not as high as with those dried at 17°C.

Since the interphase and early prophase stages are those of longest duration in the mitotic cycle in untreated embryos, and consequently more cells are in these stages at any given time, it follows that they will be of even greater duration in embryos whose development is drastically retarded, rate of development being a reflection of mitotic rate. Embryos from eggs kept dry at 17°C are now being studied to determine whether this treatment will in reality give large numbers of cells in the same stage of mitosis, and if so, whether mitosis will be normal when the embryos are returned to normal conditions.

EFFECTS OF RADIATION ON MUTATION OF FUNGI

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

Effect of Oxygen Tension on Sensitivity of Bacteria to X Rays. (Hollaender, Stapleton and Martin) Mention was made in the last quarterly report that the sensitivity of *Escherichia coli* to X rays is dependent to a very significant extent on the concentration of oxygen in the suspending medium. Differences as high as 50,000-fold in survival ratios have been observed depending on whether the bacteria have been irradiated in suspensions saturated with oxygen or nitrogen. A number of factors influencing the differential sensitivity have been investigated:

1. Effect of nitrogen, air and oxygen. — Highly significant differences in sensitivity of bacteria were found to be dependent on whether they were irradiated in a suspension saturated with oxygen, in air, or in nitrogen. (See Tables 9, and 10, Figs. 5 and 6).

2. Comparison of buffer with broth. — Bacteria are more sensitive in buffer than in broth in the presence of air. There is no difference in the sensitivity if they are irradiated in a suspension of broth or buffer in a nitrogen atmosphere. (Fig. 7 and Table 11).

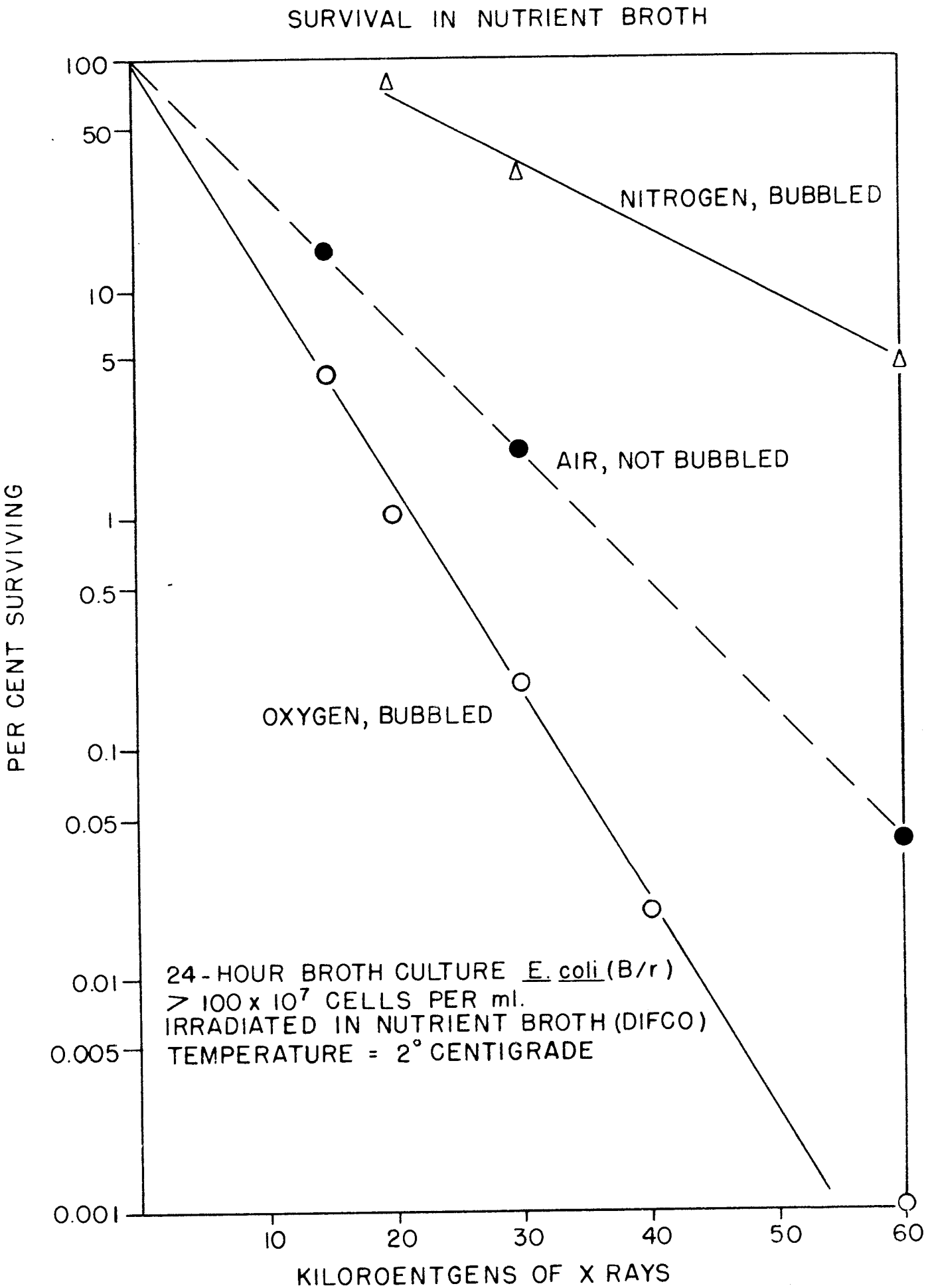

SURVIVAL IN NUTRIENT BROTH
PER CENT SURVIVING
100
50
10
5
1
0.5
0.1
0.05
0.01
0.005
0.001
NITROGEN, BUBBLED
AIR, NOT BUBBLED
OXYGEN, BUBBLED
24-HOUR BROTH CULTURE E. coli (B/r)
> 100 x 10^7 CELLS PER ml.
IRRADIATED IN NUTRIENT BROTH (DIFCO)
TEMPERATURE = 2° CENTIGRADE
10
20
30
40
50
60
KILOROENTGENS OF X RAYS

FIG. 5

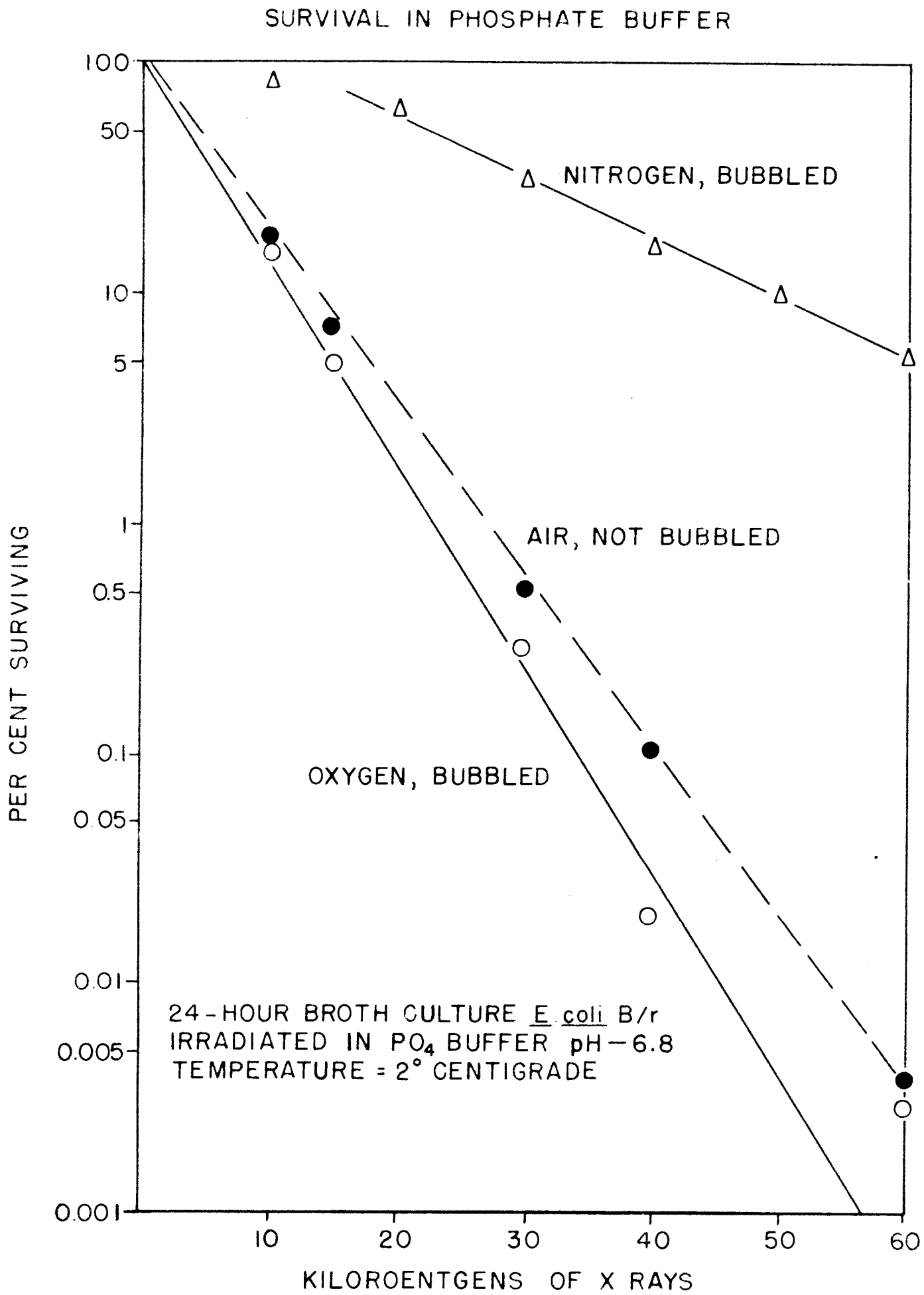

SURVIVAL IN PHOSPHATE BUFFER
NITROGEN, BUBBLED
AIR, NOT BUBBLED
OXYGEN, BUBBLED
24-HOUR BROTH CULTURE E coli B/r
IRRADIATED IN PO4 BUFFER pH-6.8
TEMPERATURE = 2° CENTIGRADE
PER CENT SURVIVING
100
50
10
5
1
0.5
0.1
0.05
0.01
0.005
0.001
10
20
30
40
50
60
KILOROENTGENS OF X RAYS

FIG. 6

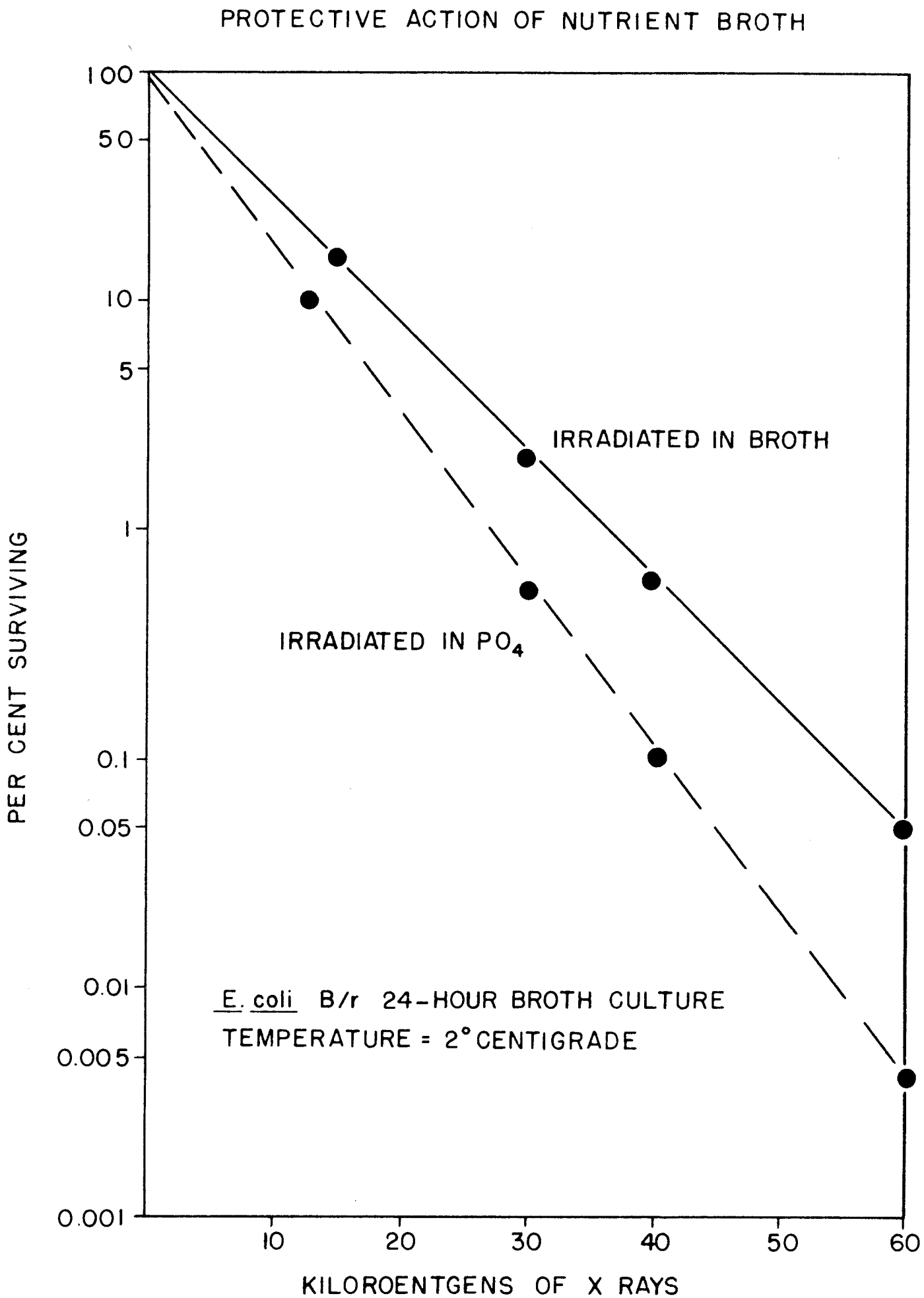

PROTECTIVE ACTION OF NUTRIENT BROTH
100
50
10
5
1
0.1
0.05
0.01
0.005
0.001
PER CENT SURVIVING
IRRADIATED IN BROTH
IRRADIATED IN PO_4
E. coli B/r 24-HOUR BROTH CULTURE
TEMPERATURE = 2° CENTIGRADE
10
20
30
40
50
60
KILOROENTGENS OF X RAYS

FIG. 7

TABLE 9

Survival of bacteria irradiated in buffer

Phosphate buffer 0.15 *M* pH = 6.8
Temperature = 2°C
Bacterial concentration = >. 100 × 10^7

DOSE IN ROENTGENS	AIR		OXYGEN		NITROGEN	
	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED
10,000					79.6	1568
15,000	10.5	959	5.11	1212		
20,000			2.51	319	66.5	4383
30,000	0.475	2121	0.626	5295	20.77	3048
40,000	0.0977	595	0.0113	1428	16.9	3004
60,000	0.0075	2446	0.0038	4326	4.66	7909

TABLE 10

Sensitivity of bacteria irradiated in broth

Nutrient broth (Difco) 8 g/*l* pH = 6.8
Temperature = 2°C
Bacterial concentration = >. 100 × 10^7 cells/ml

DOSE IN ROENTGENS	AIR		OXYGEN		NITROGEN	
	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED
15,000	14.19	1526	4.37	1653	81.0	2440
30,000	2.02	2332	0.221	2790	32.18	3692
40,000	0.602	204	0.134	876		
60,000	0.0421	1086	0.0033	643	4.49	3045

TABLE 11

Broth protection effect (in air)

Temperature ~ 2°C
Buffer = 0.15 *M* PO_4 *p*H = 6.8
Broth = 8 g/*l* *p*H = 6.8

DOSE IN ROENTGENS	IRRADIATED IN BUFFER		IRRADIATED IN BROTH	
	PER CENT SURVIVING	NO. COL. COUNTED	PER CENT SURVIVING	NO. COL. COUNTED
15,000	10.5	951	14.99	1526
30,000	0.475	2121	1.038	2008
40,000	0.0978	595	0.598	907
60,000	0.0075	2446	0.087	2436

3. Comparison of the effect of different gases. — There is very little difference in the sensitivity of the organisms if the suspension is saturated with either nitrogen, helium, hydrogen, or carbon dioxide (Table 12).

TABLE 12

Effect of various gases on sensitivity irradiated in buffer

Temperature = 2°C
*p*H = 6.8
Bacterial concentration = > 100 × 10^7

DOSE IN ROENTGENS	NITROGEN		HYDROGEN		HELIUM		CARBON DIOXIDE	
	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED
30,000	20.77	3004	17.6	2498	21.8	694	10.9	1160
60,000	4.66	7909	2.98	2132	2.76	879	1.43	1571

4. Effect of bacterial concentration. — In the range of 200×10^6 to 200×10^9 bacteria/cc there is no effect of concentration (Table 13).

TABLE 13

Bacterial concentration effect

Irradiated in PO_4 Buffer $pH = 6.8$
Temperature ~ 2°C
Treated in air

CONCENTRATION	10,000 r		20,000 r		30,000 r	
	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED	PER CENT SURVIVAL	NO. COL. COUNTED
193×10^7	16.0	2941	4.09	237	0.42	1644
193×10^6	18.32	1062	4.09	2518	1.036	60
193×10^5	13.9	807	0.512	246	0.005	706
193×10^4	3.78	219	0.134	155	0.0004	868
193×10^3	2.54	147	0.052	308		

However, at lower concentrations there is an increased sensitivity with decrease of concentration of bacteria in the suspension.

A number of organic compounds have been found to provide considerable protection to the bacteria against radiation effects. There has also been observed an interesting relation of temperature on the effectiveness of X rays, differing again with the use of oxygen and of nitrogen.

Lethal and Mutagenic Action of Ionizing Radiations on *Aspergillus terreus*. (Stapleton) As an integral part of a study of conditions and agents that alter the sensitivity of living cells to ionizing radiations, the spores of *Aspergillus terreus* were subjected to X radiation in a variety of conditions, chief among which are:

1) Low and high water content
2) Low and high oxygen tension.

A combination of (1) and (2) can be studied where one might find a relation of oxygen tension to water content.

The first condition seemed worthy of investigation in light of the probable relation of intra- or intercellular water to the indirect production of radiation effects. Spores of this fungus are well adapted to such a study since the water content of these cells can be varied over a wide range without altering their viability.

Three states, with reference to water content, are easily obtained with spores, namely:

a) Suspended, where the water content is 80-90 per cent, by weight,

b) Dry or normal, 30-40 per cent of weight in water,

c) Desiccated, 10 per cent or less in water.

Spores, exposed in these three states, show a very striking relationship of X-ray sensitivity to water where the dose necessary to produce the same quantitative lethal effect can be compared as follows:

STATE	DOSE IN ROENTGENS TO PRODUCE 99 PER CENT KILLING
(1) Suspended	50,000
(2) Normal (dry)	75,000
(3) Desiccated	120,000

It is of interest here that, from state (2) to (3), only the intracellular water content was changed, which indicates importance of intracellular water reactions with ionizing radiations.

The mutation story is somewhat perplexing, but, nevertheless, the number of morphological mutants recovered from irradiated samples, under the three conditions mentioned above, definitely decreases in the same order as the lethal effect.

From previous studies of mutation frequency as a function of X-ray dosage, there was reason to suspect some second order mutation. The nonlinearity of the curve at higher doses might be accounted for by production of chromosomal aberrations.

These second-order mutations seem to be lowered in frequency and perhaps eliminated by removal of free water from the cells previous to irradiation.

This phenomenon warrants much further study. Such treatment might provide a way of differentiating between those mutations produced by *direct* and *indirect* action of ionizing radiations.

The investigation of oxygen tension to radiation sensitivity can be summarized as follows:

1. The sensitivity of suspended spores is grossly altered when the oxygen tension is reduced. About twice the dose is necessary to produce the same quantitative effect when the oxygen tension is decreased by passing nitrogen gas through the suspension.

2. The sensitivity of normal spores, which contain less water, is also changed but not as strikingly as in the suspended spores.

3. The mutation frequency is lowered by reducing the oxygen tension, this effect being similar to that described for the desiccated spores.

MAMMALIAN GENETICS

GENETIC 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 Effects of Radiation on the Preimplantation Stages of the Mouse Embryo. (Russell and Russell) It was shown in a previous publication (in press) that irradiation of female mice on days ½, 1½, 2½, 3½ or 4½ following copulation reduced the likelihood of subsequent birth of a litter from 70 per cent to 46 per cent (not significant) and the average litter size from 6.67 ± 0.33 to 3.78 ± 0.68 (significant; t = 3.8). Young coming to term were, however, normal externally in 100 per cent of the cases and skeletally in 98 per cent at birth. Both (a) high prenatal mortality and (b) virtual absence of abnormalities were in sharp contrast to results obtained from irradiating later stages, where, with little or no prenatal mortality, young at birth show a high incidence of deformity characteristic of stage of treatment and of dose.

Since days ½ - 4½ postfertilization are cleavage stages in the mouse and since, from work on other vertebrates, disturbances during those stages may be expected to yield double-monsters, cyclopeans and otocephalics — types notably absent from our otherwise large array of abnormals resulting from treatment of later stages — it seemed of interest to examine the cases of presumed prenatal death for the occurrence of the monsters enumerated. Furthermore, since days ½ - 4½ of the mouse embryo are spent free in oviduct and uterus, litter size reduction might also have been due to faulty implantation, *i.e.*, possibly a radiation effect on the mother.

Method. Females of the C57 black strain are permitted to have one pre-experimental litter then are mated to NB strain males and examined daily for vaginal plugs (an indication of mating). On days ½, 1½, 2½, 3½ or 4½ after mating, they are taken to the X-ray machine where half of them are exposed to a single dose of 200 r (250 kvp; rate approx. 86 r/min.). The controls are put through the motions of irradiation to eliminate any effect of other disturbance (*e.g.*, noise). It is also important to pair controls with experimentals as closely as possible since there may be seasonal variations in

fertility. Groups of females are then sacrificed at various stages in pregnancy and the uterine contents examined. The stages thus far observed are day 13½ postcoitus (series almost complete) and day 10½ (series about one-third complete). It is planned to observe at successively earlier stages.

Results. Table 14 sums results thus far obtained for the two stages of observation but separates them according to stage of treatment.

TABLE 14

Results of uterine dissections on days 13½ and 10½ (summed) of pregnancy in females treated at various early stages. Tabulated by stage of treatment.

STAGE TREATED	TREATMENT	NO. TREATED	NO.[a] PREG.	PER CENT PREG.	NO. LIVE EMBRYOS	NO. RESORPTION SITES	AVER. NO.[b] LIVE EMBR.	AVER. NO.[b] RES. SITES	AVER. NO.[b] IMPLANTS.
Day ½	200 r	8	6	75	12	23	2.0	3.8	5.8
" 1½	200 r	7	3	43	5	13	1.7	4.3	6.0
" 2½	200 r	8	6	75	11	32	1.8	5.3	7.2
" 3½	200 r	6	1	17	1	6	1.0	6.0	7.0
" 4½	200 r	2	1	50	6	2	6.0	2.0	8.0
Total	200 r	31	17	54.8	35	76	2.06	4.47	6.53
Day ½	Control[c]	8	8	100	55	12	6.9	1.5	8.4
" 1½	"	7	5	71	34	8	6.8	1.6	8.4
" 2½	"	8	6	75	39	6	6.5	1.0	7.5
" 3½	"	6	5	83	35	11	7.0	2.2	9.2
" 4½	"	1	1	100	6	2	6.0	2.0	8.0
Total	Control[c]	30	25	83.3	169	39	6.76	1.56	8.32

a Showing any signs of implantation.

b Based on pregnant uteri only.

c Put through all motions of irradiation on day indicated.

Examining only the totals, certain conclusions may be drawn. (1) Irradiation greatly decreases the average number of living embryos per pregnant uterus and increases the number of resorbing bodies. (2) The total number of implantations (*i.e.*, living embryos plus resorbing bodies) averages significantly less for the irradiated uteri (6.53 ± 0.54 vs. 8.32 ± 0.34 in controls; t = 2.8). This means that some embryos either do not implant or that all traces of implantation have disappeared by days 10½ and 13½. (3) The percentage of pregnant uteri (*i.e.*, showing any signs of implantation) is significantly less in the irradiated group (t = 2.1, using Yates correction). Whether the mechanism for (3) is simply an extension of the effect mentioned in (2) needs to be elucidated by further analysis. The breakdown by day of treatment is not too meaningful with the as yet small numbers. However, for the data as they stand, it may be pointed out that day 3½ is the most sensitive.

From Table 15, which separates the results (summed for time of treatment) according to day of observation, it may be concluded the deaths very probably occur before day 10½. In the irradiated group the average number of living embryos per pregnant uterus does not decrease between days 10½ and 13½ (as a matter of fact, it increases, which is presumably due to sampling, because of the small number of living embryos in that group). The control data, somewhat more meaningful, seem to indicate a very slight degree of mortality between days 10½ and 13½, but the result becomes doubtful because of the difference in the number of implantations (whereas it seems extremely unlikely that a resorbing body present on day 10½ has disappeared without trace by day 13½). An interesting sidelight is the apparent lack of mortality between day 13½ and term, *i.e.*, day 19½ (litter sizes 6.5 and 6.7 respectively). In both the irradiated group and controls, all living embryos were externally normal.

Tentative Conclusions. Acute irradiation with 200 r of female mice during the first five days after copulation significantly decreases the number of living young born. This decrease is the result of at least two mechanisms: (1) Failure of implantation. (2) Death between the time of implantation and day 10½ of pregnancy. Survivors at day 10½ are normal and continue thus to term.

TABLE 15

Results of uterine dissections at two stages of pregnancy in females treated on days ½, 1½, 2½, 3½ or 4½ (summed). Tabulated by stage at dissection.

TREATMENT	EMBRYONIC STAGE OBSERVED	NO. TREATED	NO.[a] PREG.	PER CENT PREG.	NO. LIVE EMBRYOS	NO. RESORBING BODIES	AVER. NO.[b] LIVE EMBR.	AVER. NO.[b] RES. BODIES	AVER. NO.[b] IMPLANT.
200 r	Day 13½	22	11	50	27	46	2.5	4.2	6.64
"	" 10½	9	6	67	8	30	1.3	5.0	6.33
Control[c]	Day 13½	21	16	76	104	26	6.5	1.6	8.13
"	" 10½	9	9	100	65	13	7.2	1.4	8.67

a Showing any signs of implantation.

b Based on pregnant uteri only.

c Put through all motions of irradiation.

The Incidence of Sterility and Partial Sterility in the Offspring of X-Irradiated Mice. (W. L. Russell, Gower, Jones and Henderson.) Snell (Genetics, 20:545, 1935) and Hertwig (Biologisches Zentrablatt, 58:273, 1938) have shown that the offspring conceived shortly after acute X irradiation of either parent mouse fall into three groups: completely sterile, partially sterile and fertile. The partial sterility is inherited by descendant generations in accordance with results that would be expected from reciprocal translocations. Linkage tests and cytological observations in some of the partially sterile lines also indicate that reciprocal translocation is involved.

In attempting to obtain some partially sterile lines for certain types of investigation not tried by earlier workers, and which need not be discussed here, we have obtained additional data on the incidence of sterility and partial sterility. The total number of animals tested is not large, but as the status of each animal was established by extensive tests the data seem worth reporting for comparison with the results obtained by Snell and Hertwig.

The animals tested, 22 males and 15 females, were offspring of males irradiated in the course of another experiment. Data are still in process of collection, but the results obtained from the male offspring will be reported now since they are already adequate to establish which of these offspring are partially sterile. Results for female offspring will be presented later. The radiation factors were as follows: 250 kvp X rays, delivered by a Coolidge

self-rectifying tube with a half-value of 0.4 mm copper. Using a 3-mm aluminum filter and a current of 15 milliamperes, the intensity obtained at 84 cm was approximately 80 r per minute. The animals were exposed in individual compartments of a lucite box resting on a 10.8-cm-high masonite scatter block. Three dosages were used: 500 r, 750 r, and 1000 r.

The irradiated males were 101 × C3H F_1 hybrids. These were mated to females of a noninbred test strain. The 22 male offspring used in this experiment were conceived within from two to thirty days after irradiation of the father. Each of these offspring was mated with from three to seven vigorous and fertile 101 × C3H F_1 hybrid females. The number of litters and mean litter size for each of these test matings, and for all the matings of each male, are given in Table 16. Not included in the table are three males which produced no young though each was with three females for more than seven months after reaching the age of sexual maturity.

It is apparent from the results that four (Nos. 1, 21, 33 and 38) of the 19 males which produced offspring can clearly be classified as partially sterile. The results for all males are summarized in Fig. 8 and Table 17.

The results obtained are compared with those of Snell and Hertwig, for similar dose ranges, in Table 18. Averaging the effects over wide dose ranges is, of course, not justified as the incidence is not linearly related to dose. However, over the ranges given here, such a procedure is probably not seriously in error and, in any case, Snell does not tabulate his results in a way that can be used to calculate the incidence for each dose.

The results obtained here are in remarkably close agreement with the sum of those obtained by Snell and Hertwig. It may be concluded that approximately one third of the male offspring obtained shortly after irradiation of the male parent with doses in the neighborhood of 700 r will be either partially or completely sterile.

TABLE 16

Number of litters and mean litter size for each test mating and for all matings of each male

SERIAL NO. OF ♂ AND DOSE GIVEN TO ♂ PARENT	RESULTS OF EACH MATING: NO. OF LITTERS	RESULTS OF EACH MATING: MEAN LITTER SIZE
1 (500 r)	6	3.8
	4	4.0
	3	4.7
	5	2.8
	4	3.5
	6	3.5
	1	3.0
	Total 29	3.6
4 (1000 r)	1	8.0
	2	8.5
	6	8.8
	Total 9	8.7
6 (500 r)	6	10.3
	4	10.3
	1	9.0
	1	9.0
	Total 12	10.1
8 (750 r)	4	5.3
	5	9.2
	3	8.7
	2	7.5
	2	7.5
	Total 16	7.7
9 (750 r)	5	9.4
	2	7.5
	6	8.7
	Total 13	8.8
10 (750 r)	5	9.4
	7	8.3
	6	10.0
	1	7.0
	Total 19	9.1
11 (750 r)	3	9.3
	3	10.0
	8	9.4
	Total 14	9.5
12 (750 r)	6	8.7
	7	8.6
	6	10.0
	Total 19	9.1
20 (500 r)	6	8.5
	8	6.4
	5	10.2
	Total 19	8.1
21 (500 r)	6	5.0
	6	4.5
	6	3.2
	5	4.4
	5	4.6
	5	4.4
	Total 33	4.3
31 (500 r)	7	8.4
	5	8.4
	5	8.2
	Total 17	8.4
33 (500 r)	6	3.5
	6	4.2
	4	4.8
	Total 16	4.1
34 (500 r)	5	8.8
	7	7.7
	6	9.0
	Total 18	8.4
35 (500 r)	3	10.0
	6	9.3
	5	8.4
	Total 14	9.1
36 (500 r)	6	8.8
	5	8.4
	6	7.8
	Total 17	8.4
38 (750 r)	5	4.8
	5	4.6
	6	4.8
	Total 16	4.8
41 (750 r)	5	9.2
	6	8.5
	5	10.8
	Total 16	9.4
42 (750 r)	5	10.4
	6	9.0
	6	8.7
	Total 17	9.3
43 (750 r)	1	10.0
	1	6.0
	1	6.0
	Total 3	7.3

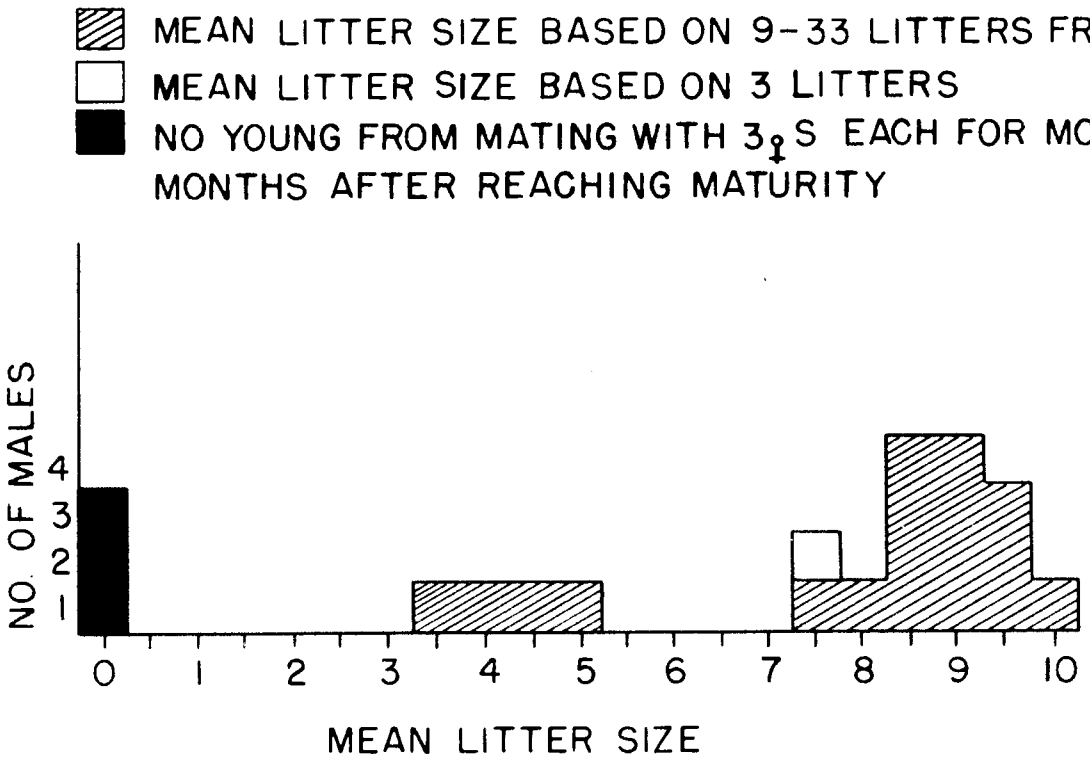


FIG. 8 MEAN SIZE OF LITTERS PRODUCED BY MALE OFFSPRING OF IRRADIATED MALES

TABLE 17

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

DOSE TO ♂ PARENT (r)	OFFSPRING			
	STERILE	PARTIALLY STERILE	FERTILE	TOTAL
500	2	3	6	11
750	1	1	8	10
1000	-	-	1	1
Total (Mean dose 636r)	3	4	15	22

TABLE 18

Comparison of results with earlier data on incidence of sterility and partial sterility in male offspring of irradiated males

INVESTIGATOR	DOSE TO ♂ PARENT (r)	OFFSPRING			
		PERCENTAGE			TOTAL NUMBER
		STERILE	PARTIALLY STERILE	FERTILE	
Snell	400 - 1200 (mostly 600 - 800)	10.2	35.6	54.2	59
Hertwig	500 - 1000 (Mean 740)	12.3	12.3	75.3	81
Total, Snell and Hertwig		11.4	22.1	66.4	140
W.L. Russell	500 - 1000 (Mean 636)	13.6	18.2	68.2	22

MICROBIOLOGY

TRACER STUDIES ON METABOLISM

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D. S. Anthony
J. W. Foster (Res. Participant)
E. F. Phares
W. J. Jefferson
Mary V. Long

Dr. J. W. Foster, Professor of Bacteriology at the University of Texas, has just completed a five-month period of resident collaboration with this group, a most fruitful project for the Biology Division. Dr. Foster brought with him results of several long-term investigations on biochemistry of fungi, which had been advanced to the crucial point of exceeding amenability to attack by carbon tracer methods. These methods, brought to successful development in our laboratory during the past two years, paved the way for a number of quite important findings in fundamental biochemistry.

Below three papers are summarized — one has been published, and the remaining two are in press.

Aerobic Formation of Fumaric Acid in the Mold *Rhizopus nigricans*: Synthesis by Direct C_2 Condensation. (Proc. Nat. Acad. Sci., 35:663, 1949) (Foster, Carson, Anthony, Jefferson, Long) Conditions for obtaining high yields of fumaric acid from ethanol by *Rhizopus nigricans* are specified. Yields of fumaric acid were so high that the tricarboxylic acid cycle as a mechanism of formation of fumarate is excluded. Yields of fumarate from acetate are one-third those from alcohol. Experiments with unlabeled ethanol and C^{14}-labeled carbon dioxide eliminate a carbon dioxide fixation mechanism of fumarate synthesis. The mold possesses an enzyme which oxidizes succinate to fumarate.

Fumarate formed from C^{14} methyl-labeled ethanol contains the same specific activity in the methine-carbon as that of the methyl group from the starting ethanol. The carboxyls contain a small amount of radioactivity due to a reversible decarboxylation reaction probably involving oxalacetate decarboxylase.

Fumarate from C^{14} carbinol-labeled ethanol contained no radioactivity in the methine-carbons, but abundantly in the carboxyls. All the evidence proves unequivocally that the C_4 dicarboxylic acid synthesis occurs by direct $2C_2$

condensation as hypothesized originally by Thunberg. The implications of this reaction in the tricarboxylic acid cycle of respiration and in synthesis of tracer intermediates for biochemistry and synthetic organic chemistry are discussed.

Metabolic Exchange of Carbon Dioxide with Carboxyls and Oxidative Synthesis of C_4 Dicarboxylic Acids. (Carson, Foster, Phares, Anthony, Long and Jefferson) In the preceding section of this report, evidence was advanced for the biosynthesis of fumaric acid by *direct* condensation of $2C_2$ moieties. Fumaric acid formed from unlabeled ethanol in the presence of labeled carbon dioxide was found to contain labeled carbon in the carboxyl groups. Hence it was indicated that a "carbon dioxide metabolic exchange" with preformed C_4 acids can occur, and this would have broad significance for all tracer studies involving labeled carbon dioxide and C_4 acids.

Experiments were conducted on fumarate synthesis from labeled ethanol with varying atmospheric carbon dioxide tensions. As the carbon dioxide tension was increased, the specific radioactivity of the fumaric acid carboxyls decreased. This finding strongly suggests that a carbon dioxide exchange reaction takes place with fumaric acid. In one experiment, with 5-per-cent carbon dioxide in the atmosphere (labeled with C^{13}), an exchange amounting to 21 per cent occurred.

The results of a number of such experiments also indicated that a C_4 respiratory cycle may be at least partially responsible for the movement of C^{14} from methyl to carboxyl. This is based partly on the fact that the C^{14}-labeled methine carbons of fumarate have exactly the same specific activity as the C^{14}-methyl-labeled ethanol precursor. Since the conclusions require that there be no mixing of methine and carboxyl carbon (in fumarate), this point has been most carefully re-examined. Three independent methods of fumarate degradation were used.

If the interpretation of the data obtained relative to $2C_2$ condensation is correct in its main conclusion, it opens a direct oxidative pathway to the C_4 dicarboxylic acids in addition to the Wood-Werkman reaction. Indeed, demonstration in normal tissues of the Wood-Werkman formation of C_4 acids by locating the labeled carbon dioxide in carboxyls of these acids would not, in view of these experiments, constitute unequivocal proof of *net* synthesis involving carbon dioxide, for metabolic exchange of the carbon dioxide with carboxyls of acids *preformed, and by a routine independent of carbon dioxide fix-*

ation, may yield the same results. It would seem that, in the absence of evidence excluding the operation of other mechanisms of organic acid synthesis, the incorporation of labeled carbon dioxide into carboxyl groups would have to be interpreted with considerable reserve.

Citric Acid Formation by *Aspergillus niger* Through Condensation of $3C_2$ Moieties. (Carson, Foster, Phares, Anthony, Jefferson, Long) Biogenesis of citric acid through condensation of oxalacetate and acetate (C_4 dicarboxylic acid + C_2) is a reasonably well-accepted hypothesis. The C_4 dicarboxylic acid is generally presumed to originate by way of the Wood-Werkman reaction (pyruvate and carbon dioxide) though now the C_2 condensation must also be considered (Foster *et al.*, Proc. Nat. Acad. Sci., 35:663, 1949). The following experiments were intended to demonstrate the relative participation of the two modes of genesis of the C_4 moiety.

Radioactive citrate was produced from sucrose by washed *Aspergillus niger* submerged mycelium (200 mg dry weight) in the presence of 2 mg of high specific activity methyl-C^{14}-labeled acetate and carbon dioxide containing 19.2 atom per cent $C^{13}O_2$. To the 38 mg of citric acid produced in 40 hours (at which time considerable unconsumed sucrose remained) 350 mg of carrier citric acid was added; calcium citrate was isolated, and purified by precipitation and twofold reprecipitation from hot solution.

The radioactive citric acid was converted to pentabromoacetone, which represents the noncarboxyl carbons of the citric acid. The noncarboxyl carbons were also obtained in the form of acetone, by dilute acid-dichromate oxidation of another portion of citric acid. The acetone was further degraded to iodoform and acetic acid; the acetic acid was then degraded (Phares, to be published) to methyl amine and carbon dioxide. Specific activity measurements were made on barium carbonate obtained by wet combustion.

The mean C^{13} content of the atmospheric carbon dioxide [(19.2 - 10.5)/2 = 14.9 atom per cent] permitted the calculation that carbon dioxide carbon from the atmosphere entered citrate to the extent of 1.3 per cent of the total citrate carbon; if the Wood-Werkman reaction were entirely responsible for net citrate synthesis the figure should be 16.7 per cent. Theoretically, unlabeled intracellular carbon dioxide from sucrose could also account for some net synthesis; it has not yet been possible to conceive a definitive experiment on this point. On the other hand there is an equally good possibility that the C^{13} in citrate entered by simple metabolic exchange (Foster and Carson, in press).

TABLE 19

C^{13} and C^{14} Values

FRACTION	SPECIFIC ACTIVITY[a]	ATOM PER CENT C^{13}
1. Total citric acid	0.16	1.107 ± 0.005[c]
2. Noncarboxyl carbons:		
Pentabromoacetone	0.15	1.084 ± 0.002[c]
Acetone	0.15	
Iodoform	0.17	
Acetic acid	0.16	
Methyl amine	0.15	
Carbon dioxide	0.17	
3. Carboxyl carbons:		
Primary carboxyls	0.12	1.132 ± 0.009[c]
Secondary carboxyl	0.16	1.090 ± 0.010[c]
4. CO_2 in atmosphere		
Initial	0.00	19.2 ± 0.1
Final	0.43[b]	10.5 ± 0.1

[a]Counts/sec/mg $BaC^{14}O_3$ (measured on citrate diluted with carrier).

[b]Measured as 4.3 counts/sec/mg $BaC^{14}O_3$, but calculated as if diluted same amount as the citrate.

[c]Measurements made on citrate diluted with carrier, and its degradation products.

The essentially equal specific activity of the noncarboxyl carbon chain is interpreted to mean that it arose by condensation of methyl groups of acetate, probably thus: $2C_2 \longrightarrow C_4$; $C_4 + C_2 \longrightarrow C_6$. Isotope dilution experiments with this organism have demonstrated the synthesis of C_4-dicarboxylic acids from ethanol by the $2C_2$ condensation reaction (unpublished data). The high C^{14} specific activity of the final carbon dioxide (three times that of citrate even though diluted heavily with unlabeled carbon dioxide from substrate) indicates a very active C_4-dicarboxylic acid respiratory cycle. Such a cycle moves methyl activity to carboxyl, and thus one finds C^{14} in all three citrate carboxyls; whereas C^{13} from $C^{13}O_2$ enters primary carboxyls only (carbon dioxide fixation and/or exchange).

RADIATION BACTERIOLOGY

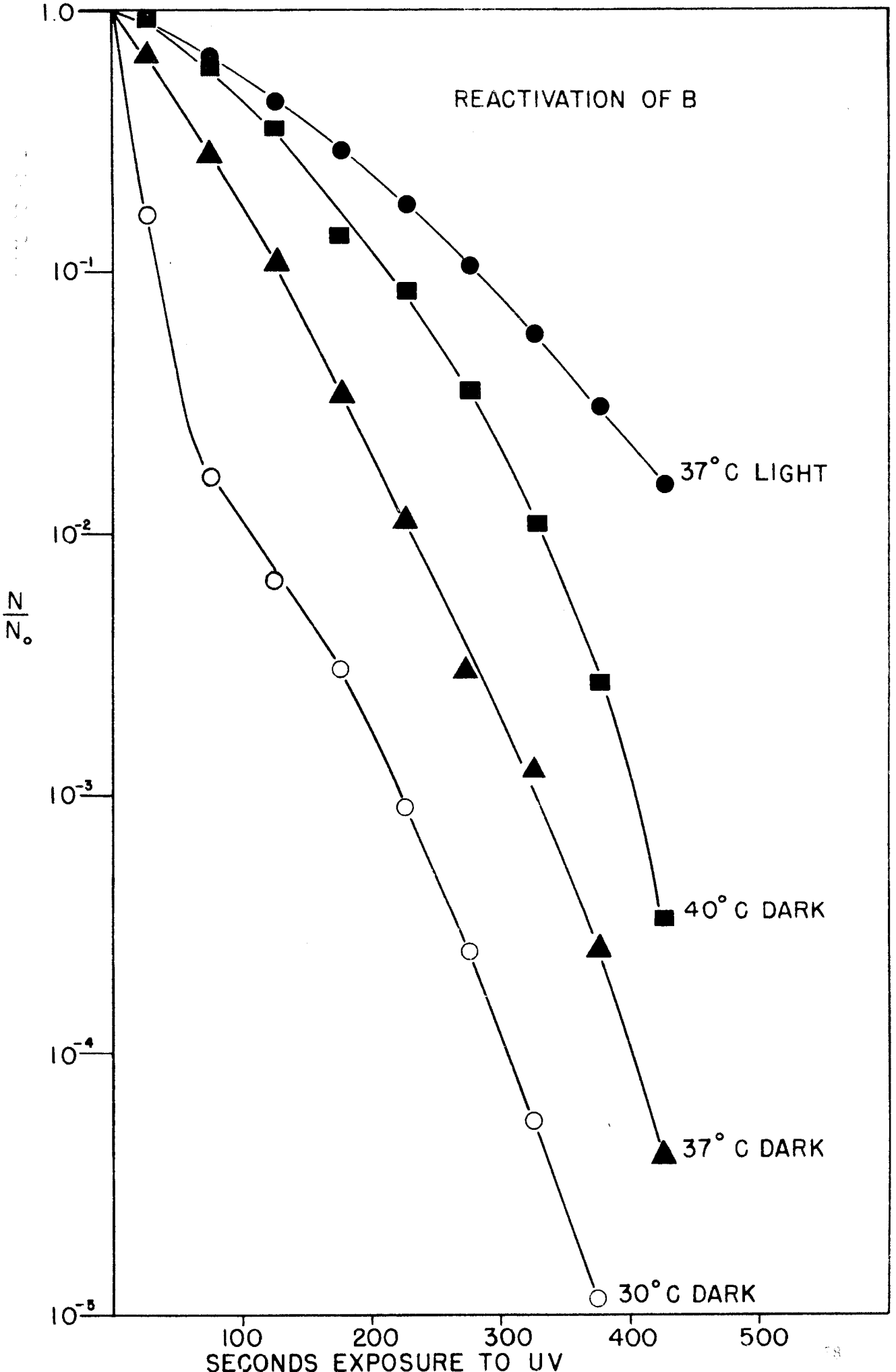
E. H. Anderson (Leader) Ruth W. Whittle

Reactivation of Ultraviolet Irradiated Bacteria. (Anderson, Whittle) A method of reactivating ultraviolet-inactivated bacterial cells by heat treatment was described in the last quarterly report. These studies have been continued and show that the B and B/r strains of *Escherichia coli* differ very greatly in their response to postirradiation conditions affecting survival.

Ultraviolet-inactivated cells of both *coli* strains may be reactivated to a very high degree by exposure to visible light for short periods. Survival curves of strain B in which aliquot plates were incubated at 30, 37 and 40° C in the dark show that the temperature at which ultraviolet-inactivated cells of this organism are incubated has a very pronounced effect on their survival. Data from one experiment is presented in Fig. 9. Although unirradiated cells show no difference in assay values over the temperature range used it may be seen that a ten-degree rise in incubation temperature between 30 and 40°C produces a reactivation of ultraviolet-inactivated cells of strain B of the same order of magnitude as does photoreactivation. Strain B/r does not respond to heat reactivation to an unusual degree. The same incubation temperature increment produces a three- to fourfold increase in survival with this strain, but only at the longer ultraviolet exposures. This is in sharp contrast to the B strain which shows a rapid rise in heat reactivation at short ultraviolet exposures.

The difference between strains B and B/r in their response to postirradiation treatment affecting survival is not limited to heat reactivation. Although both strains are capable of being photoreactivated to a similar degree the two strains respond to reactivation with visible light in a different manner. A study of the ratios of light survivors at 37°C to dark survivors at 37°C at various lengths of ultraviolet exposure showed that the degree of photoreactivation of B increases more or less exponentially with the length of exposure to ultraviolet, and therefore with the amount of inactivation. Strain B/r, however, shows a pronounced lag in its degree of photoreactivation at the shorter ultraviolet exposures and only at the longer exposures, at dark survival ratios of 1×10^{-4} or less, does B/r exhibit a degree of response to photoreactivation equal to that obtained with strain B.

FIG. 9


REACTIVATION OF B
1.0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
$\frac{N}{N_o}$
37° C LIGHT
40° C DARK
37° C DARK
30° C DARK
100
200
300
400
500
SECONDS EXPOSURE TO UV

In studies with X-ray-inactivated cells of both strains it was found that neither strain responded to treatment with visible light even at dark survival ratios of 10^{-5}.

Aliquot assay plates for X-ray survival curves for strain B incubated at 30, 37 and 40°C did, however, show that the temperature at which X-ray-inactivated cells of strain B are incubated has an appreciable effect on the survival values obtained. At exposures giving survival ratios of 1×10^{-4} at 37°C incubation a ten- to fifteenfold increase in survival was obtained on plates incubated at 40°C over that obtained at 30° incubation. No temperature effect was found for X-ray survival curves of strain B/r. Under carefully controlled incubation temperatures, X-ray survival curves of uniform slope were obtained with B for the three temperatures used.

Although the degree of heat reactivation obtained with X-ray-inactivated cells of B is of an order of magnitude lower than that obtained with ultraviolet treated cells it may explain the change in slope at about 1 per cent survival that is shown in published X-ray survival curves for strain B.

It is not yet known whether or not heat reactivation of ultraviolet-inactivated cells is as general a phenomenon as photoreactivation appears to be for microorganisms. Preliminary studies carried out with several haploid and diploid strains of *Saccharomyces cerevisiae* have given negative results. Various strains of bacteria are now being investigated for their ability to respond to heat reactivation.

BIOCHEMISTRY

BIOCHEMISTRY OF NUCLEOPROTEINS, 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

Partial Purification of a Nonphosphorylytic Pyrimidine Nucleosidase from Yeast. (Carter) The phosphorylytic cleavage of the riboside linkage in guanosine and inosine by an enzyme purified from extracts of rat liver reported by Kalckar and the previously described requirement for phosphate and arsenate in the cleavage of purine and pyrimidine ribosides by enzymes extracted from several mammalian tissues reported by Klein, raises the problem of whether all the enzymatic cleavages of purine and pyrimidine ribosides are of the phosphorylast type. An enzyme system has been purified by ammonium sulfate fractionation from yeast which hydrolyzes the pyrimidine riboside, uridine, in the absence of phosphate, arsenate or activating cation.

The enzymatic hydrolysis of uridine at the ribose linkage was followed by a spectrophotometric method based on the observation that whereas uracil in alkaline solution has a high molecular extinction coefficient at 290 mμ, uridine in alkaline solution exhibits very little absorption at this wave length. From these data, the conversion of uridine to uracil at a concentration of 19 μg per ml in 1/100 normal sodium hydroxide causes an increase in absorption at 290 mμ of D = 1.0 or delta D/μg uracil form = 0.0525. Uridine nucleosidase of yeast exhibits pH optima at 7 in phosphate, glycine and veronal buffers. The comparative rates of hydrolysis in these buffers are identical. Unlike the purine nucleosidase of liver, yeast pyrimidine nucleosidase exhibits no requirement for inorganic phosphate and is not inactivated by prolonged dialysis. Uridylic acid is not hydrolyzed at the riboside linkage by the purified uridine nucleosidase. No purine or pyrimidine nucleoside other than uridine was hydrolyzed by the enzyme. When ribose was added to the enzyme substrate system in a molar ratio of ribose to uridine of approximately ten to one, a 40 per cent inhibition of the rate of reaction was found.

Due to the low pyrimidine nucleosidase activity of the mammalian tissues a satisfactory enzyme preparation which could be rigorously examined for nonphosphorylytic uridine nucleosidase activity has not been obtained.

Nucleic Acid Metabolism of Microörganisms. (Morse and Carter) It has been shown that cells of the radiation-resistant B/r strain differ from those of the radiation-sensitive B strain by desoxyribonucleic acid (DNA) content. When compared in 24-hour broth cultures the radiation-resistant strain contains three to four times more DNA per cell. However, cells from these two cultures behave differently upon transfer to fresh broth. During the lag phase of both cultures the DNA content of the cells doubles; with the onset of division the DNA content per cell decreases. In the case of B the DNA content per cell returns to the value for 24-hour B cells, while with B/r the DNA content per cell falls to less than that present in 24-hour B/r cells. After three generations the DNA content per cell of B/r approximates that of B. It has been found in addition that the B/r cells are larger in 24-hour broth cultures. Studies indicate that B/r during growth has been found to follow the same course as the DNA per cell of B/r. When cells of B are transferred to fresh medium the volume per cell increases and during growth remains approximately twice the 24-hour volume. Ultraviolet survival curves of cells from cultures of B and B/r with the same DNA and volume per cell still show that differences in resistance to radiation exist between the two strains.

Enzymatic and Ion-exchange Analysis of Desoxyribonucleic Acid. (Volkin, Cohn, Khym and Carter) The preparation of desoxyribonucleotides, in particular, and desoxyribonucleosides has been carried out employing enzymatic digestion of the nucleic acid, followed by separation of the nucleic acid digest products by anion-exchange chromatography.

Desoxyribonucleic acid is first hydrolyzed by desoxyribonuclease, then by intestinal alkaline phosphatase. During the latter process, arsenate is added to inhibit the dephosphorylation of nucleotides by the phosphatase. The reaction rate is followed by titration with alkali. After the reaction has progressed to the stage where one-mole base has been added per mole of released titratable phosphate group the reaction is stopped by the addition of acid to *p*H 4.

The table (Table 20) gives a summary of the ion-exchange products resolved from three such digests. Experiments II and III represent small aliquots of digests prepared for the isolation of large amounts of the desoxyribonucleotides. It can be seen that, under the conditions employed, about 70 per cent of the reaction products are desoxyribonucleotides. Longer periods of enzymatic digestion result in increasing amounts of the corresponding desoxyribo-

sides, whereas shorter digestion periods yield considerable quantities of polynucleotides.

TABLE 20

PRODUCTS	I MILLIGRAMS	I PER CENT OF TOTAL DIGEST	II MILLIGRAMS	II PER CENT OF TOTAL DIGEST	III MILLIGRAMS	III PER CENT OF TOTAL DIGEST
Total digest	150		20		43	
Base + desoxyribosides	27	18	1.5	7	3.0	7
Cytidylic	29	19	2.1	10	6.2	14
Thymidylic	39	26	3.1	16	6.5	15
Adenylic	15	10	3.9	20	9.7	23
Guanylic	28	19	3.2	16	8.7	20
Total nucleotides	111	74	12.3	62	31.1	72
Polynucleotide fraction	13	8	6.2	31	8.9	21

Effect of Gamma Radiation on Chymotrypsin. (Doherty) Reconstruction and calibration of the cobalt source has slowed the progress of this problem. Previous work has been repeated confirming earlier observations as to the resistance of chymotrypsin to gamma radiation. The enzyme concentration has been reduced by a factor of one hundred with no increase in sensitivity noted. The enzyme concentration will be reduced as far as possible commensurate with the stability of the protein and the precision of measurement of enzyme activity. A search is in progress for more sensitive synthetic substrates for this purpose.

Synthesis and Identification of Adenylic Acids "A" and "B". (Doherty) A. M. Michelson and A. R. Todd (*J. Chem. Soc.*, Oct., 1949, p. 2476) have pub-

lished the synthesis of adenosine-2′-phosphate. Their procedure was essentially the same as that reported here previously although experimental details differ somewhat. A comparison of compounds shows good agreement up to the stage of the removal of the protecting benzyl and benzylidene groups from the 3′5′ benzylidene adenosine-2′-dibenzyl phosphate. Their conditions were more drastic and in the process rearrangement took place yielding adenosine-5′-phosphate rather than adenosine-2′-phosphate as expected. A study of mild catalytic removal of these groups has been in progress for some time but so far has not met with success.

Synthesis of ribose 2 phosphate has been attempted by two procedures. α Aniline ribofuranoside has been prepared and attempts made to condense it with benzaldehyde to yield 3,5 benzylidine α aniline ribofuranoside. Several dehydrating agents have been tried and conditions varied widely, but in every case an intractable black tar was obtained. Therefore α methyl ribofuranoside was prepared and condensed with benzaldehyde to yield presumedly α methyl 3,5 benzylidene ribofuranoside. This compound will be phosphorylated and compared with the ribose phosphate from adenylic "A". Several ribose derivatives were also prepared and submitted to Dr. Vaslow for comparative infrared spectrophotometric analysis.

Enzyme Substrate Equilibria. (Doherty and Vaslow) The dissociation constant for the reaction N (acetyl dibromtyrosine) + chymotrypsin = (acetyl dibromtyrosine)$_n$ chymotrypsin has been determined at 20°C and *p*H 5.4 in 1 per cent chymotrypsin solution. Under these conditions the value of K is $(4.3 - .5) \times 10^{-3} \times N$. N, the number of binding positions on the enzyme could not be determined because of the limited solubility of the substrate, but it is at least two and probably greater. Variations of K were noted with *p*H, change of buffer (phosphate to citrate), and possibly with the amount of substrate combined.

Static deflections of the electrometer cannot be determined to an accuracy better than .5 per cent. Since only about 5 per cent of the substrate is bound, the value of K could not be determined to better than 10 per cent. In order to obtain the accuracy necessary for determining the entropy and enthalpy of the enzyme-substrate combination, apparatus for rate of drift measurements has been designed and installed with an increase in accuracy by a factor of about five.

Infrared Spectroscopy. (Vaslow) In order to determine the difference in structures between adenylic acids A and B, the infrared spectrograms of

these and related compounds have been obtained. Preliminary evidence indicates that the adenine part of A is present as a salt; also, there appears to be a strong resemblance between the spectra of A and that of desoxyribonucleic acid.

RADIOBIOCHEMISTRY

C. W. Sheppard (Leader)
W. T. Burnett, Jr.
Leon Wish
Maryann Huddleston
E. B. Darden, Jr.
Gertrude Beyl

Health Physics Trainees. During the previous quarter Lieut. Col. C. S. Maupin and Capt. Ellis Oster, Health Physics trainees in the Armed Forces Special Weapons Project, worked with the group on a project involving the checking of Victoreen condenser-type r-meters against a standard air chamber. The results of their work plus some additional comments on the Victoreen instrument are included in a Division Memorandum by Sheppard, Maupin, Oster and Conger entitled "X-ray Calibration in the Biology Division of Oak Ridge National Laboratory". This work has put our X-ray standardization on a much firmer footing. As a result dosimetric services have been provided to the NEPA project, Oak Ridge Institute of Nuclear Studies and Oak Ridge Hospital through the Health Physics Division, Oak Ridge National Laboratory.

Two Health Physics Fellows, Mr. Robert Sodaro and Mr. L. C. Emerson, are now working with the group as part of their training course.

Training Course in Radiation Physics. The course in radiation physics, revived at the request of Dr. Gaulden and Dr. Furth, meets with Dr. Sheppard once a week for one hour. Some success appears to have been achieved in reducing this subject to elementary nonmathematical terms; the instruction is being well received.

Radiological Physics. (Darden) The calibration of the gamma-ray thimble chamber has been completed and a report is being written. In using this instrument to study the variation of the intensity of the gamma rays in the lead shield containing the cobalt source in room 127, anomalous results were obtained. This was traced to nonuniformity in the activity of the cobalt producing an azimuthal variation of the radiation intensity. With the assistance of Mr. Hembree (Engineering and Maintenance Division) and the Operations Divi-

sion the source was removed and disassembled. A new cap for the brass cartridge was then provided. This cap clamps the cobalt slugs securely with a spring so that no rotation of the slugs is possible inside the container. By mechanically modifying the air cylinder so that no rotation of the piston can occur, the variations were successfully eliminated and extremely reproducible radiation intensities within the lead shield are now observed.

An investigation of the radiation field close to the radiation source is now in progress to improve the accuracy of the exposure data for the studies of enzyme inactivation by the Biochemistry group. These studies have also indicated that under certain conditions scattered radiation cannot be ignored. An investigation of the reliability of the Victoreen condenser r-meter for gamma-ray studies is being included. This is of considerable importance in interpreting earlier work done in this laboratory, in which this instrument was used to measure hard radiation.

Erythrocytes. (Beyl) Further studies of the "prolytic" disturbances of human erythrocytes by X rays confirm earlier observations that irradiated cells leak potassium to the plasma more than the controls. The threshold for the effect seems to be in the neighborhood of 1000 r. The loss of potassium is nearly compensated by a gain of sodium. The effect thus suggests that the cell no longer can distinguish between the two cations. Under existing conditions little osmotic disturbance is observed. The effect is thus considerably more subtle than the classical "radiation hemolysis", and also more clear-cut. By the use of isotopic potassium, the potassium effect is found to be an acceleration of the loss of potassium to the plasma rather than an impaired uptake. In the case of sodium, isotope studies strongly suggest that the inflow of this element occurs through an accelerated uptake rather than an impaired loss.

Mineral Metabolism. (Burnett) Chemical, polarographic, flame photometric, and spectrographic methods have been investigated for possible use in the determination of trace quantities of manganese in biological material. The spectrographic method appears at present to offer the most promise. Interfering substances in the methods tried out include sodium, potassium, iron and zinc. Through the work of Dr. Bigelow, successful techniques have been developed for establishing temporary and permanent types of pancreatic fistulas in dogs. Manganese-54 is being recovered from residues left from a partial separation of the isotope from a cyclotron target. A group of mice has

been injected with ^{54}Mn for the purpose of obtaining radioautographs of certain tissues. A definitive experiment, using the above methods and techniques, is planned for the near future to obtain information relative to the metabolism of manganese in the dog.

Through the careful work of Mr. Davidson of the Instruments Department, a twelve-channel count-rate meter has been put in working order for circulatory mixing studies. This has involved a complete overhaul. The feasibility of using the instrument was demonstrated by giving a dog an oral dose of ^{131}I, recording its disappearance from the stomach and its appearance in the thyroid. The shielding required to count beta and gamma activities close to background in the presence of high gamma activities is being determined by Mr. Sodaro. Thus a number of Geiger-Muller tubes may be used simultaneously and the possibility of recording the level of isotope in the circulation becomes less remote.

Further assistance has been provided to the Pathology and Physiology Group in the injection of mice with ^{131}I.

Colloids. (Wish, with the cooperation of the Pathology and Physiology Group) Since the phagocytic activity of the reticulo-endothelial (macrophage) cells is an important subject for studies in mammalian radiobiology, investigations of the disappearance from the circulation of radioactively-labeled gold colloids are in progress. Gold sols containing ^{198}Au were injected intravenously in mice. The animals were killed at various intervals and the activity of various organs determined. Although 85 - 90 per cent of the activity was found in the liver, in one to three hours the rate of disappearance was significantly less than in other species. More thorough studies involving the preparation of sols of carefully controlled particle size and of more precisely defined properties are now under way. With such materials, studies of the effect of particle size and other parameters on the rate of removal will be investigated.

PLANT PHYSIOLOGY

G. R. Noggle (Leader)
R. A. Bolomey* Eleanor Schumacher

Production, Isolation and Purification of C^{14} Labeled Carbohydrates. (Noggle, Bolomey, Schumacher) A column separative technic for separating glucose and fructose worked out by Dr. Bolomey is as follows: 50 to 100 gm of Magnesol (regular industrial grade obtained by courtesy of Westvaco Chemical Division, Ford Machinery and Chemical Corp., South Charleston, W. Va.) were suspended in 95-per-cent ethanol. The slurry was poured into a column 3.14 $cm^2 \times 100$ cm long fitted with a glass wool plug. The Magnesol particles were allowed to settle as the excess alcohol filtered through the column.

The free volume of the column under operating conditions was determined as follows: a weighed amount of Magnesol was uniformly suspended in a known volume of 95-per-cent ethanol contained in a stoppered graduated cylinder, and the total volume of the alcohol plus the Magnesol was noted. The slurry was then quantitatively transferred to a column of known dimensions and allowed to settle under operating conditions. Particles adhering to the graduated cylinder were washed into the column with more alcohol (the volume of alcohol is not important). The bed volume was measured from the dimensions of the column after equilibrium had been reached and the free volume was then calculated. The solid volume of the adsorbent may be calculated from its density or by substracting the volume of alcohol employed to suspend the adsorbent from the total volume measured in the graduated cylinder. The free volume then becomes the difference between the solid volume and the bed volume after equilibrium has been reached under operating conditions.

Inasmuch as the glucose and fructose were passed through the column dissolved in 95-per-cent ethanol, some method had to be devised for determining the amounts of each sugar in the various eluted fractions. Alcohol interferes with ordinary copper reduction methods so advantage was taken of the α-napthol and sulphuric acid colorimetric test. Ten milliliters of concentrated sulphuric acid were rapidly added to a mixture of 1 ml of 1 per cent α-napthol in 95-per-cent ethanol and 1 ml of an aliquot of the alcohol eluate. The solution was mixed and allowed to stand for one hour at room temperature and the color intensity then compared against a similarly prepared standard in an Eve-

*Transferred to Chemistry Division.

lyn colorimeter (54 filter). The intensity and purity of the color was found to change with time. The instability of the color renders the method only semiquantitative, however the method is simple, rapid and sufficiently accurate to follow the elution of the sugars from the column.

Using the column preparation and the sugar analysis methods described, the following results were found.

Known amounts of C. P. glucose and fructose were dissolved in a minimal amount of 95-per-cent ethanol and added to the top of the Magnesol column. Then 95-per-cent ethanol was added to move the sugars through the column. It is essential that the flow rate be very slow. The first fraction of the eluate (0 to 1.4 free volumes) contained as a rule insignificant quantities of glucose. The fraction represented by 1.4 to 2.6 free volumes contained 70 to 80 per cent (depending on the flow rate) of very pure glucose. Once the main portion of the glucose band has been eluted the glucose in the remaining eluate approaches zero concentration asymptotically and will be a contaminant in the fructose fraction. No fructose impurity could be detected in the major glucose band.

The fraction 2.6 to 3.4 free volumes contained both glucose and fructose. When the fraction was concentrated to a small volume *in vacuo* at 50°C and rechromatographed it was further resolved into glucose and fructose.

The last fraction (3.4 to 10 free volumes when 95-per-cent ethanol was employed throughout or 3.4 to 5.6 free volumes when 80-per-cent ethanol was used to elute the fructose) contained mainly fructose and a small amount of glucose. The main factors affecting the glucose content of this fraction were related to the glucose content of the sample and to the flow rate. Thus, a sample containing initially more glucose than fructose may be expected to give a rather impure fructose fraction especially if the rate of elution is fast.

An appreciable amount of Magnesol dissolves in the 95-per-cent ethanol and interferes with the crystallization of the glucose and fructose. The Magnesol impurity can be removed by passing the alcohol-free extracts through cation and anion exchangers (Duolite A-3 and C-3).

The glucose impurity in the fructose fraction can be removed by periodate oxidation methods. Following the oxidation the sample is passed through Duolite C-3 and A-3 to recover the pure fructose.

It was found that sucrose behaved as glucose on the Magnesol column so that plant extracts containing sucrose, glucose and fructose must first be hy-

drolyzed with either invertase or acid before passage through the column. An investigation is under way at present to determine whether or not it is possible to make an initial separation of the disaccharides from the monosaccharides by the use of an adsorbent different than Magnesol. Several runs have been made with charcoal (Darco G-60) and Celite (535) mixtures but only partial success has been achieved.

Several batches of C^{14} labeled sugars have been prepared from detached sugar beet leaves that had undergone photosynthesis in a chamber containing $C^{14}O_2$. There are indications that sugars other than sucrose, glucose and fructose are present and a study is being made on their identification and isolation.

BIOPHYSICS

PHOTOSYNTHESIS

W. A. Arnold (Leader) Imogene A. Brooks*
A. S. Holt R. W. Kozs

Effects of Ultraviolet on Green Algae. (Holt, Arnold) Previous work on the effect of 2537 A on *Chlorella pyrenoidosa* had shown that ultraviolet had a deleterious effect on photosynthesis and relatively little effect on endogenous respiration. This work was extended to include the effects on other algae, specifically, those capable of utilizing molecular hydrogen during carbon dioxide reduction; the effect on oxygen production in the absence of carbon dioxide; and glucose oxidation. It was found that a straightforward first-order relation between dosage and survival of photosynthesis was not the prevailing case but that with both *Chlorella* and *Scenedesmus* an additional type of inactivation was evident which caused a curvature in the log survival versus dosage curve and indicated the presence also of higher order inactivation. Specific effects dependent on the type of suspending medium used for the algae were evident with *Chlorella* but not with *Scenedesmus*. This was made evident by the fact that fractionated doses with periods of photosynthesis between successive exposures, caused much less inactivation than did complete doses if alkaline carbonate buffers were used. No such effect was found when neutral phosphate was used as the suspending medium. Inactivation of photoreduction, and of oxygen evolution in the absence of carbon dioxide but with *p*-benzoquinone added, was found with *Scenedesmus*, the amount of inactivation being close to that found for photosynthesis. Where poisons such as hydrogen cyanide or hydroxylamine, presumably specific for the dark fixation of carbon dioxide and oxygen evolution respectively, were added to exposed *Scenedesmus* it was found that the inactivation was increased, showing that these poisons could not be causing their effect on a chain of different successive reactions.

Glucose oxidation by *Chlorella* was found to be far more sensitive than photosynthesis, while survival, as measured by ability to produce colonies on nutrient agar was far more sensitive than any of the other reactions studied. In addition, a comparison of *Chlorella* and *Scenedesmus* using suspensions of

*Terminated.

equal age, cell density, chlorophyll concentration and absorption at 2537 A showed that the photosynthetic mechanism in *Chlorella* has much greater sensitivity than that of *Scenedesmus*.

Effects of Ultraviolet on Enzyme Systems in Chloroplast Preparations. (Holt, Brooks) Isolated chloroplasts and chloroplast fragments obtained from disintegrated leaves of higher plants are known to possess activity to effect a portion of the over-all process of photosynthesis, *i.e.* oxygen evolution but not carbon dioxide reduction. In addition, it is known also that many such preparations contain oxidases such as polyphenol oxidase, cytochrome oxidase, and also catalase. The inactivation of the photochemical production of oxygen, measured either manometrically or by reduction of an indophenol dye, showed that (just as with photosynthesis by green algae) the relation by dosage and survival is predominantly first order. Also as with green algae, it was shown that the damage by ultraviolet was to a component of the photochemical mechanism since the amount of inactivation was independent of the light intensity used to determine the residual activity of the chloroplast fragments.

Comparison of the sensitivities of the various enzyme systems present in spinach preparations showed that, whereas no photochemical activity was evident after a ten-minute exposure, and only 50 per cent of the cytochrome oxidase activity remained after a one-hour exposure, no damage was evident either to polyphenol oxidase or to catalase.

No evidence of a change in the absorption spectrum in the ultraviolet region was found with a suspension irradiated to the point where no photochemical activity could be determined.

Photochemical Reduction and Dark Reoxidation of Cytochrome c by Chloroplast Preparations. (Holt) A previous investigation by the author several years ago to extend the list of substances known at that time to serve as hydrogen acceptors in the photochemical splitting of water by chloroplasts, had revealed no evidence of reduction of cytochrome c. This was reinvestigated and a reduction was found which is rapidly reversed as the result of the presence of a thermolabile constituent in such chloroplast preparations, requiring that time lags between illumination and spectrophotometric determination be reduced.

This reoxidation is due to the presence of an oxidase which reoxidizes reduced cytochrome c in darkness with an uptake of oxygen. The rate is re-

duced by hydrogen cyanide, carbon monoxide and azide, the common poisons used to show the presence of cytochrome oxidase. Reversal of the inhibition by carbon monoxide in light was not shown, possibly due to the use of too thick chloroplast preparations, with a consequent result that chlorophyll absorbed the major portion of the light intended to effect reversal of the iron-carbonyl complex. A temperature coefficient of 2 was found for the region 15-35°C indicating similarity in temperature optimum with the cytochrome oxidase in animal preparations, since little loss of activity during the first 40 minutes of measurement was found at 35°C.

Attempts to isolate highly active fractions from such chloroplast preparations by centrifugal procedures revealed that little difference in oxidase activity, based on chlorophyll concentration, was obtained.

The Preparation of Ethyl Chlorophyllide. (Holt) Ethyl chlorophyllide, a product resulting from the action of an enzyme, chlorophyllase, present in the leaves of certain species of higher plants, is found when this enzyme acts upon chlorophyll in the presence of ethanol. This compound has been introduced into the quantum yield problem as a sensitizer for the photochemical oxidation of thiourea, a reaction found to have a quantum yield of 1. Thus, by measuring the rate of oxygen uptake manometrically one can determine the energy equivalent of the light beam. Previous descriptions of how to isolate this compound were found to be confusing and the plants with leaves rich in this enzyme scarce. Kudzu *(Pueraria thunbergiana)* was found to be adequate for the purpose and modifications in the isolation of the compound were introduced to simplify the procedure.

Photometric Estimation of Dye Reduction by Illuminated Chloroplast Preparations. (Holt) A method, utilizing the change in transmission resulting from the photochemical reduction of indophenol dyes by illuminated chloroplasts, has been standardized such that routine determinations requiring but two to five minutes duration are possible with dye concentrations ca. $2 \times 10^{-5} M$ and chlorophyll concentrations ca. 0.01 mg. The same light effecting the photochemical change is used to register the change in transmission. This change causes an increase in current output from a photovoltaic cell, situated behind the reaction cuvettes, and enclosed in a light-tight housing. Color filters are used to isolate the band absorbed by the dye and are placed directly in front of the photronic cell. The housing and holder for the reaction cuvette are immersed in a constant temperature thermostat and the progress of the re-

action is recorded on a Brown Recorder. By the use of Beer's Law, previously found to apply to such conditions, the reaction can be followed quantitatively.

PATHOLOGY AND PHYSIOLOGY

Jacob Furth (Leader)

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Mary Knochulzen
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J. J. Lane
Germaine Click
Arthur Burke
Eschol Ledford

Slow Neutron Studies. (In cooperation with Drs. Spyker and Andrews with technical assistance of J. J. Lane and W. D. Gude) Reactor exposure of mice is being continued with matched X-ray exposures. The LD-50 is about 80 minutes with mice in the bottom position of the "safe". The equivalent LD-50 of X rays is about 600 r administered during the same time period. The total number of mice thus far exposed for aging, numbers 531 (365 reactor, 166 roentgen exposures) with 134 controls. The dose ranges from LD-50 to 1/16 of this which is the assumed minimal neoplasia-inducing dose.

After an initial weight loss during the first week following exposure the exposed animals rapidly gain weight almost at the rate of their control siblings. Their postexposure leukocyte curves are similar to the well known X-ray depression curves. The reticulocyte level is high in normal mice (2-4 per cent); after exposure this drops to zero within three to six days. Recovery begins at tenth to fourteenth day with a great rise of reticulocytes (8-10 per cent) on about the eighteenth day (Andrews).

Groups of mice of both sexes exposed in the 80-minute series are being sacrificed at various intervals for a thorough comparative hematological and histologic study. The eyes are being studied at the Army Institute of Pathology.

Blood Volume and Clearance Studies. (Wish and Huddleston—of Radiobiochemistry,—Storey and Beale) These studies are being conducted in X-rayed and normal rabbits with cooperation of the Radiobiochemistry section of Dr. Sheppard (Mr. Wish). After preliminary experiments the arbitrary ratio between dye-isotope concentration in 6- and 30-minute samples was taken as the most useful disappearance value. There is a greater disappearance in X-rayed rabbits of injected homologous and heterologous plasma tagged with ^{131}I and of tagged red cells than in normal rabbits. Every value for the ten rabbits of both sexes tested with heterologous plasma from two to thirteen days after irradiation was above the average of normal. A much larger number of rabbits tested with homologous plasma and a small number with tagged erythrocytes behaved similarly. The trend was the same for Evans blue except in the three

rabbits tested four to seventeen hours postirradiation, in which there was a much decreased disappearance rate. The factors involved are numerous and the meaning of this difference is not clear; both are currently occupying the attention of this group.

An attempt was made to study, in the same rabbit, the rate of disappearance of Evans blue and tagged plasma before and at various intervals after irradiation. In normal rabbits of this series there was some irregular fluctuation of Evans blue disappearance rates averaging, however, a horizontal line at about 10 per cent (Fig. 10). In X-rayed rabbits, on the contrary, after an early (four to seventeen hours) drop postirradiation there was a marked rise to a peak of 31 per cent at nine days postirradiation and a decline thereafter. This is in contrast to the maximum disappearance value in normal rabbits of this series amounting to 18 per cent. Averages of the blood volume values were likewise horizontal in normal rabbits of this series while in X-rayed rabbits there was a decline reaching the depth of 3.0 per cent at nine days postirradiation. This value is in contrast to the lowest value of 3.6 per cent and average of 4 per cent body weight in normal rabbits of this series. Although only about 1.8 cc of blood was withdrawn from rabbits of this series every four days, the X-rayed rabbits developed a marked anemia, their hematocrit values dropping to 20 per cent below the initial value while those of normal rabbits dropped to about 3.5 per cent below the initial value.

No great blood volume changes follow exposure of rabbits to 800 - 1000 r. The Evans blue blood volume values are slightly higher in rabbits two to nine days after irradiation than in control rabbits as indicated in Fig. 11, but these values may represent greater loss of Evans blue from the blood of X-rayed animals than in controls rather than differences in blood volume. The plasma volumes determined by Evans blue are greater than those of ^{131}I-tagged plasma. In mice there is a constant ratio (1.2 : 1) between Evans blue and ^{131}I values even in presence of marked hypervolemia. Iodinated plasma is not withdrawn appreciably by the thyroid during the period of observation. These and anatomical observations make it evident that the Evans blue values are incorrect, though useful because of the constant ratio. It seems that this dye is rapidly withdrawn from the blood by certain tissues which have an affinity for Evans blue and that these tissues include but are not identical with the reticulo-endothelial system, as is generally supposed.

Efforts are being made to devise a dependable procedure for assay of reticulo-endothelial cell activity in both normal and irradiated animals. To

Note to Fig. 10

Rabbits were given intravenous injections of 1 cc of 2-per-cent T1824 at intervals indicated and the per cent of disappearance of this dye between 6 and 30 minutes after injection plotted. The 6-minute samples approximate the greatest concentration of T1824 attained in the blood. These rabbits received 800 r (250 kvp, 15 ma, 71 cm t.s.d., with only inherent filtration).

DISAPPEARANCE RATES OF T 1824 IN NORMAL AND X-RAYED RABBITS (1000 r)

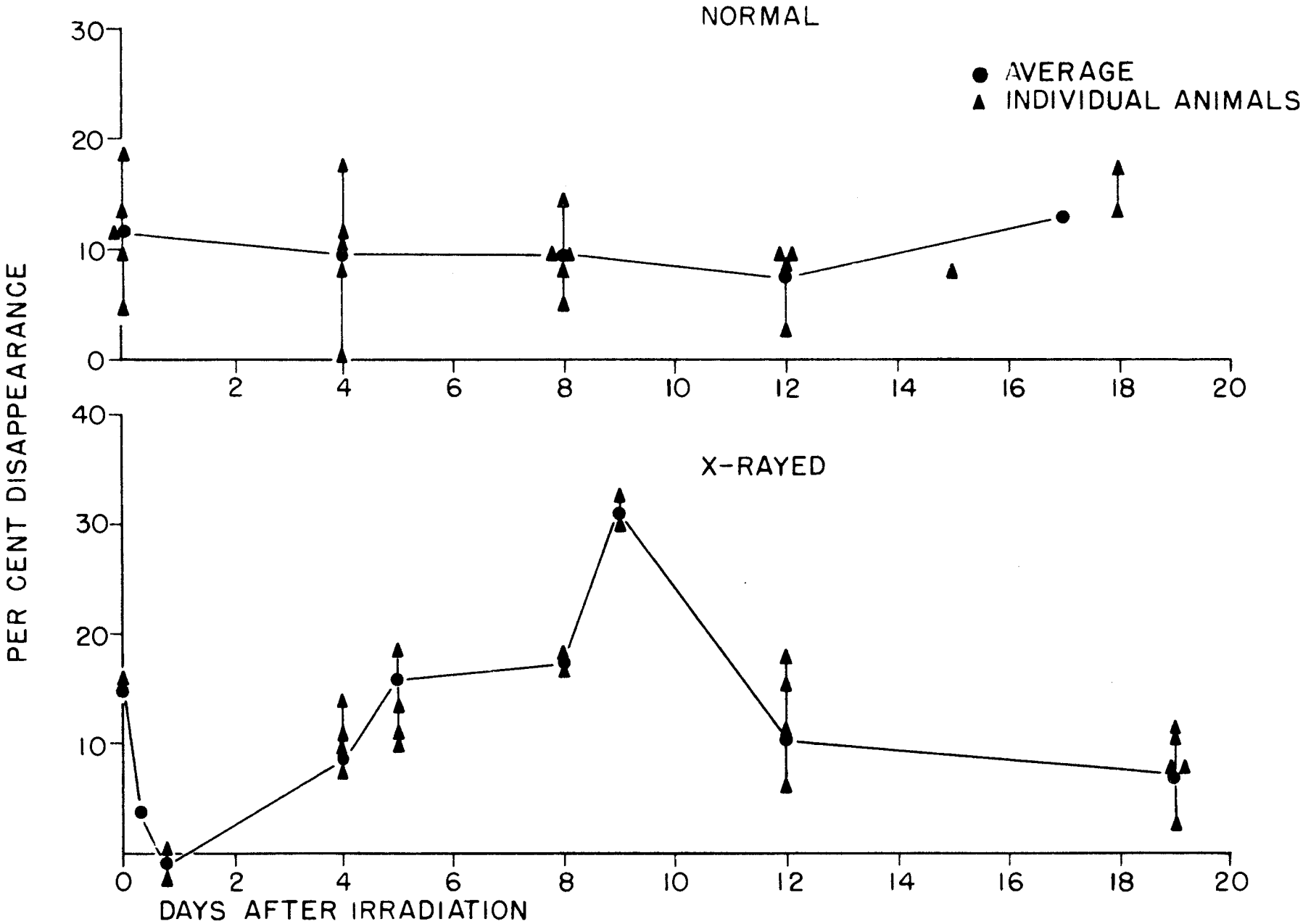

FIG. 10

Note to Fig. 11

These rabbits received 1000 r (factors as those in the figure). Each point is obtained on a different rabbit. The determinations were made in pairs of rabbits (irradiated and normal, matched for sex, weight and hematocrit values). The solid line indicating the difference between the pairs, the broken line between the X-rayed rabbit and the average normal value.

EFFECT OF X RAYS ON PLASMA VOLUME IN RABBITS (1000 r)

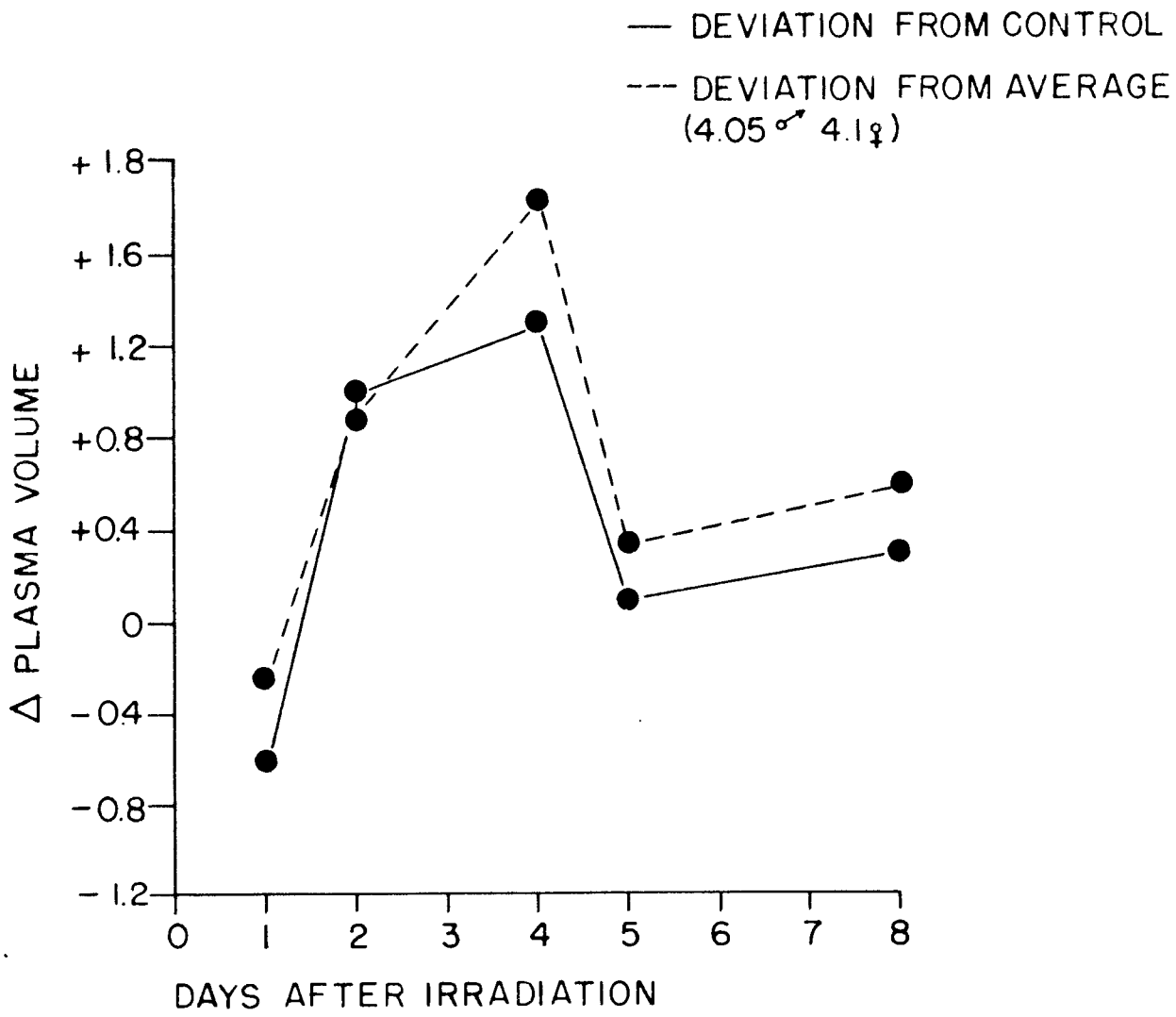

FIG. 11

[redacted]

this system is attributed new formation of hemopoietic cells as well as riddance of foreign matter from the circulation; and antibody production.

Figure 12 is illuminative in this connection. It gives the comparative disappearance rate of different materials thus far assayed. (^{198}Au colloids are also discussed by Dr. Sheppard.) It may be that disappearance of heterologous cells or colloidal particles will prove more useful in the study of reticulo-endothelial function and of body compartments in X-rayed animals than tagged plasma.

Organ blood volume determinations are worked out on mice in order to assay precisely the blood distribution in different compartments of irradiated animals, as well as to find out the primary site of hypervolemia in granulosa tumor bearing animals. An incidental finding to be followed up is that the tumors are markedly ischemic (anoxic) as measured after the usual 6-minute mixing time, a finding probably due to inefficient circulation through the newly formed capillaries of tumors.

X-ray Induced Ovarian Tumors. (Knoohuizen and Burke) One growth arising in a mouse exposed to 50 r developed an ovarian growth which, on successive passages made during the past four years, yielded a fast growing adenocarcinoma which seems to have arisen from a very slow growing adenomatous downgrowth of the germinal epithelium (studied earlier with Dr. Bali). This strain (XIX) is associated with slight to moderate hypervolemia but with no estrogenic change. There are two possibilities: this cell (the supposed precursor of the estrogen producing granulosa cells) produces or causes the production of plethorin, or (less likely, on the basis of earlier work) hypervolemia is a nonspecific change occurring to variable extent with different neoplasms. Experiments now in progress are aimed to answer these questions.

Two other types of X-ray induced neoplasms described in earlier publications are still under observation: a) blood volume raising and estrogen producing granulosa tumors and b) masculinizing and polycythemia producing luteoma. The identification of the hormones secreted and the pathogenesis of the secondary changes are the main objects of this study. Other unusual tumors and leukemias occurring in our colony (normal and irradiated) are being kept alive in the frozen state for possible future investigation.

[redacted]

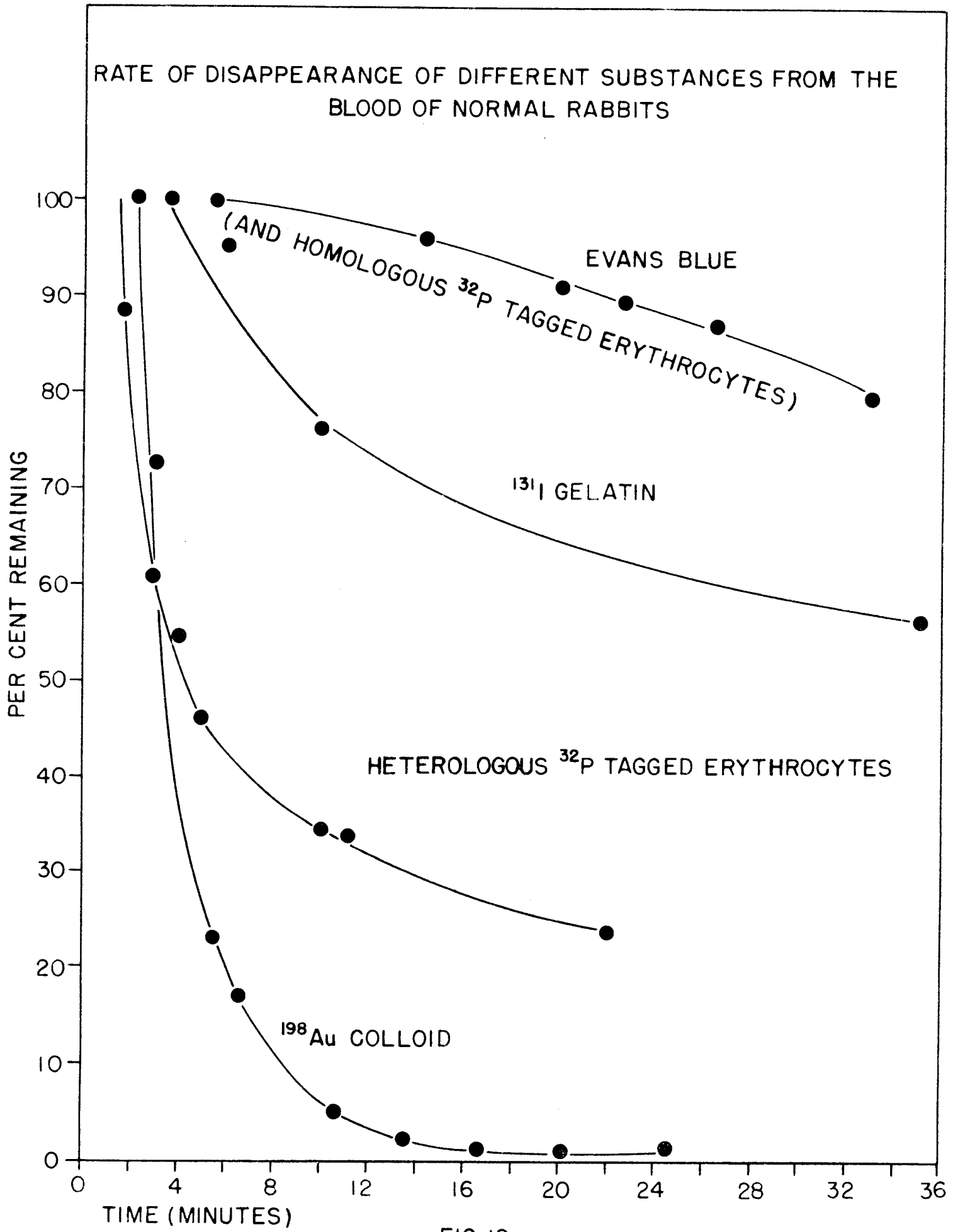
RATE OF DISAPPEARANCE OF DIFFERENT SUBSTANCES FROM THE BLOOD OF NORMAL RABBITS
PER CENT REMAINING
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
32
36
TIME (MINUTES)
EVANS BLUE
(AND HOMOLOGOUS 32P TAGGED ERYTHROCYTES)
131I GELATIN
HETEROLOGOUS 32P TAGGED ERYTHROCYTES
198Au COLLOID

FIG. 12