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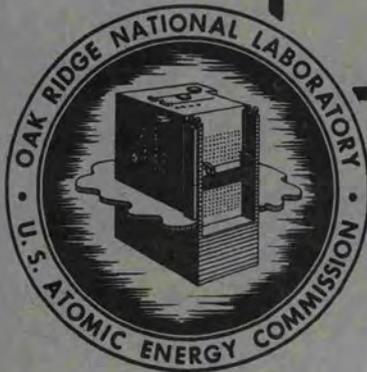
BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT FOR
PERIOD ENDING AUGUST 15, 1950

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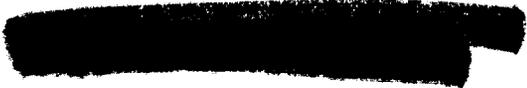
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BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT
for Period Ending August 15, 1950

Alexander Hollaender, Director

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INTRODUCTION

Review of research progress. This quarterly report is somewhat colored by the work of the twelve Research Participants who have been temporary members of this Division for the past three to eight months. All these visitors have done noteworthy work and have brought to the Division the influence of their own research interests. Some investigations discussed in this report will not be continued here since they are somewhat removed from the main interest of this Laboratory. It should also be pointed out that there is a stronger tendency in our Laboratory toward shifting of some of the broad aspects of radiation effects to problems of more immediate interest in this field. It is our intention to continue investigations of basic aspects of radiation effects; however, we feel that, during the impending national emergency, the thinking of our group should include problems which could be valuable in the near future in ameliorating the possible damage resulting from use of atomic weapons.

The proceedings of the first open Information Meeting for Biology and Medicine held in Oak Ridge have appeared as a supplement to the Journal of Cellular and Comparative Physiology. It is hoped to bring out, in similar form, the proceedings of the two succeeding meetings which have taken place in Oak Ridge.

The study of the effect of oxygen tension on the sensitivity of living cells to X radiation has become somewhat widespread in the Division, embracing not only the activities of the cytology groups, but also the interest of the Biochemistry and Microbiology sections as well. Early observations demonstrated that the response to X rays in the presence of oxygen is more pronounced if the irradiation is done at *low* temperatures. Later findings brought out the fact that, in the presence of nitrogen, the sensitivity of cells is increased if the radiation is accomplished at *higher* temperatures. This phenomenon was first noted in regard to the sensitivity of bacteria, and lately, in regard to the sensitivity of *Tradescantia* chromosomes. Different type deletions respond differently to irradiations done in oxygen and in nitrogen, thereby opening to us a clue to some possible mechanism involved. It was reported first by English workers, and also on the basis of findings in this laboratory, that if the irradiation is done with α rays, the oxygen tension has relatively little effect. Work with fast neutrons has produced evidence that they are intermediate between alpha and X rays in their effectiveness in producing chromosomal changes as modified by oxygen tension.

It was mentioned in the previous quarterly report that isolate bacteriophage are not influenced by oxygen tension during irradiation, since bacteriophage are considered to have no independent respiration.

It was found by members of our group that the mutation production in *E. coli* is affected differentially for different biochemical mutants, depending on whether or not the irradiation is done in oxygen. It appears that reverse mutations in the streptomycin-dependent strain respond in the same proportion as the survival ratio. However, a purineless strain does not follow this survival ratio. This may prove to be an excellent tool for separating mechanisms for different types of biochemical mutations.

It was found that the effect of ultraviolet on photosynthesis has no photoreactivation.

The major experiment on determination of mutation rate in certain chosen loci in mice, influenced by X rays, has given somewhat unexpected results. The mutation rate of the chosen loci appears to be greater than expected results from *Drosophila* studies. This finding may shorten somewhat the long-term study of the mutation rate in mice as produced by X rays. Careful studies continued with energy values as low as 50, or even 25 r, on the embryonic development in pregnant mice show very significant results. This study, which is nearing completion of its first phase, is believed to be very important in regard to the present practice of promiscuous X-ray photographing of pregnant women.

The metabolism group has found that crystalline cytochrome c and vitamin B₁₂ have very similar effects on B₁₂-deficient cells, a rather surprising finding which may be of considerable importance in nutrition work. Investigations in this laboratory indicate that there is a new biosynthetic mechanism for the formation of lactate which involves an oxidative pathway through C₂ condensation and C₄ dicarboxylic acids, and which is to be contrasted with the usual glycolytic cleavage to pyruvate and subsequent reduction.

The new adenylic acid which has been isolated by the ion-exchange method appears to be an isomer of the adenylic acid which had been known previously.

A small project is under way using firefly luminescence factors as a tool for studying the effect of adenosinetriphosphate in biological reactions.

The distribution of isotopic formate in the nucleotides of ribo- and desoxyribonucleic acids of rats, and control and folic-acid-deficient chicks was investigated.

Considerable effort was spent in standardizing our new X-ray machine, but its use is still beset with difficulties.

Studies on potassium exchange in living cells are being extended, especially through the participation of a summer visitor who is interested in the exchange rate of plasma and tissue potassium.

An interesting investigation of the nucleic-acid content of anthers of *Lilium longiflorum* is being carried out. The nucleic acid concentration was followed during growth of the anthers.

A study of the effect of X rays on peanuts, initiated in cooperation with a North Carolina State College geneticist, was reopened with emphasis on seed germination. The very striking results obtained are evident in the photograph (Fig. 20).

Satisfactory progress has been made in the study of the effects of slow neutrons on cataract formation and leukemia production, and interesting data have been obtained. Determination of plasma, cell and organ-blood volumes by isotope technics in normal and tumor-bearing mice brought out the low blood volume of the tumors examined. This may give rise to some interesting new problems. A number of other projects in the Pathology and Physiology group have progressed to a publishable stage.

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications: Symposium on Radiation Genetics. Publication of the papers and discussions of the first Information Meeting for Biology and Medicine to be held in Oak Ridge has been accomplished. The Wistar Institute of Anatomy and Biology issued these proceedings as a supplement (June 1950) to the Journal of Cellular and Comparative Physiology, one of the nine journals published by the Institute. Bound reprints of the complete collection are now being distributed by the Biology Division. Persons who desire copies of this publication may request them of the Division Director or the Division editor. This seems to be an excellent way of presenting these symposia. It is planned to bring out successive ones in the same general form. Contents of the 210-page volume follow.

SYMPOSIUM ON RADIATION GENETICS

Introduction	Alexander Hollaender
Some present problems in the genetic effects of radiation	Herman J. Muller
The effect of X rays on chromosome structure	Karl Sax
Chromosomal interchanges induced in <i>Tradescantia</i> microspores by fast neutrons from uranium fission	Norman H. Giles, Jr. and Alan D. Conger
Effects of radiation on mitosis	J. Gordon Carlson
Cytological and phenotypical effects induced in maize by X rays and the Bikini Test Able atomic bomb	L. F. Randolph
Effects of radiation on fungi	E. L. Tatum
Some effects of ultraviolet irradiation on microorganisms	Orville Wyss, Felix Haas, J. B. Clark and Wilson S. Stone
Radiation induced mutations in chemical requirements in <i>Salmonella typhimurium</i>	Harold H. Plough
The effect of radiations on genetic mechanisms of <i>Paramecium aurelia</i>	Richard F. Kimball

Inactivation of enzyme-substrate film by small doses of X rays	Daniel Mazia and Gertrude Blumenthall
Discussion on population genetics and radiation	Sewall Wright

Publications: General list. Publications by members of the Biology Division in open and Project literature for the past quarter are listed. Many additional papers are being reviewed or are in final preparation. Proceedings of the 1949 Information Meeting for Biology and Medicine and the material presented at the 1950 Biology Conference are also in process of collection preparatory to editing. Both these collections will contain contributions from outstanding scientists in the fields of microbiology and biochemistry.

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
<u>Rene' A. Bolomey</u> and G. R. Noggle	A Chromatographic Method for the Separation of Photosynthesized Radiofructos and Radioglucose (Abstract)	Proc. of the Assoc. of Southern Agricultural Workers 47th Annual Convention, 1950	
<u>Rene' A. Bolomey</u> and Leon Wish	The Application of Anion-exchange Resins to the Chromatographic Separation of Yeast Ribonucleic Acid from its Smaller Degradation Products	Accepted by J. Am. Chem. Soc.	ORNL-627
J. G. Carlson	Effects of Radiation on Mitosis	J. Cell. & Comp. Physiol., Supp. 1, 35:89 June, 1950	ORNL-586
<u>C. E. Carter</u> and W. E. Cohn	The Enzymatic Degradation of Ribonucleic Acid by Crystalline Ribonucleotides	J. Am. Chem. Soc., 72:2604, 1950	
W. E. Cohn	Heterogeneity in Pyrimidine Nucleotides (Communication to Editor)	J. Am. Chem. Soc. 72:2811, June, 1950	
<u>W. E. Cohn</u> and C. E. Carter	The Separation of Adenosin Polyphosphate by Ion Exchange and Paper Chromatography	J. Am. Chem. Soc. In press	ORNL-610

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
<u>W. E. Cohn</u> and <u>C. E. Carter</u>	The Preparation of Uridylic and Cytidylic Acids by an Ion-Exchange Procedure	J. Am. Chem. Soc., 72:2606, June, 1950	
<u>Alan D. Conger</u> and <u>N. H. Giles, Jr.</u>	The Cytogenetic Effect of Slow Neutrons	Genetics, 35: 397, July, 1950	ORNL-409
<u>N. H. Giles, Jr.</u> and <u>Alan D. Conger</u>	Chromosomal Interchanges Induced in <i>Tradescantia</i> Microspores by Fast Neutrons from Uranium Fission	J. Cell. & Comp. Physiol., Supp. 1, 35:83, June, 1950	ORNL-586
<u>N. H. Giles, Jr.</u> and <u>H. P. Riley</u>	Studies on the Mechanism of the Oxygen Effect on the Radiosensitivity of <i>Tradescantia</i> Chromosomes.	Proc. Nat. Acad. Sci., 36:338, June, 1950	
<u>R. F. Kimball</u>	The Effect of Radiation on Genetic Mechanism of <i>Paramecium aurelia</i> .	J. Cell. & Comp. Physiol., Supp. 1, 35:157, June, 1950	ORNL-565
<u>H. I. Kohn</u>	Changes in the Composition of the Blood Plasma of the Guinea Pig During the Acute Radiation Syndrome	Am. J. Physiol. In press	
<u>P. B. Lowrance</u> and <u>C. E. Carter</u>	The Effect of Nitrogen Mustards on the Metabolism of Nucleic Acids in the Hematopoietic Tissue of the Rabbit	J. Cell. & Comp. Physiol., 35:387, June, 1950	ORNL-318
<u>C. W. Sheppard</u> and <u>W. R. Martin</u>	Cation Exchange Between Cells and Plasma of Mammalian Blood: I. Methods and Application to Potassium Exchange in the Human Being	J. Gen. Physiol., 33:703, June, 1950	
<u>C. W. Sheppard</u> and <u>A. S. Householder</u>	The Mathematical Basis of the Interpretation of Tracer Experiments in Closed Steady State Systems	J. Gen. Physiol. In press	ORNL-716
<u>R. H. Storey</u> <u>Leon Wish</u> and <u>J. Furth</u>	Changes in Cell and Plasma Volumes Produced by Total Body X Radiation	Proc. Soc. Exp. Biol. & Med., 74: 242, June, 1950	

AUTHOR (S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
P. C. Tompkins, W. T. Burnett, Jr. and Leon Wish	A Method of Estimation of Beta Activities from Bremsstrahlung Measurements in an Ionization Chamber	Anal. Chem., 22:672, May, 1950	

Traveling seminars and scientific society lectures. Early summer is a slack season for this activity. The following eleven lectures constitute the Division's contribution to this phase of research.

W. E. Cohn	University of Tennessee Sch. of Biological Sci., Memphis	The Application of Ion Ex- change to Nucleic Acid Prob- lems	
W. E. Cohn	University of Arkansas School of Medicine, Little Rock	Same subject	
W. E. Cohn	Bowman-Gray Sch. of Med. Wake Forest College, Wake Forest	Same subject	
W. E. Cohn	Marine Laboratory Duke University, Durham	Same subject	
S. F. Carson	Brookhaven Nat. Lab., Brookhaven	Metabolic Exchange of CO ₂ with Carboxyls and Oxidative Syn- thesis of C ₄ Dicarboxylic Acids	
J. Furth (Paper given by A. Hollaender)	Fifth Internat. Congr. for Cancer Research, Paris	Experimental Radiation Induced Hormone Secreting Ovarian Tu- mors; Adenocarcinoma with Hy- pervolemia	
N. H. Giles, Jr.	Symposium on Radiobiology Oberlin College, Oberlin	Recent Evidence on the Mech- anism of Chromosome Aberration Production by Ionizing Radia- tions	
Alexander Hollaender	Symposium on Radiobiology Oberlin College, Oberlin	Physical and Chemical Factors Modifying the Sensitivity of Cells to High Energy and Ul- traviolet Radiation	

Alexander Hollaender	Sixth Internat. Congr. of Radiology London	Factors Modifying the Sensi- tivity of Living Cells to Ion- izing Radiation
M. L. Morse	Soc. of Am. Bacteriol., Baltimore	Resistance to Ultraviolet Radiation in <i>E. coli</i> , Strains B and B/r
C. W. Sheppard	Soc. of Gen. Physiol., Woods Hole	The Disturbance by X Rays of Selective Potassium Accumula- tion in Human Erythrocytes

The first part of September will see an upswing in scientific society participation. Thirteen papers will be given on Biology Division research by the following investigators:

American Chemical Society, Chicago	1, 2. W. E. Cohn
	3. J. R. Totter (E. Volkin and C. E. Carter)
	4. F. Vaslow (D. G. Doherty)
	5. D. G. Doherty
American Society of Plant Physiologists, Columbus	6. G. R. Noggle
American Physiological Society, Columbus	7. W. T. Burnett (C. W. Sheppard and R. R. Bigelow)
	8. W. G. Walker (W. S. Wilde)
Genetics Society of America,	9. W. L. Russell
	10. Liane B. Russell
	11. A. V. Beatty (N. H. Giles, Jr.)
	12. R. F. Kimball
	13. Marjorie Nix (Mary E. Gaulden)

Lectures to Health Physics Training Class. In cooperation with the Health Physics Division's training course for visiting investigators, members of the Division have delivered a series of lectures on biological subjects. The series, beginning May 10 and ending July 5, consisted of the following lectures.

E. H. Anderson	Bacteriology
A. H. Doermann	Bacteriophage
A. D. Conger	Cell Structure
M. E. Gaulden	Cell Division
R. F. Kimball	Introductory Genetics
N. H. Giles, Jr.	Chromosome Changes
N. H. Giles, Jr.	Mutations
S. Pomper	Fungus Genetics
W. L. Russell	Mammalian Genetics
L. B. Russell	Mammalian Development
G. R. Noggle	Plant Physiology
J. B. Kahn	Pharmacological Aspects
C. W. Sheppard	Permeability and Radiation
S. F. Carson	C-14 Biochemistry
W. E. Cohn	Nucleic Acids in Biology

Lectures to ORINS Isotope Training Classes. Beginning on January 10, 1950, monthly lectures to the Isotope Training classes have been given by Division members. Six lectures dealing with Effects of Radiation on Cells have been delivered by Alexander Hollaender and one lecture on Cellular Effects of Ionizing Radiation has been given by Alan D. Conger. On August 15, the entire class was given a conducted tour of Biology Division Facilities.

Visiting Lecturers. In continuance of the Division policy of bringing in scientists of established reputation for lectures and consultation, the following seminars have been given during this quarter by speakers from other laboratories.

Dr. Robert P. Wagner - University of Texas - "What is a Genetic Block?"

Dr. Peter P. H. DeBruyn - University of Chicago - "*In vivo* Affinity of Diaminoacridines for Nuclei"

Dr. Norman H. Horowitz - California Institute of Technology - "Genes and Enzymes in *Neurospora*"

Dr. H. S. Mayerson - Tulane University Medical School - "Exchange of Albumin Between Plasma and Lymph"

Dr. John H. Hampton, Jr. - Tulane University Medical School - "The Biological Significance of Ferritin"

Dr. A. J. Goldforb - College of the City of New York - "Relation of Science to Publication"

Dr. J. Oliver Lampen - Western Reserve University - "Enzymatic Degradation of Pentose and Desoxypentose Derivatives"

CYTOGENETICS

CYTOGENETICAL EFFECTS OF RADIATIONS

N. H. Giles, Jr. (Leader)	Mary E. Case
A. H. Doerman	D. S. Daniels
A. D. Conger	Lucille M. Fairchild
Seymour Pomper	Dorothy L. Giles
A. V. Beatty*	Betty B. Hill
H. E. Wheeler*	Kathryn Meyer
H. P. Riley*	N. T. Brumfield*

Newton Underwood*

Tradescantia studies on the oxygen effect with fast neutrons and X rays. (Giles, Beatty, Riley, Fairchild) *Tradescantia* inflorescences have been exposed to fast neutrons from uranium fission, utilizing the special exposure cart in the Oak Ridge pile. The inflorescences were placed inside an airtight lucite exposure chamber which contained either oxygen or nitrogen (or in two instances, helium) during the irradiation. The neutron dose was monitored with the same Victoreen thimble ionization chamber (100 r, #2) used in previous experiments in air performed with A. D. Conger. The results for both chromosome and chromatid aberration types are presented in Figs. 1 and 2. In all instances, a linear relationship between dose and aberration frequency was found. For chromosome interchanges (Fig. 1), the removal of oxygen results in a decrease (by about one-third) in aberration frequency. This decrease is markedly less than that obtained in similar experiments with X rays, but it is apparently more than that found when irradiation is carried out with particles in the absence of oxygen (Thoday and Read, *Nature*, 163:133, 1949; Conger, unpublished). There is no difference in aberration frequencies when helium is substituted for nitrogen.

Data are now available for chromatid aberration types induced with X rays in oxygen and in helium (Figs. 3 and 4), and these may be compared with the neutron results (Fig. 2). All X-ray-induced aberration types are reduced in frequency when oxygen is removed, but the magnitude of the effect seems to depend on the particular type of aberration studied. Thus chromatid deletions (Fig. 4) seem to be relatively slightly affected, whereas isochromatid types (Fig. 3) are almost three times as frequent in oxygen as in helium. When fast neutrons are used, all chromatid aberration types decrease in frequency when oxygen is removed (Fig. 2), but the degree of this change is not so great as that noted with X rays, except for the chromatid deletions.

* Research Participant

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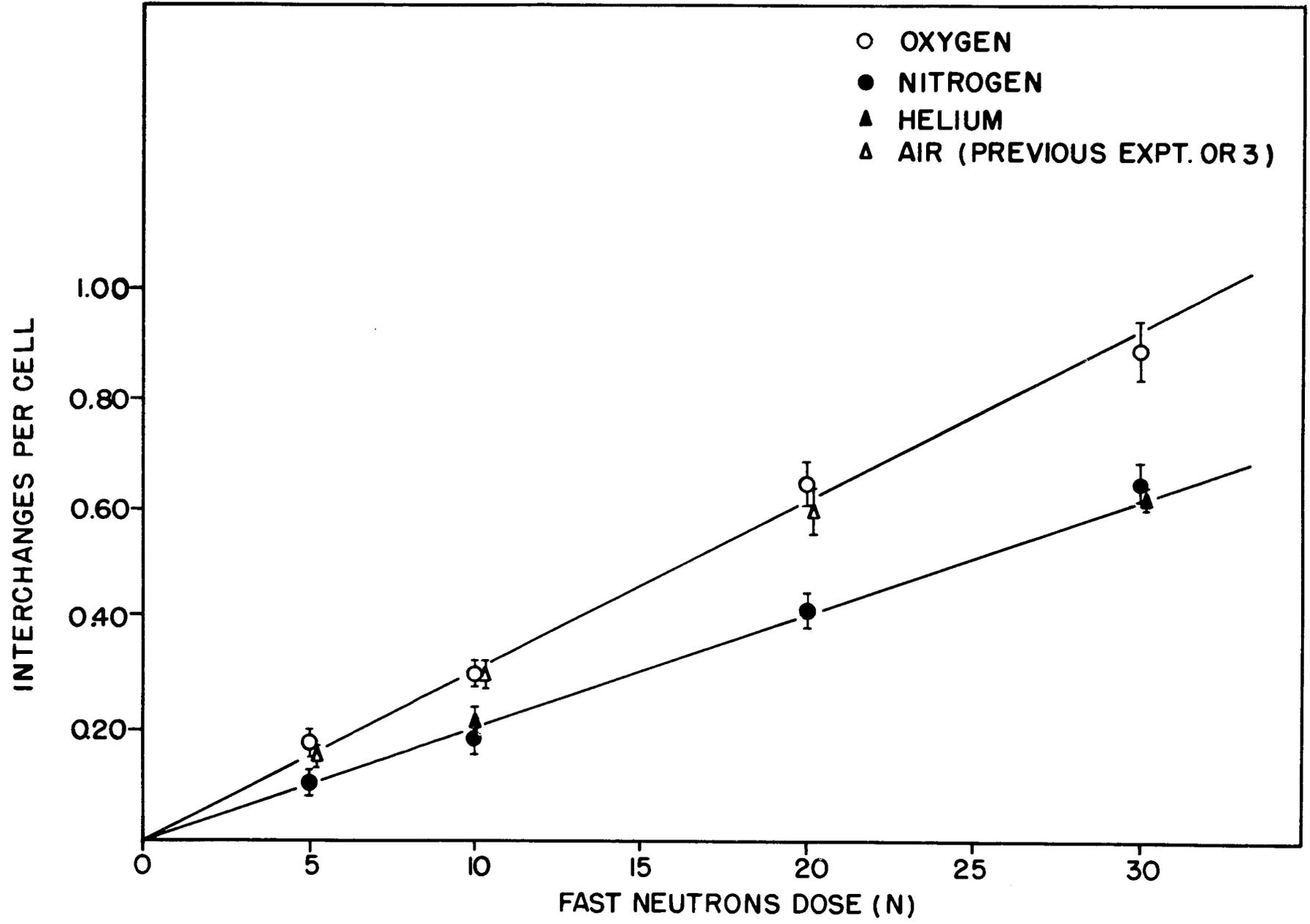


FIG. 1

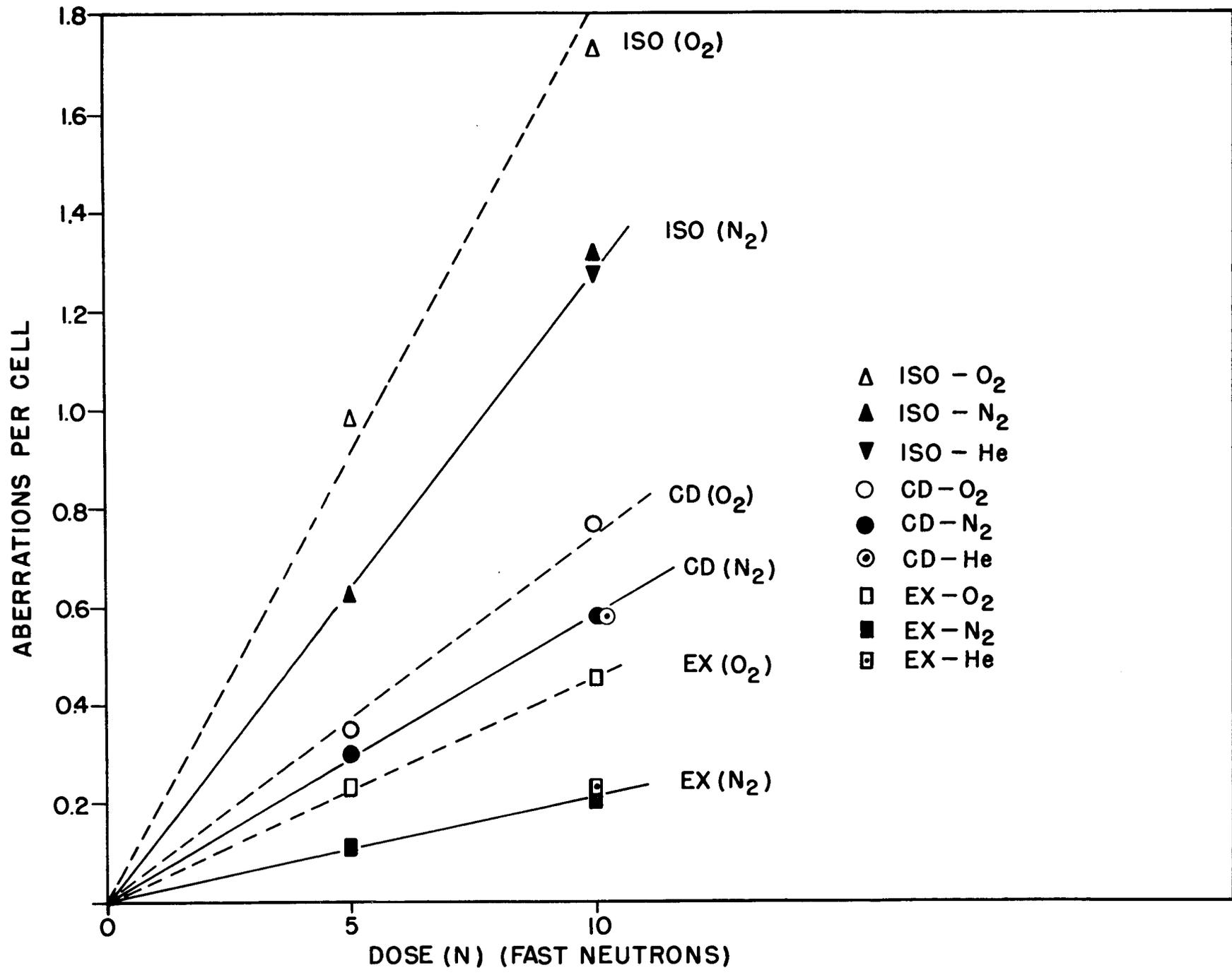


FIG. 2

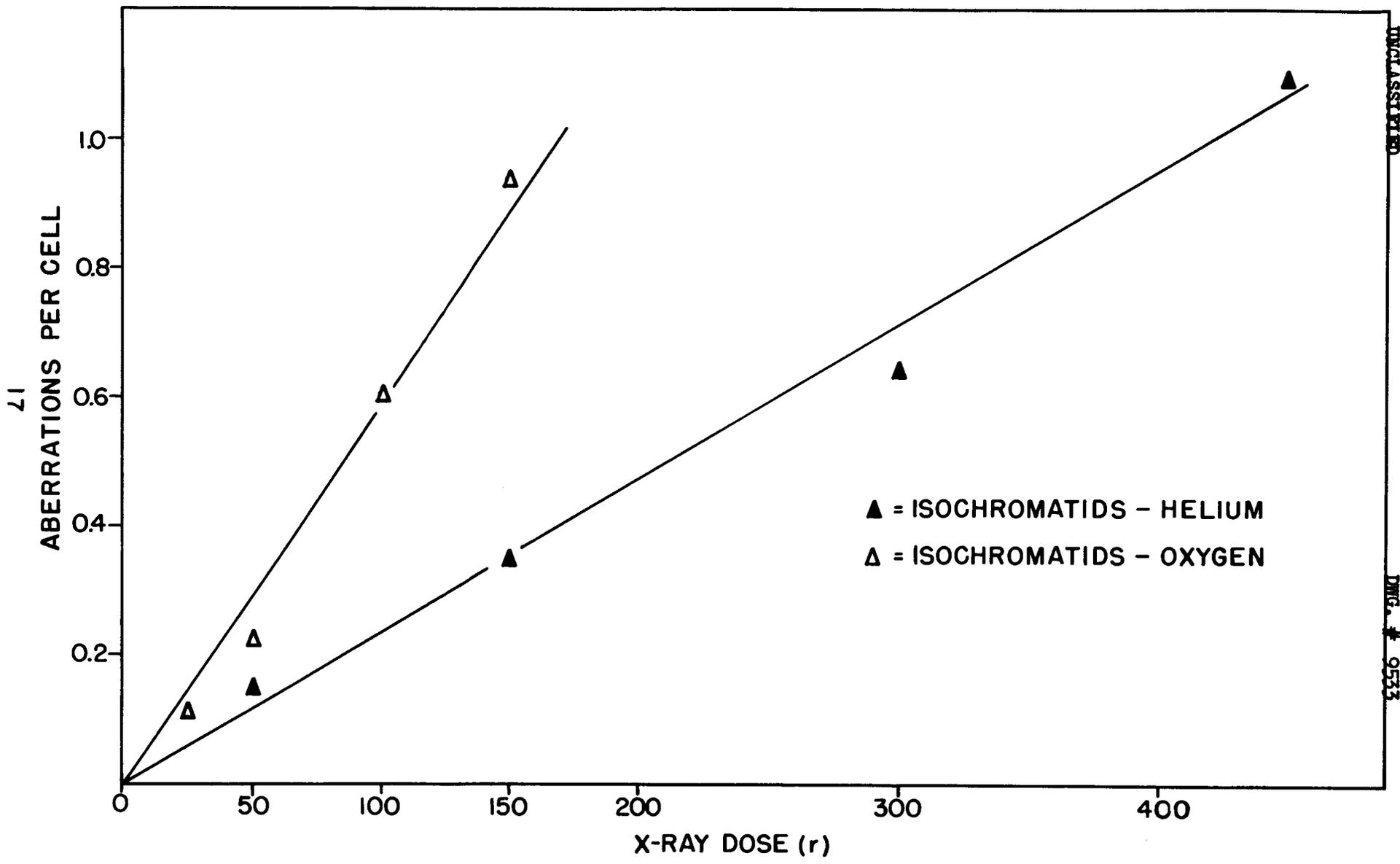
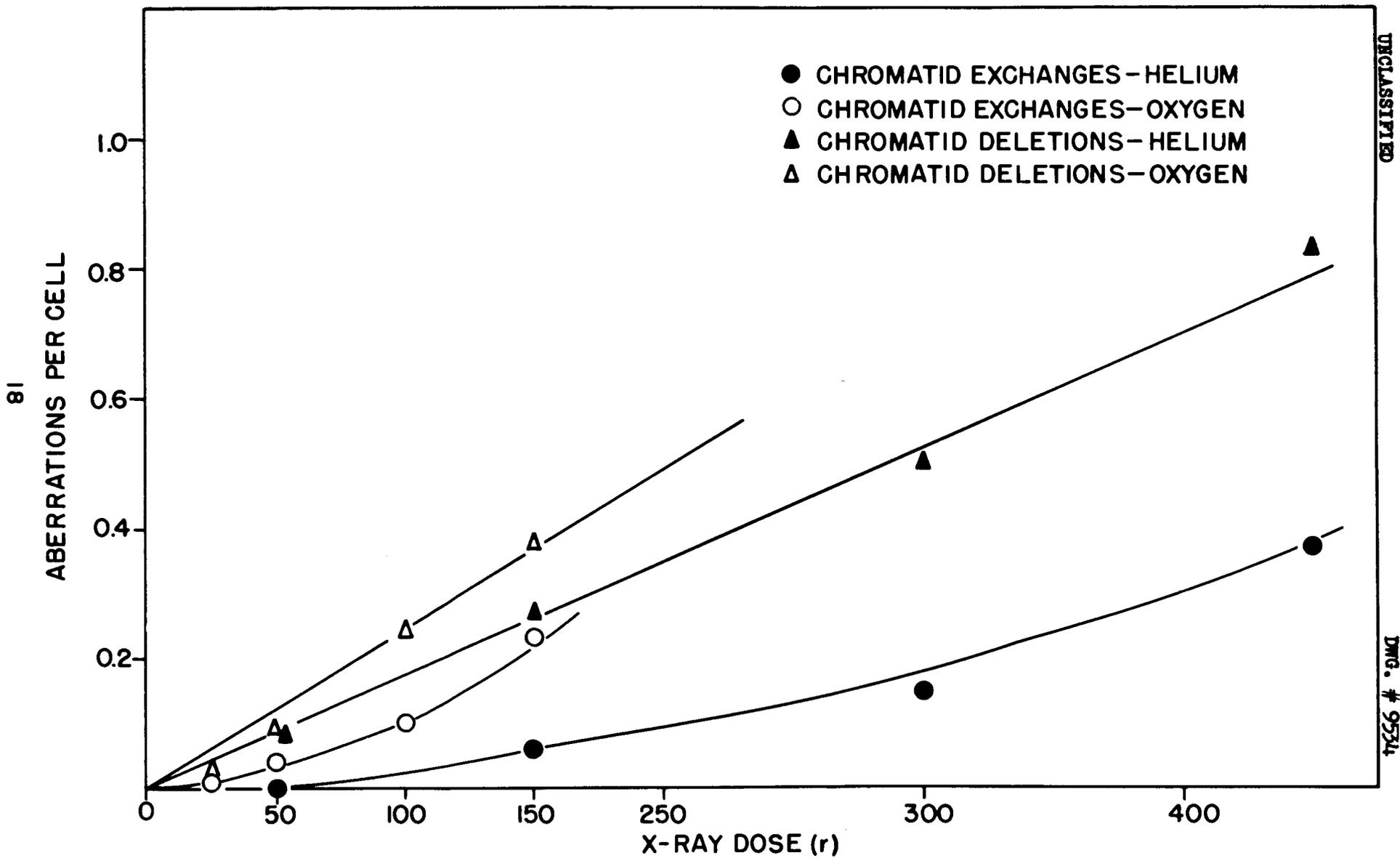


FIG. 3

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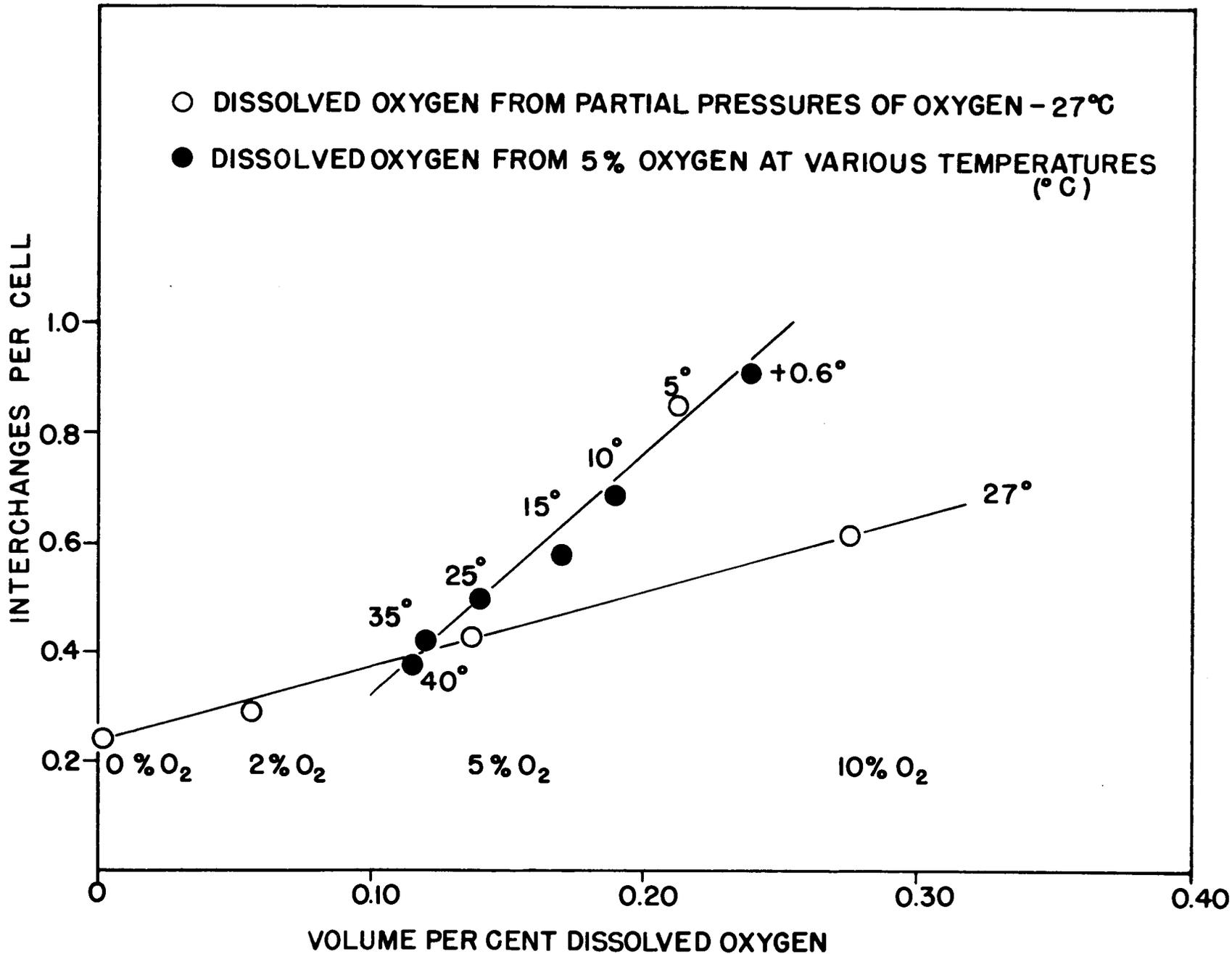
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FIG. 4

These results indicate that the magnitude of the effect of oxygen in increasing aberration frequencies in *Tradescantia* microspores is inversely related to the specific ionization of the radiation utilized. There is experimental evidence (Toulis, Report UCRL-583) that protons are intermediate between α and X rays in the decomposition of H_2O to form H_2O_2 and thus the present results are compatible with the hypothesis that H_2O_2 may be the substance responsible for chromosome break production.

Temperature effects on chromosome aberration frequency. A number of experiments have been performed to determine the relationship between the oxygen effect and the effect of temperature on aberration frequency. Previous investigators (Sax, Catchside) have demonstrated that X-ray-induced aberrations are considerably more frequent at low temperatures in *Tradescantia*. It seemed possible that this effect might be due to the increased solubility of oxygen in water at low temperatures. Consequently, irradiations have been performed at various temperatures in the presence and absence of oxygen in order that the resulting aberration frequencies could be compared with previous results obtained with irradiation in various partial pressures of oxygen at room temperature ($\sim 27^\circ C$). The most extensive results obtained to date are those with 5 per cent oxygen (at 400 r at 50 r/min) and with helium (at 900 r at 300 r/min). These results are summarized in Figs. 5 and 6. In Fig. 5, interchange frequency has been plotted against the calculated volume of dissolved oxygen present during X radiation. When exposures are made at $27^\circ C$, utilizing various percentages of oxygen, the lower curve in Fig. 5 is obtained. If the effect of temperature is due entirely to changes in oxygen availability resulting from an effect on solubility, then aberration frequencies obtained at various temperatures when exposures are made in 5 per cent oxygen should fit onto this curve. The data obtained in the experiment performed with 5 per cent oxygen, at various temperatures are plotted in the upper curve (Fig. 5). Although the aberration yield at $25^\circ C$ is actually somewhat higher than expected on the basis of previous results, it is clear that even if this difference is compensated for, the two curves are certainly not parallel. It thus appears that the effect of temperature in modifying aberration frequency can be ascribed only in part to a modification of oxygen solubility. There is an additional effect of temperature itself. It is not evident at the present time whether this effect is due to an influence of temperature on the formation or effectiveness of some intermediate product (such as H_2O_2) responsible for chromosome breakage, or whether the effect is on the behavior of broken chromosome ends during the recovery process.



VOLUME PER CENT DISSOLVED OXYGEN

FIG. 5

Another exceedingly interesting observation, which further complicates the analysis of the temperature effect, at least for the moment, has been made in preliminary experiments on the effect of temperature on aberration frequency when irradiation is performed in the absence of oxygen. In this experiment, exposures of 900 r (at 300 r/min) were made in helium at various temperatures. The results are shown in Fig. 6. It is clear that there is a substantially higher aberration yield at higher temperatures, although the shape of the curve cannot be satisfactorily determined from these results. For comparison, the curve obtained when irradiation is performed in 5 per cent oxygen at various temperatures has been included in the figure. There is as yet no experimental evidence concerning the mechanism of this reversed effect of temperature in the absence of oxygen. A similar effect had been previously obtained in studies on survival of *E. coli* following X irradiation (Hollaender, Stapleton, Martin).

Studies on reverse mutations in Neurospora. (Giles, Giles, Case) A considerable amount of genetic data is being accumulated on the linkage relationships of genes in the V-linkage group near the inositolless locus. Recently several experiments have been performed utilizing genetically marked stocks to test more adequately the hypothesis that the differences in frequencies of reverse mutation in various inositolless mutants of independent origin are due to conditions at the inositolless locus itself rather than to modifying genes at other loci. The results of two types of crosses will be discussed briefly. Crosses of inositolless 37401 carrying pabless (1633) and asparagineless (1007) markers, respectively two and seventeen crossover units distant from the inositolless locus, were made to inositolless mutants 5202 (which have a high spontaneous reversion rate) and 89601 (which has a low ultraviolet-induced reversion rate). Tests of spontaneous mutability were made in the segregants from ten complete asci in the cross with 5202. In all asci, two spore pairs yielded cultures with high spontaneous reverse-mutation rates and two with low rates. The cultures with high rates were all pab-independent and 65 per cent were asparagine-independent. Similar tests of ultraviolet-induced reverse-mutation frequencies are being completed with asci from the cross with 89601. Here the mutable cultures to date are all pabless and most are asparagineless. These results furnish conclusive proof that the differences in mutability are in fact associated with the inositolless locus (or with some very closely linked locus) and are not due to various modifying factors at other distant loci which influence mutability.

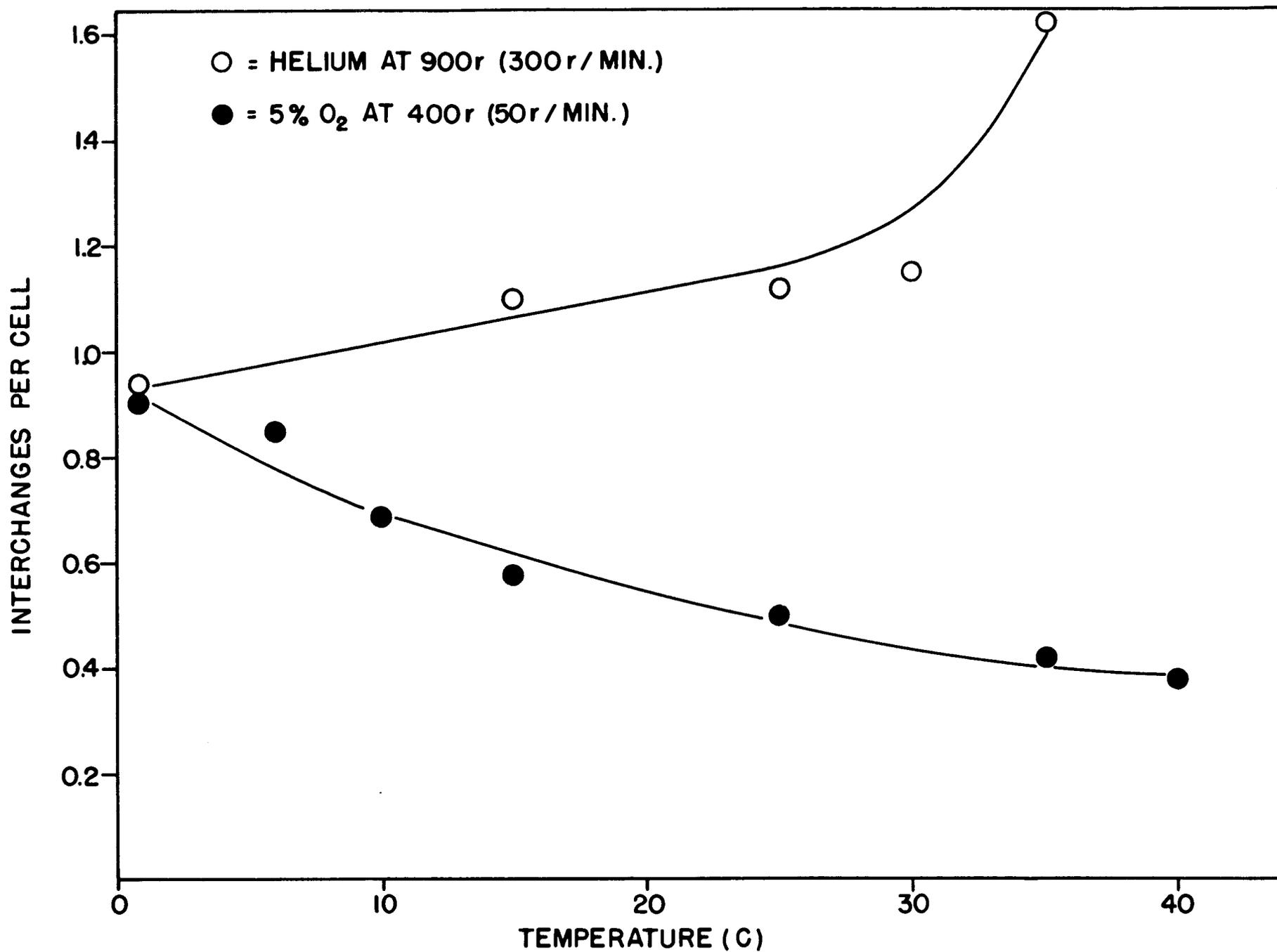


FIG. 6

Glomerella genetics. (Wheeler) Genetic analysis of morphological and biochemical mutants, initiated during the last quarter, has been continued. Tetrad analyses of approximately 1000 asci, involving 39 mutant genes, have been carried out. This brings the total number of asci which have been analyzed in this fungus to slightly more than 1700. The more important results of these studies are summarized in Table 1 and the linkage groups which have been established are shown in Fig. 7. In Table 1 the asci have been grouped into three classes: class I - those from which the eight spores segregated in a 1:1 ratio for the two parental types (parental ditype); class II - those from which a 1:1 segregation for the two recombination types was obtained (recombination ditype); class III - those from which four types segregated in a 1:1:1:1 ratio (tetratype). In nearly all cases, regular Mendelian ratios were obtained. However, a few asci from a cross involving a methionine-requiring mutant and three asci from one involving a cystine-requiring mutant segregated irregularly. A recheck of the latter indicated that the irregularities were due to reversions to cystine independence. The results obtained would seem to furnish an adequate background for more precise studies of sexuality and mutability in this fungus and serve to establish the suitability of this organism as a tool for genetic investigations.

The results of exploratory experiments using C¹⁴-labeled sucrose (supplied by G. R. Noggle) to trace the development of the sexual organs of this fungus in crosses by "labeling" the mycelium of one strain have been extremely encouraging. This technique offers a direct approach to certain problems concerning sexuality in this fungus which otherwise could be attacked only by indirect and very time-consuming methods. The results obtained also suggest that similar techniques may prove very useful in attacking a number of problems in the field of plant pathology.

The effect of ultraviolet radiation on root growth. (Brumfield) The effect of ultraviolet radiation on living roots of *Phleum pratense* is being studied in an attempt to obtain evidence concerning the factors controlling cell growth. By a technique described elsewhere (Brumfield, Am. J. Bot., 29:533, 1942) the rate of elongation and division of the surface cells of the living root can be determined. Cell elongation proceeds at constant rates in these root meristems. Cells near the root apex elongate at a relatively low rate and, since cell division occurs in the region, it is apparently a region of protoplasmic synthesis. A second region of constant elongation occurs

TABLE I

Tetrad analyses from crosses

GENES TESTED	NUMBER OF TETRAIDS IN CLASS*				P VALUE	RATIO OR PER CENT RECOMBINATION [†]
	I	II	III	TOTAL		
A and B	160	119	605	884	.014	47.7
A and F	17	10	63	90	.216	.300
A and ad	7	4	24	35	.382	.314
A and arg	20	24	40	84	.558	.524
A and bi	21	22	46	89	.843	.483
A and cy	27	18	57	102	.185	.441
A and leu	15	16	45	76	.874	.408
A and ly	15	19	78	112	.495	.304
A and ni	18	3	30	51	< .010	35.3
A and ni-a	4	9	23	36	.174	.565
A and or	44	48	92	184	.683	.500
A and py	12	15	50	77	.579	.351
A and st	8	6	26	40	.616	.350
A and th	37	0	15	52	< .010	14.4
A and try	106	6	99	211	< .010	26.6
B and F	12	13	42	67	.849	.373
B and ad	5	1	26	32	.095	.188
B and arg	18	15	51	84	.612	.393
B and bi	17	15	57	89	.723	.359
B and cy	24	10	68	102	.017	43.1
B and leu	9	13	45	67	.411	.328
B and ly	18	19	74	111	.875	.333
B and ni	16	5	30	51	.017	39.2
B and ni-a	6	7	23	36	.398	.361
B and or	33	27	123	184	.452	.326
B and py	15	16	50	81	.865	.383
B and st	6	5	26	37	.758	.299
B and th	6	11	35	52	.230	.327
B and try	20	35	156	211	.045	...
arg and bi	3	6	2	11	.320	.818
arg and leu	17	1	21	39	< .010	29.5
arg and or	40	0	8	48	< .010	8.3
bi and cy	3	6	10	19	.320	.474
bi and try	5	6	16	27	.356	.407
cy and try	8	8	22	40	.999	.400
leu and or	22	4	34	60	< .010	35.0
ly and or	9	9	26	44	.999	.409
ni and cy	19	0	15	34	< .010	22.1
ni-a and or	5	3	18	26	.485	.308
or and py	10	11	39	60	.834	.350
th and try	29	0	11	40	< .010	13.8

KEY TO SYMBOLS

A	F	mating reaction
ad	adenine	
arg	arginine	
bi	biotin	
cy	cystine	
leu	leucine	
ly	lysine	
ni	nicotinamide	
ni-a	nicotinamide (2nd locus)	
or	orange	
py	pyridoxin	
st	streaked	
th	thiamine	
try	tryptophan	

* Class I - parental ditype; class II - recombination ditype; class III - tetratype.

[†] Ratio refers to the value obtained from $\frac{I + II}{I + II + III}$; values less than 1 are ratios, those greater than 1 are recombination percentages.

Figure 7

Figures above the solid lines are recombination percentages; those below represent gene-centromere distances calculated by Lindegren's graphical method for asci having monlinear spore arrangement.

LINKAGE GROUPS IN GLOMERELLA

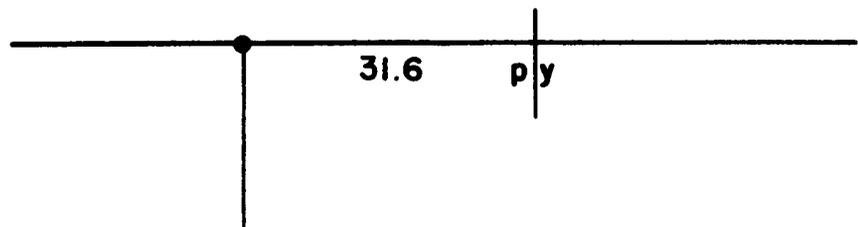
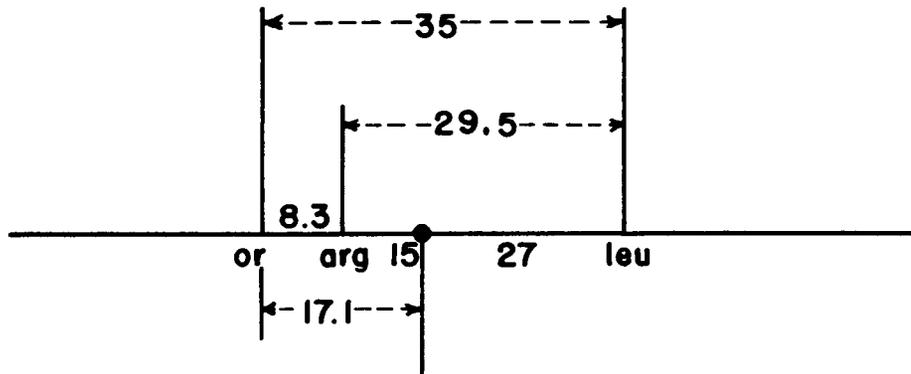
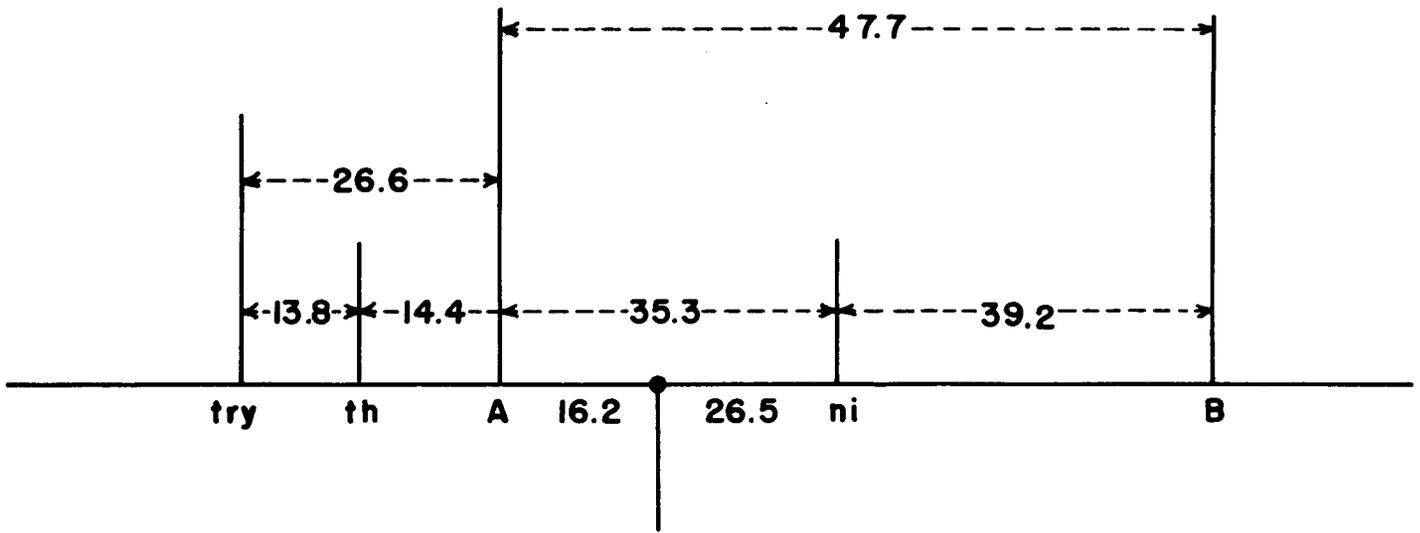


FIG. 7

It will be recalled that the genetic analysis permitted classification of Ad₄ as following, and Ad₂ and Ad₃ as preceding Ad₅ (the pigment-accumulating mutant). Ad₁ had not been classifiable because of poor germination. The accumulation by Ad₃ of material which had no activity for Ad₂ (or for Ad₅ and Ad₄), but which has activity for Ad₁ suggests the above sequence for the biochemical steps in which the mutants are blocked.

Various experiments have been carried out, are in progress, or in prospect to further characterize the active substance or substances. A positive imidazole test has been obtained suggesting that this may be a lead in fractionation procedures. The color was yellow, not pink as it would likely be if histidine itself were given the test. Heating the supernatant does not seem to affect its activity. The absence of heat-precipitable material suggests that there is no appreciable amount of protein present.

Irradiation experiments have been initiated using marked haploids and diploids. The haploid cultures were similar in that they both required adenine and uracil, and the diploid was synthesized from them in such a way that it was homozygous recessive for adenine and uracil, and heterozygous for tryptophan and methionine. The genes studied with regard to mutagenic action were the Ad₃ and the Ur₁ loci.

Ultraviolet is the only radiation studied thus far. A number of experiments have been carried out with the diploid yeast. In Fig. 8 are presented the data for "reversion" to adenine independence, and in Fig. 9, the data for "reversion" to uracil independence for the diploid. The term "reversion" is used here nonspecifically since the appropriate tests to establish whether we are dealing with a true gene reverse mutation at the same locus, or suppressor mutation at an independent locus are not yet complete. It can be seen in Figs. 8 and 9 that there is a noticeable increase in mutation rate even with the smallest increment of exposure (1 minute), and that the ratio of mutants to total number of cells increases with increasing ultraviolet exposure to 16 minutes (the longest exposure reported). Of considerable interest is the fact that, during this interval of time when the radiation is causing tremendous mutational effects, there is no significant killing, as measured by plate (colony) counts (Fig. 10). This is rather strongly suggestive that the mechanism of killing is by recessive lethal mutations, which are not effective in diploid unless they are homozygous. Experiments with one of the haploids have been started, but the data are still quite tentative. A difficulty still to

"REVERSION" TO ADENINE INDEPENDENCE, DIPLOID YEAST

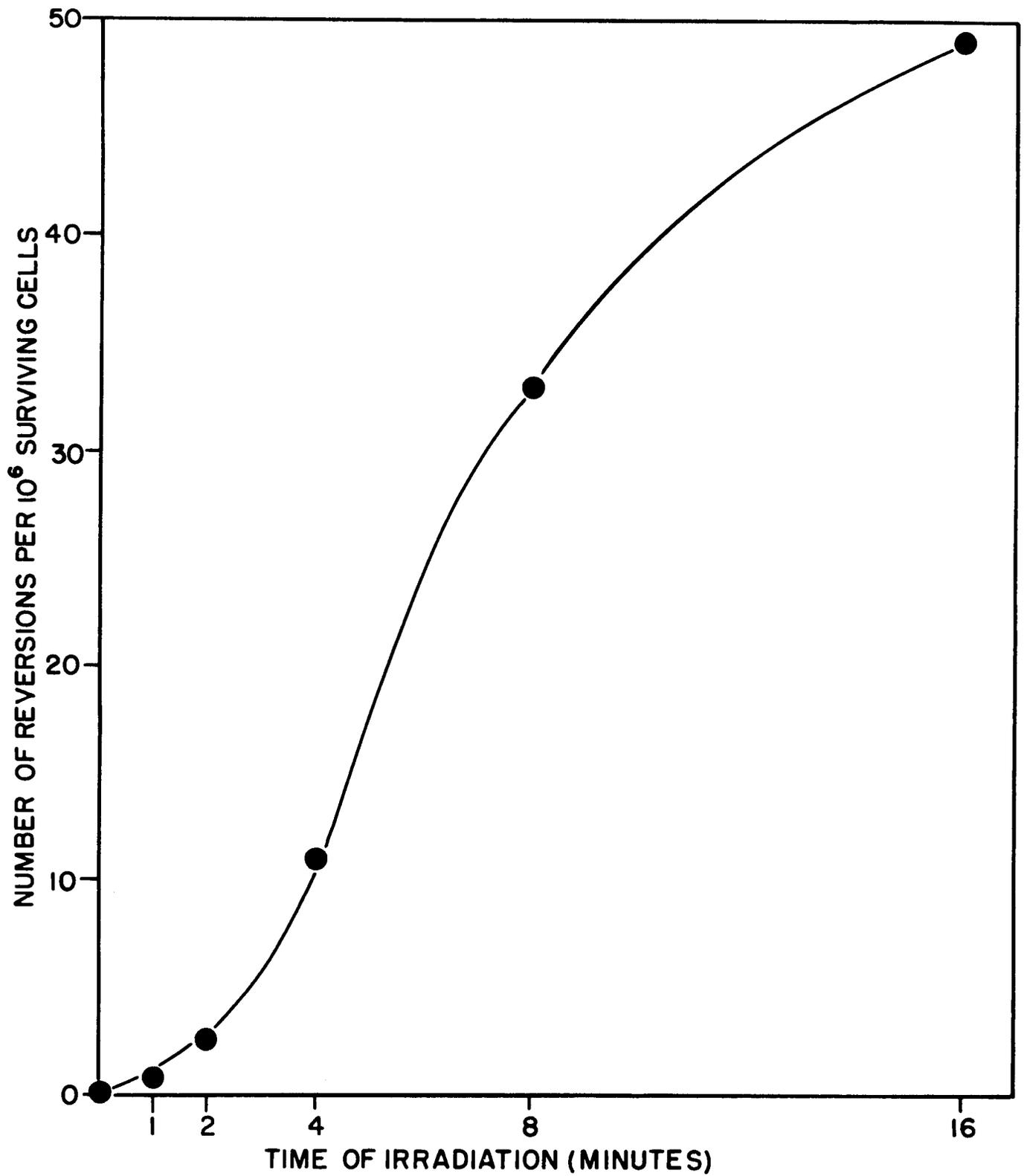


FIG. 8

"REVERSION" TO URACIL INDEPENDENCE, DIPLOID YEAST

DWG. # 9539

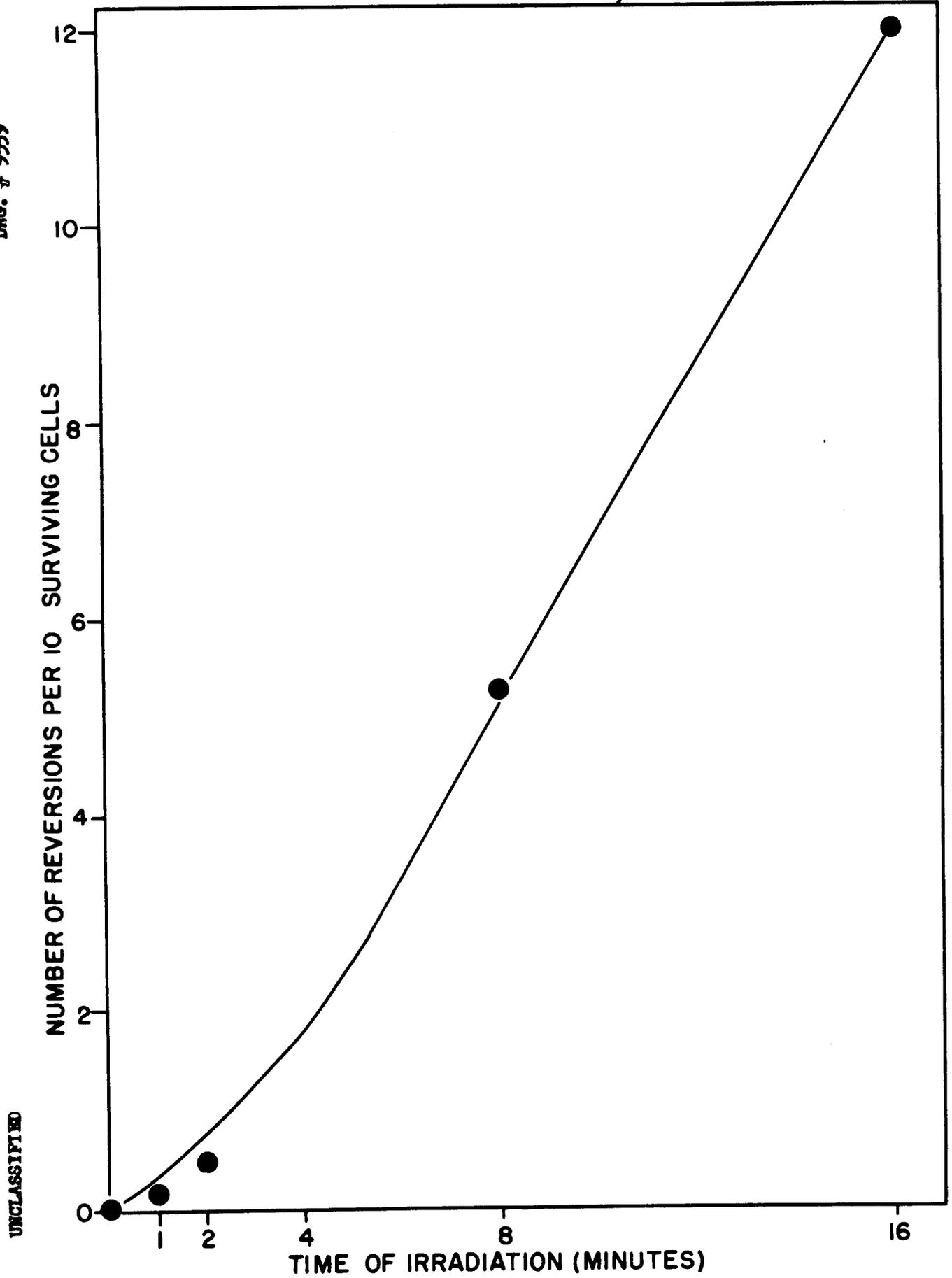


FIG. 9

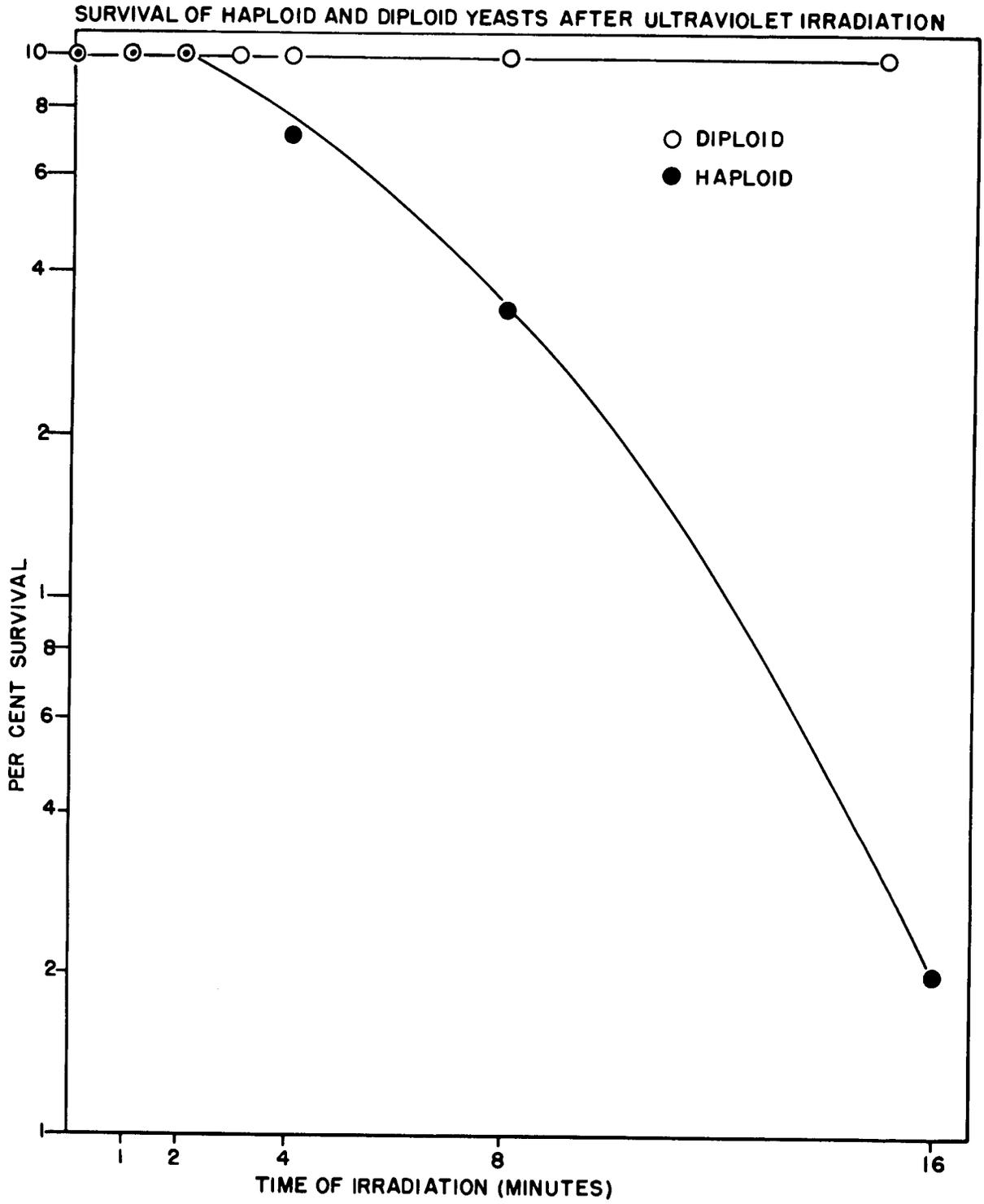


FIG. 10

be overcome with the haploid is the tendency to aggregate in small clusters, thus decreasing the apparent kill and increasing the apparent mutation frequency. The curves shown in Figs. 10, 11 and 12 should be examined with these limitations in mind. Fig. 10 is a semilogarithmic plot, showing the per cent survival with different periods of irradiation. Figs. 11 and 12 are mutation data for reversion from adenine and uracil requirements. It can be seen that the haploid is clearly more sensitive to the killing action of the ultraviolet rays than is the diploid, inasmuch as there is appreciable (98 per cent) killing in 16 minutes with the haploid, while there is little or none with the diploid (in some experiments it has approached 50 per cent). There seems to be some tendency for the mutation rates of the haploid to be higher than those of the diploid at the lower doses, but this may be an experimental artifact. It is planned to extend this work to ionizing radiations.

The effect of oxidizable substrates on X-ray sensitivity of Escherichia coli. (Doermann, Underwood, Hill) The remarkable effect of oxygen tension in altering the effects of X radiation has been subject to considerable investigation in the Biology Division for the past 18 months. Recently we tested the lethality of X radiation on bacteriophage both in the presence and the absence of oxygen. Surprisingly enough, no effect of oxygen tension was found (Quarterly Report, ORNL-644:25-26). In these experiments, oxygen tension was shown to affect neither lethality of direct hits on the virus particles, nor the indirect lethality produced by irradiation of the suspending medium. (Details of the analysis of direct and indirect action of X rays on bacteriophage may be found in the Ph.D thesis of J. D. Watson, Indiana University, 1950.) Since neither direct nor indirect effects of X rays are here modified by oxygen tension, one must look to some difference between the bacterial viruses and the other organisms studied in order to explain the unusual condition existing with phage. The most striking difference in this connection is that bacteriophage suspensions in the absence of host cells do not exhibit any oxygen consumption. The hypothesis obviously suggests itself that a considerable portion of the lethal effects of X rays on these organisms which consume oxygen is due to action on the respiratory metabolism, and, moreover, that this is the site where oxygen tension affects X-ray sensitivity.

With this hypothesis in mind, we have begun a series of experiments in the hope of linking oxidative respiration and X-ray sensitivity. The rationale behind the experiments is the following: If low oxygen tension is causing

"REVERSION" TO ADENINE INDEPENDENCE, HAPLOID YEAST

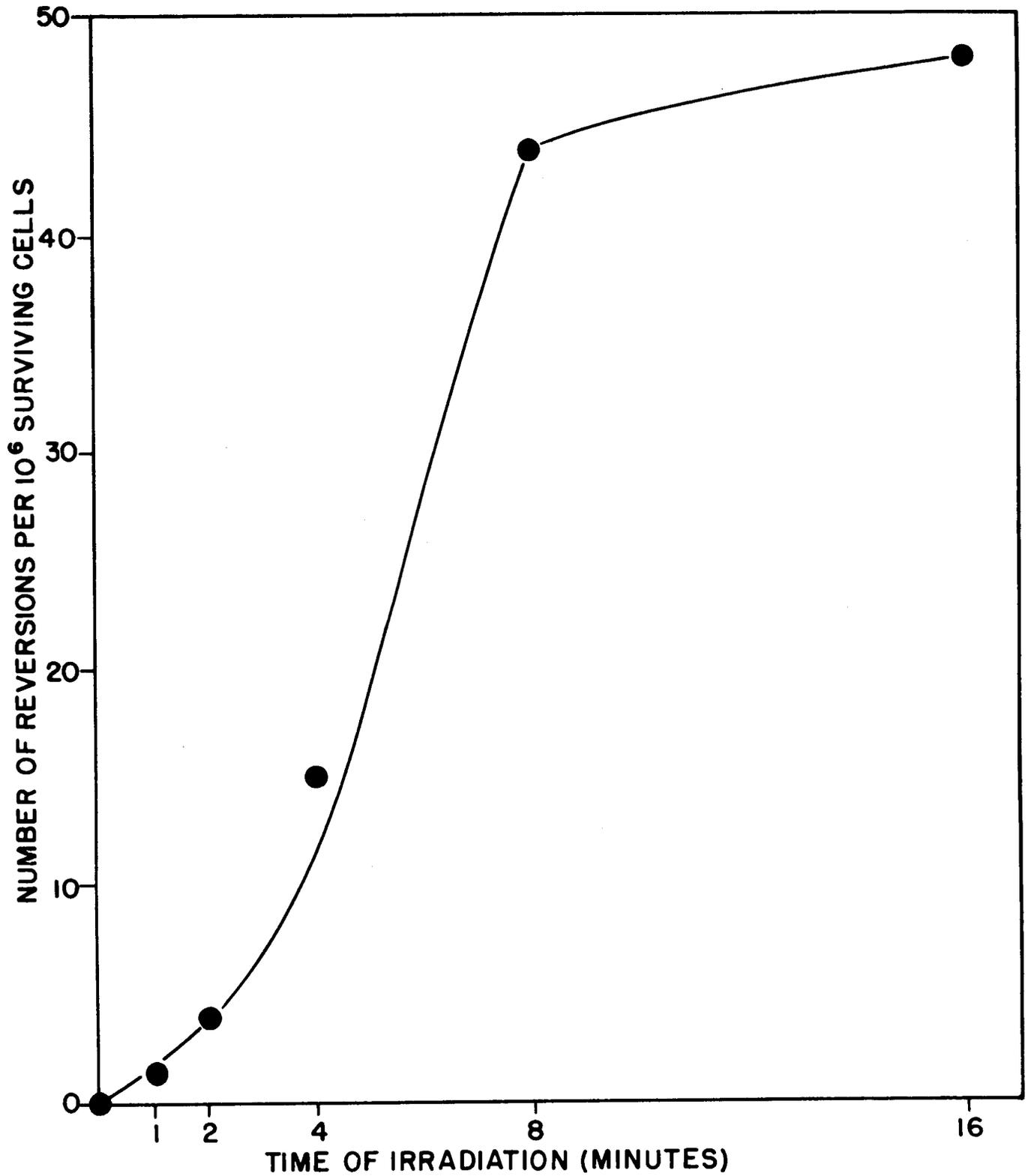


FIG. II

"REVERSION" TO URACIL INDEPENDENCE, HAPLOID YEAST

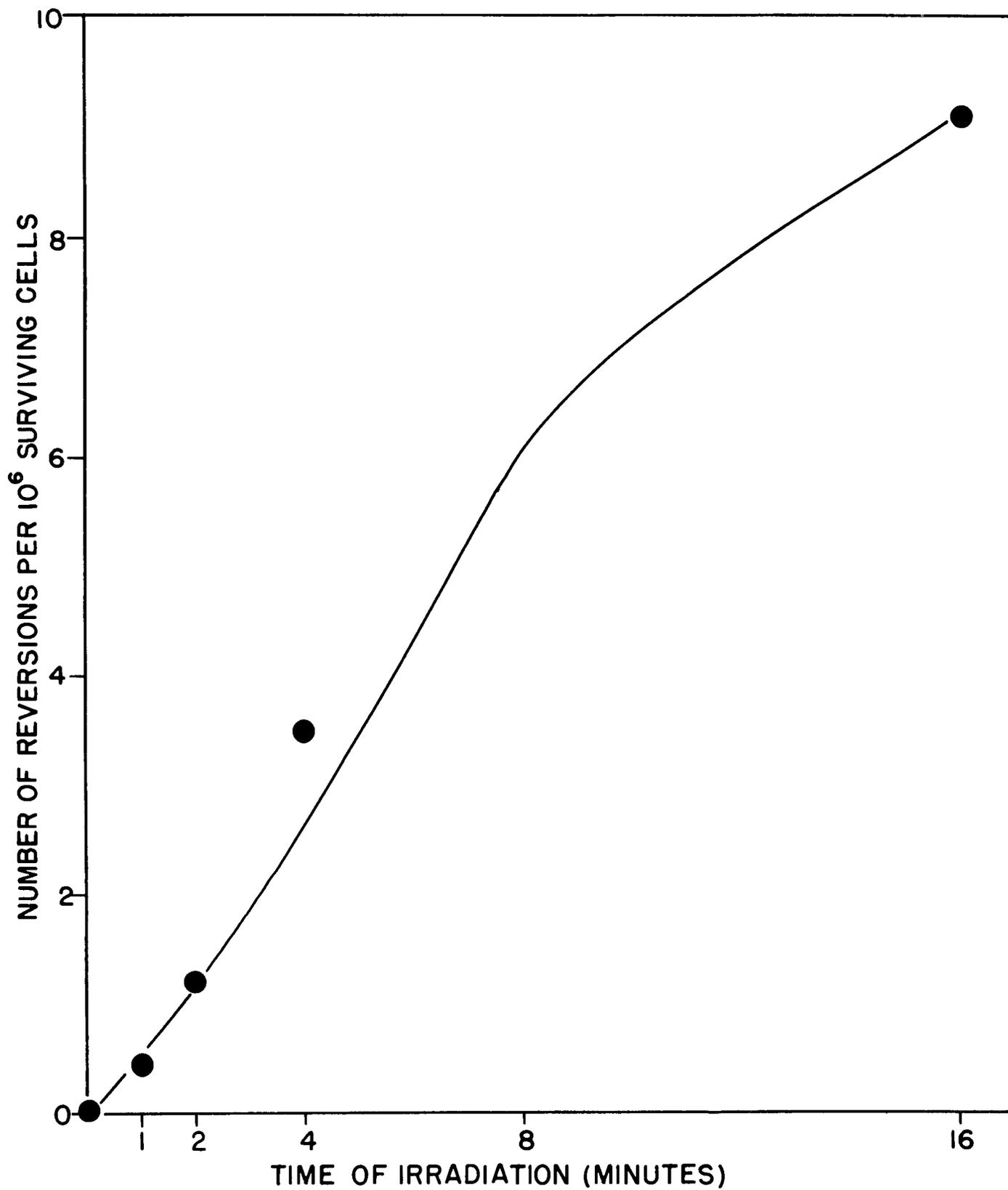


FIG. 12

increased X-ray resistance through its effect on the respiratory metabolism, it seems reasonable that it is accomplishing this result by keeping some carrier of the system in the reduced state; it should be possible to accomplish this in another way if one could supply such an over-abundance of an oxidizable substrate so that it could keep the carrier in the reduced state. The experiments were made with cells of a purine-dependent, radiation-resistant mutant of *E. coli*. The purine-dependent mutant was used so that in the future it would be possible to control growth without limiting the usual respiratory substrates. The cultures used were 18- to 20-hour cultures in a medium containing limiting amounts of glucose and acid-hydrolyzed casein. The cells were thoroughly washed before irradiation. The results of exposing cultures of such cells to 50 kr of X rays in the presence of the two oxidizable substrates, glucose and glycerol, are shown in Table 2. In the case of

TABLE 2

The effect of the presence of oxidizable substrates on the X-ray sensitivity of Escherichia coli (B/r, purine-dependent mutant).

EXPERIMENT	SUBSTRATE PRESENT DURING IRRADIATION	SURVIVAL AT 40 KR
1	buffer control (air)	0.0022
	1% glycerol (air)	0.0044
	3% glycerol (air)	0.016
	10% glycerol (air)	0.056
2	buffer control (air)	0.0034
	0.33% glycerol (air)	0.0006
	1.0% glycerol (air)	0.0019
	3.0% glycerol (air)	0.0105
	9.0% glycerol (air)	0.084
	27.0% glycerol (air)	0.30
3	buffer control (air)	0.0034
	0.33% glucose (air)	0.0005
	3.0% glucose (air)	0.0003
	9.0% glucose (air)	0.0009
4	buffer under air	0.0049
	buffer under nitrogen	0.23

glucose, the cells, instead of being protected, were sensitized to X rays. With glycerol, although low concentrations sensitized, high concentrations protected the cells against the lethal action of X rays. In fact, 27 per cent glycerol protected as efficiently as nitrogen used in a parallel experiment.

No conclusions can be drawn from these results, for they might be due to several causes. They do, nevertheless, fit in with the hypothesis proposed. Rigorous proof might be obtained by finding a compound which could be oxidized only after the cell had become adapted to its utilization. Protection of the adapted but not the unadapted cell would furnish strong evidence in support of the hypothesis.

Designing a nephelometer adapted to bacteriophage experiments. (Underwood) A nephelometer has been developed which has three main advantages over those available commercially. (1) The light intensity is quite low. The power supplied to the bulb is a fraction of a watt. When it is desirable to minimize even further the effect of light (possibly where photoreactivation would affect results) a K-2 yellow light filter can be inserted. (2) The nephelometer part has been made small, and in separate units from the control and indicating devices. It can easily be put into an incubator. Thus the progress of the experiment can be followed at short intervals without disturbing the specimen under observation (except that aeration must be cut off during a reading). (3) Four or more nephelometers may be operated from the same indicating device, making it possible to have at least three experiments with one control.

One of the important problems connected with standardization of a nephelometer is that of preparing standards with stable properties. This has been attempted by suspending very fine particulate matter in a chemoplastic prior to addition of the "setting" agent.

Kinetics of genetic recombination among bacteriophages. (Doermann, Hill) The phenomenon of recombination of genetic material appears to be one of the few clues available for investigating what occurs in the synthesis of a bacteriophage particle before it becomes a mature infectious unit. We have undertaken a few experiments in recombination kinetics. The cross used was described in ORNL-644. This cross has in our experiments yielded about 37 per cent recombinant and 63 per cent parental types, the two alternative recombinant types occurring in about equal amount, and the parental types appearing approximately in the ratio in which they originally infected the cells.

The first experiments were designed to test whether the first newly formed phage particles have the same probability of being recombinants as those particles appearing later in the latent period. The cyanide-lysis technique was used to induce lysis of the cells at various stages of the latent

period. (Earlier experiments indicated that the first matured phage particles might be recombinants, but the tests were made with a cross yielding only 2 per cent recombinants, and this made estimation of the probabilities experimentally impractical.) The results of the most extensive experiment are given in Table 3. The first formed phage particles (that is, when there is less than one particle per cell) have about two-thirds the probability of being a recombinant as those particles found in the total crop which are yielded after the latent period has been allowed to go to completion. Several hypotheses could account for the result. The most likely, in the light of present theories of phage synthesis, would be that mixing of the genetic subunits is less efficient in the cell where small numbers of them are being circulated. Other possibilities are being considered and experiments are planned for obtaining more information on this point.

TABLE 3

Estimation of intracellular recombinants in the cross $T4r_{48} \times T4m_{41}$

TIME OF CELL DISRUPTION (min)	PHAGE PER CELL	TOTAL RECOMBINANTS OBSERVED	PERCENTAGE RECOMBINANTS
15	0.0058	155	24.9 ± 3.4*
16	0.054	1046	25.5 ± 0.4
17	0.34	720	24.4 ± 1.2
18	1.1	881	24.8 ± 0.9
19	2.3	1093	27.9 ± 0.7
21	10	1151	27.5 ± 0.6
24	34	1308	30.1 ± 0.4
28	91	1032	32.6 ± 0.6
33	172	1246	36.0 ± 0.9
38	321	1198	37.0 ± 0.6
Control burst	313	1690	37.3 ± 0.5

* Standard error of the mean.

A single experiment was made to test whether the total multiplicity of infection affects the percentage of recombinants. Average multiplicities were 9.2 and 11.6 of the two parental types in the control experiment, and in the parallel experimental culture they were 2.1 and 2.7. When corrections were

made for those bacteria which were not infected with one or the other of the parental types (Poisson distribution), the recombinant percentages were 37 and 35 per cent, respectively. Clearly the total multiplicity of infection has little effect on the amount of recombination.

EFFECTS OF RADIATION ON *PARAMECIUM*

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R. P. Geckler* Mary King

Photoreactivation of the effects of ultraviolet irradiation. (Kimball, Gaither, King) The analysis of data on this problem has been completed and a detailed paper has been prepared. Only a brief review of the major findings and conclusions will be given here. Some of these are based upon work included in previous reports.

Delay in cell division by 2804 A ultraviolet was found to be significantly less when the animals were exposed to visible light immediately after ultraviolet. Delay in cell division by 2378, 2537, and 2650 A ultraviolet all appear to be decreased by visible light. However, the decreases are not established as statistically meaningful. Nevertheless, the regularity with which decreases occur suggests that the effect is real. It is possible that differences exist between groups treated with different wave lengths in their response to visible light, but such differences are not established as significant.

Exposure to visible light considerably decreases the amount of death produced by 2804 A ultraviolet. The amount of death before the first division after irradiation is less affected than is the amount after the first division. The latter category is closely associated with some of the processes involved in cell division delay and so it is not surprising that division delay by 2804 A ultraviolet is more affected by light than is death before the first division. These results establish that different effects of ultraviolet can be subject to different degrees of photoreactivation.

Reduced vigor after autogamy (presumably due in considerable part if not entirely to gene mutations) brought about by 2650 A ultraviolet is decreased by exposure to light, but the very similar reduced vigor brought about by X irradiation is not decreased by exposure to visible light. This finding suggests that, despite the apparent similarity of ultraviolet and X-ray-induced

mutations, the mechanisms by which they are produced must be quite different. Either the ultraviolet-induced mutations are produced by some indirect mechanism subject to photoreactivation and capable of acting some time after irradiation ceases, or the mutations produced must themselves be reversible by light for some time.

The fact that such a variety of ultraviolet effects is subject to photoreactivation indicates a possibility that they are all secondary consequences of some primary action of ultraviolet which is itself subject to different degrees of photoreactivation. However, the fact that different effects are subject to different degrees of photoreactivation makes it seem probable that there must be further complications in the mechanism.

The role of cytoplasmic inheritance in reduced vigor after autogamy. (Kimball, Geckler, Gaither, King) Kimball (Genetics, 34:412-424, 1949) has presented evidence that suggests that much of the reduced vigor which appears after autogamy (self-fertilization) following exposure to X or beta irradiation is due to gene mutation, though it is pointed out that the experiments were not especially designed to detect cytoplasmically inherited changes. Geckler (Genetics, 35:253-277, 1950) has presented evidence that the similar reduced vigor resulting from exposure to nitrogen mustard is due partly to gene mutation but partly to cytoplasmically inherited changes. An experiment has been designed to investigate this matter more closely, using both X rays and nitrogen mustard. Several preparatory investigations have been carried out, and the main experiment is now well along, but since results and analyses are incomplete, a detailed discussion will be deferred until the next report.

Cell division delay by nitrogen mustard. For comparison with work upon division delay by irradiation it seemed desirable to carry out experiments upon division delay by nitrogen mustard. For this purpose stock 90 of variety 1 of *Paramecium aurelia* and a stock of *Paramecium caudatum* were used. Detailed analyses of the data have not been completed but there is clear indication that the delaying action lasts for several divisions after treatment as it does following ultraviolet. However, it does not seem probable that the delay will be distributed to the various division intervals in the same way as it is for ultraviolet.

EFFECTS OF RADIATION ON RATE OF MITOSIS

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

Effect of oxygen tension on radiation-induced mitotic inhibition. (Carlson, Gaulden, Harrington, Nix) This study has been extended to include 5 per cent oxygen. It has been shown that irradiation with 64 r of X rays at this tension, as at 2 and 0 per cent oxygen, results in a shorter mitotic inhibition period and a more rapid recovery from the inhibition than when cells are irradiated in 21 and 100 per cent oxygen. Work is now in progress to establish the relationship of the effects of radiation in 10 per cent as compared with those in 5 and 21 per cent oxygen.

Mitotic rate in cells in grasshopper egg. Extensive experiments have been made which confirm the preliminary results reported in May. With the time at which the maximum number of mid-mitotic cells occurs after irradiation serving as criterion, it has been shown that mitotic rate in embryos removed from the egg and grown in culture preparations is slower than in embryos which are not removed from their natural environment. The maximum number of mid-mitotic cells occurs in culture preparations at 240 to 265 minutes after treatment, while in embryos which remain in the egg after treatment the maximum number of mid-mitotic cells occurs 190 to 220 minutes after treatment.

Effects of low intensity gamma radiation on mitotic rate. An extensive and thorough study is now in progress on the effects on mitotic rate of exposure of neuroblasts to 0.78 r/hour gamma radiation for 6 days.

EFFECTS OF RADIATION ON MUTATION IN MICROORGANISMS

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

Effects of oxygen tension on sensitivity of bacteria to X rays. (Hollaender, Stapleton, Martin) A good part of the time of this group was devoted to completing work which was discussed in the previous Quarterly Report (ORNL-727). The following interesting results were obtained in regard to certain aspects of the influence of oxygen tension on the sensitivity of *E. coli* to X rays.

1) It has been found that the sensitivity of coli cells to X rays, in the presence of oxygen, showed greater increase if the irradiation is done at 2° rather than at 27° C. Similar results have also been obtained by W. K. Baker in regard to *Drosophila* and by other investigators. It was observed several months ago that if the bacteria are X-irradiated in nitrogen, the sensitivity increases with higher temperatures, the exact opposite of the response in oxygen. The interpretation of this is somewhat difficult at the present time. However, it points out the possibility that the effect of X rays on living cells in the absence of oxygen is not necessarily a direct effect on an essential structure, but rather is an effect which initiates a chemical change leading to the destruction of an essential part of the cell. Further work on this point is necessary.

2) Earlier investigation suggested that bacteria irradiated with X rays are more sensitive if the irradiation is given in a suspension containing only a few bacteria. This effect has been verified.

3) Several attempts were made to influence bacteria by growing them in a variety of media after exposure to X rays. As yet, it has not been possible to influence the survival ratios by this means.

4) Possible effects of oxygen tension on sensitivity to ultraviolet have been sought in several attempts. All the experiments have been negative or inconclusive.

5) In the preceding Quarterly Report, it was mentioned that bacteria grown in glucose broth display higher resistance to X rays than those grown in beef broth. The X-ray resistance increases manifold if bacteria grown anaerobically in glucose broth are irradiated in the absence of oxygen. Bacteria which have been grown in beef broth under the same conditions were found to have somewhat increased resistance to X rays, but much less than bacteria grown in a glucose broth.

6) An intensive investigation is in progress to determine the variation of the sensitivity of bacteria to X rays during different phases of their growth cycle.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION ON MICE

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Louis Wickham
J. C. Kile
A. B. Grobman*

Josephine S. Gower
Jane Crowell
Gloria Jones
Mary Henderson
Patricia A. Sarvella

Determination of mutation rates at certain chosen loci. (W. L. Russell, Gower, Henderson, Jones) Important results have been obtained since the latest progress report (Report, ORNL-244) on this long-term project. A pilot experiment with acute X irradiations was undertaken while the stocks of mice were being built up to the size necessary for the large-scale program. The findings from this experiment have settled many of the problems, particularly effects on fertility, involved in an economical operation of the large project. On the basis of these findings, irradiations in the main project were started several months ago.

Data on the incidence of sterility and partial sterility in the offspring of irradiated males were also obtained from the pilot experiment and have already been reported (Reports, ORNL-644 and ORNL-727).

On the basis of mutation rates in *Drosophila*, few, if any, mutations at the chosen loci were expected in the pilot experiment. Six possible ones, involving four of the seven loci, have, however, been observed. Two of these have already been adequately tested and confirmed genetically, one has been partially tested, one died young and two are still too young to have had offspring. Since more data, particularly from controls, should soon be available, it would be premature to report the mutation rate calculated from the present figures. The results do indicate that the large-scale program should yield enough mutations to give a reliable figure on the induced rate. The anxiety involved in having to rely primarily on *Drosophila* data in the planning of this large project with mice is thus somewhat reduced.

The induction of developmental abnormalities with low doses of X rays. (Russell, Russell, Henderson, Jones) As stated in the last report (Report, ORNL-727) an experiment has been undertaken to determine whether relatively

* Research Participant

low doses of X rays—possibly low enough to fall within the human fluoroscope range—would produce abnormalities if administered at embryonic stages critical in the development of certain characters. These critical stages had been determined in an earlier extensive investigation using higher doses (Report, ORNL-595).

Two strains are being irradiated at three stages of pregnancy and with four doses (200, 100, 50, and 25 r). Since some of the results expected are quantitative shifts, large numbers are required. To date we have obtained and processed for skeletal study 1,144 animals. With the tabulations still incomplete, a sample report will be given at this time, covering only a few of the stage-dose groups and characters studied in only one of the strains. (See Table 4).

It should be noted that, even with 50 r, the incidence of certain severe structural abnormalities is high, some characters being more sensitive at 7½ days, others at 8½ days. For shift in quantitative characters, see Table 5 (B alb C and 129 strains). It may be pointed out that 50 r falls into the range of doses used in human fluoroscopy.

TABLE 4

Percentage incidence of certain abnormalities of the axial skeleton following low dose irradiation of B alb C mouse embryos at certain sensitive stages in development

	CONTROL	7½ DAYS	8½ DAYS	
		<u>50 r</u>	<u>50 r</u>	<u>100 r</u>
No. observed	32	22	38	27
Cervical				
"jumbling"	0	9.1	0	-
atlas abn.	0	31.8	22.7	-
centra unoss. or small	9.4	40.9	31.8	-
Thoracic				
"jumbling"	0	0	0	11.1
prejumbling ?	0	31.8	9.1	-
Lumbar				
double centra	9.4	50.0	59.1	-
Sternum				
jumbling	0	13.6	2.6	29.6
Costal cartilages				
miscellaneous	0	4.5	4.5	-
Ribs				
fusion	0	0	5.3	33.3

Changes in the relative proportions of different axial skeletal types within inbred strains of mice brought about by X irradiation at critical stages in embryonic development. (Russell, Russell) This experiment, as yet incomplete, is designed to elucidate threshold relations existing in different strains with regard to certain quantitative characters. Some of the characters chosen are: The thoracolumbar border, the extent of the thoracic basket (as expressed by number of costal cartilages articulating with the sternum and the number of sternal elements), and the lumbosacral border. The (C57xNB) F_1 hybrid is normally quite stable with regard to these characters, strain 129 is quite variable at the lumbosacral border and very slightly at the thoracolumbar, while B alb C is variable with regard to all the characters listed.

Without, at the present, going into detailed interpretations of the results so far obtained (see Table 5), it may be pointed out that the more naturally variable the strain, the more easily it is shifted by irradiation at the critical time. Thus, a significant posterior shift in the thoracolumbar border (for which day 8½ is the most sensitive stage) occurs even with 50 r in the B alb C's where it is accompanied by an extension of the thoracic basket; but in strain 129 there is only a small shift, even with 100 r, and the number of sternal elements is not increased. On the other hand, strain 129, which normally shows slight variability of the thoracolumbar border in an anterior direction, is considerably more sensitive to that shift than the (C57xNB) F_1 hybrid on day 9½. (maximum critical period, as determined earlier, is day 11½). At the lumbosacral border, both the variable strains (B alb C and 129) are shifted toward the higher component of their natural complement (26-or-27 and 25-or-26 respectively), while the (C57xNB) F_1 hybrid is apparently so far removed from the threshold that it is barely shifted. This is in contrast to its apparent position with regard to the threshold involving the thoracolumbar border.

TABLE 5

Percentage incidence of various quantitative changes in the vertebral column, ribs, and sternum following irradiation of three different strains at embryonic stages sensitive to the production of these changes

	STRAIN	CONTROLS	7% DAYS			8% DAYS			9% DAYS	
			50 r	100 r	200 r	50 r	100 r	200 r	100 r	200 r
Thoracic rib no.										
> 13	C57×NB	0(274) ^b	-	-	83(52)	-	-	100(66)	-	24(66)
	B alb C ^a	41(116)	52(44)	100(10)	-	90(76)	100(54)	100(10)	-	-
	129	0(192)	-	3(68)	-	-	2(50)	46(22)	0(70)	0(37)
< 13	C57×NB	0(274)	-	-	0(52)	-	-	0(66)	-	2(66)
	B alb C	0(116)	0(44)	0(10)	-	0(76)	0(54)	0(10)	-	-
	129	4(192)	-	0(68)	-	-	0(50)	0(22)	0(70)	46(37)
Cost. cart. artc.										
> 7	C57×NB	0(137)	-	-	18(50)	-	-	80(64)	-	0(66)
	B alb C	28(110)	23(44)	100(10)	-	86(76)	87(54)	80(10)	-	-
	129	1(98)	-	4(54)	-	-	8(38)	-	0(28)	-
Sternal element										
> 6	C57×NB	0(137)	-	-	21(26)	-	-	68(31)	-	0(33)
	B alb C	35(58)	18(22)	0(5)	-	63(38)	77(27)	80(5)	-	-
	129	37(49)	-	19(27)	-	-	37(19)	-	7(14)	-
Presacral no.										
> 26 bilat.	C57×NB	0(137)	-	-	0(26)	-	-	3(33)	-	0(33)
	B alb C	33(58)	41(22)	100(5)	-	74(38)	100(27)	100(5)	-	-
	129	0(104)	-	0(34)	-	-	0(25)	0(11)	0(39)	0(19)
< 26 bilat.	C57×NB	0(137)	-	-	4(26)	-	-	0(33)	-	-
	B alb C	0(58)	0(22)	0(5)	-	0(38)	0(27)	0(5)	-	-
	129	62(104)	-	35(34)	-	-	8(25)	18(11)	23(39)	16(19)

^aIn this strain, percentages represent grades greater than 132 (i.e., approximately half development of a 14th rib).

^bFigure in parentheses indicates the number on which percentages are based. This is the number of animals observed in case of the last two items (sternal elements and presacral number), and the number of sides observed for the others (thoracic ribs, costal cartilages).

MICROBIOLOGY

TRACER STUDIES ON METABOLISM

S. F. Carson (Leader)

D. S. Anthony

E. F. Phares

E. H. Mosbach

W. J. Jefferson

J. W. Foster*

Mary V. Long

H. C. Lichstein[†]

Separation of organic acids by ion exchange. (Anthony, Long) This investigation was undertaken in order to supplement the partition-chromatographic separation of organic acids developed by Phares.

A column one meter long packed with about 30 g of IRA-400 resin in the sulfate form permitted complete separation of a mixture of lactic, succinic, pyruvic, and fumaric acids in amounts of about 0.5 mM each. The acids were put on the column at about pH 6 as dilute solutions of their ammonium salts. Elution was accomplished with dilute sulfuric acid, starting at 0.005 or 0.01 N and shifting to 0.025 N, then 0.075 N as the more readily eluted acids were removed. The acids came off in the order mentioned above. Incomplete data indicate that malic acid elutes at about the position of succinic but the degree of separation of this pair is in doubt; partition chromatography, however, readily separates this pair of acids. Malonic acid comes off the column close to fumaric, while citric and α -ketoglutaric acids are more firmly adsorbed than fumaric. Quantitative information on these latter separations is not complete.

Experiments are in progress to complete the above data and to ascertain the performance of maleic and malonic acids. By a combination of the partition-chromatographic and ion-exchange techniques, it is possible to separate a mixture of lactic, pyruvic, malonic, succinic, fumaric, malic, maleic, α -ketoglutaric, and citric acids.

A combination of the two techniques has already been successfully used to completely separate mixtures of lactic, pyruvic, succinic, fumaric, and malic acids in studies on intermediary metabolism.

* Research Participant

† Consultant

Bio-organic chemistry. (Phares, Mosbach) Attempts to improve separation of acid mixtures containing lactic and succinic acids by partition chromatography have resulted in the use of ethyl ether as the mobile phase with the usual 0.5 N sulfuric acid on celite as the inside phase. The ethyl ether caused many "vapor patches" to show on the column but this apparently did not interfere with the separations.

Propyl ether overcame the objections which arose from the high volatility of ethyl ether, but more than twice as much solvent volume was required for elution. Hence, ethyl ether was the solvent of choice.

Trace amounts of acids from fermentations were detected and isolated by use of 4 × 150-mm celite column. With chloroformbutanol as the organic solvent, 0.5-mg amounts of lactic acid gave a peak twenty-five times the blank titration. With ethyl ether 0.1-mg amounts of succinic and lactic acids were separated, the peaks being about three times the blank titrations. Titrations were made with a microburet, using 0.1 N alkali.

Function of vitamin B₁₂. (Lichstein, Carson) Certain mutant strains of *E. coli* require vitamin B₁₂ for normal growth, but will grow reasonably well when B₁₂ is replaced by methionine. B₁₂-deficient cells were prepared by growing the special strain of *E. coli* (designated *E. coli* M) in a synthetic medium containing methionine.

The rate of oxidation of a number of organic substrates by B₁₂-deficient "resting-cell" suspensions was greatly stimulated by the addition of B₁₂. Cells grown anaerobically (stationary) responded to B₁₂ somewhat differently from those grown aerobically (shaken); as one might expect, the aerobic cells grow much faster. The anaerobic B₁₂-deficient cells exhibited a very long lag toward the oxidation of substrates; B₁₂ stimulated the rate, however, there was a lag between the time of B₁₂ addition and the observed accelerated rate of oxidation. Aerobic B₁₂-deficient cells, on the other hand, rarely exhibited a lag upon addition of the substrate, and more striking indeed was the *immediate* acceleration of rate of oxidation upon addition of B₁₂.

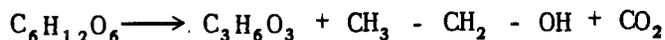
The B₁₂ stimulation was noted with a variety of substrates, e.g., acetate, fumarate, malate, succinate, and pyruvate. The magnitude of stimulation for these various substrates was not significantly different. The enzyme systems in these cells were quite stable; cells aged 21 days at 5° C were quite

active, and exhibited the B₁₂ stimulation as readily as fresh cells. RQ measurements on the various substrates, with and without B₁₂, yielded little additional information.

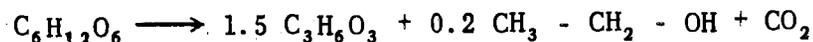
A most interesting phenomenon was observed, however, in connection with the addition of minute amounts of crystalline cytochrome c. This substance apparently stimulated B₁₂-deficient cells in a manner *exactly parallel* to that observed with B₁₂. A large number of runs were made with cytochrome c, with B₁₂, and with both substances added together. It was quite encouraging that no "additive" effect was noted. Further, B₁₂-deficient cells after incubation with B₁₂ showed no B₁₂ or cytochrome response, and such deficient cells after treatment with cytochrome exhibited no cytochrome or B₁₂ response. The specificity of the B₁₂-cytochrome effect is thus a very real one.

It seemed reasonable to assume, as a working hypothesis, that B₁₂ is concerned with the synthesis of cytochrome, perhaps at the level of porphyrin. With the aid of Dr. Totter (Biochemistry), some cytochrome and porphyrin analyses have been initiated. The total amount of porphyrins is apparently quite small, but nevertheless the preliminary determinations indicated a small increase in total porphyrins in these B₁₂-deficient cells to which B₁₂ was added. Subsequent spectrophotometric measurements on whole cells grown in the absence and in the presence of B₁₂ showed a definite increase in both porphyrin and cytochromes in the cells harvested from the B₁₂ medium. It is still too early, however, to state with absolute certainty that B₁₂ is involved in porphyrin and/or cytochrome synthesis. Should these results be repeated and extended they would demonstrate a metabolic function of B₁₂ which fits in well with the observed clinical effects of this vitamin in pernicious anemia.

A new mechanism of lactic acid formation. (Foster, Jefferson, Carson)
Lactic acid formation is a general metabolic characteristic of most species of *Rhizopus*. Under *anaerobic* conditions, resting-cell suspensions of a strain of *Rhizopus* (MX) convert one mole of glucose to one mole each of lactic acid and ethanol:



aerobically, however, one finds that the yield of lactic acid per mole of glucose consumed is substantially higher:



In view of our present understanding of "anaerobic glycolysis", this increase in lactic acid formation under aerobic conditions can be construed to be quite anomalous. It certainly indicates that the extra lactic production must be due to an oxidative (dehydrogenative) process. The problem, therefore, is to attempt to find the participation of an oxidative mechanism in the formation of lactic acid by MX.

If MX were able to produce a precursor of lactic acid by a series of oxidative reactions, then one would have a good case for the extra lactate which is produced under oxidative conditions.

Previous published reports from this laboratory bearing on the intermediary metabolism of *Rhizopus* and *Aspergillus* species have clearly demonstrated the prominent role of the C_2 condensation reaction in the formation of C_4 dicarboxylic acids (Proc. Natl. Acad. Sci., U.S., 35:663-672, 1949; Ibid., 36:219-229, 1950; J. Am. Chem. Soc., 72:1865, 1950).

Most important is the fact that these reactions, leading to the formation of C_4 dicarboxylic acids, are *oxidative*. Hence, if lactate formation can be found to proceed from C_4 acids formed by such an oxidative process, then an entirely new mechanism will have been established for the biosynthesis of lactic acid.

The experiments reported here were performed with resting-cell suspensions of pregrown mycelia of a lactic-acid-producing strain of *Rhizopus*, designated MX.

(1) Aerobic; tracer, methyl-labeled pyruvate; glucose substrate.

Carbon dioxide in the final gas phase was quite active and degradation of the lactate formed showed that 6 per cent of the methyl activity had moved to the alpha and carboxyl positions. These two facts indicate that quite active "oxidative cycling" was in progress.

Fumarate was isolated and found to have a specific activity substantially higher than the lactate (by a factor of 3). This finding leaves little doubt that an active C_4 dicarboxylic acid metabolism was present; and closely connected to the C_3 intermediate, pyruvate and/or its split products.

- (2) Aerobic; tracer, carboxyl-labeled pyruvate; glucose substrate.

Results were substantially the same as stated for the methyl-labeled pyruvate. Twelve per cent of the activity originally in the alpha position moved out to the carboxyl.

- (3) Aerobic; tracer, methyl-labeled ethanol; glucose substrate.

Here one finds the most convincing evidence for

- (a) the formation of C_4 dicarboxylic acids by C_2 condensation, (b) production of lactate from the C_4 acid, and (c) very active oxidative cycling, as evidenced by the large amount of active carbon dioxide produced.

These interpretations are based upon the following facts. Degradation of the lactate showed equal specific activities in both the alpha and beta carbons; the carboxyl contained 20 per cent of the total activity. A very small amount of exceedingly active malate was isolated; complete degradation of this compound into its four carbon atoms showed that the two central carbons had equal specific activities; the two carboxyls had equal specific activities; the ratio of specific activities of the central carbon atoms to the carboxyls was 3:1, which was quite close to that observed with the lactic (i.e., ratio of the α or β , which were equal, to the carboxyl).

The aerobic alcohol tracer experiment gives strong evidence for an aerobic lactate-forming mechanism involving C_2 condensation and C_4 dicarboxylic acids. One may predict, therefore, that under anaerobic conditions, very little lactate, if any, should be formed from tracer ethanol.

Following are results of such a test, in which lactate formation from glucose was tested with methyl-labeled ethanol, both anaerobically and aerobically.

	Total lactate formed, mM	Total counts/sec in lactate	Specific activity of lactate*
Aerobic	2.6	172	0.11
Anaerobic	1.1	0	0.00

* c/s/mg $BaCO_3$

Very high specific activity succinate was also isolated from the aerobic process but the amount was so small that an accurate activity could not be measured.

All these results point strongly to the concept that there is a new biosynthetic mechanism for the formation of lactate which involves an oxidative pathway, by C_2 condensation and C_4 dicarboxylic acids, and which is to be contrasted with the usual glycolytic cleavage to pyruvate and subsequent reduction.

A function of zinc in C_3 and C_4 acid metabolism. (Foster, Carson, Jefferson) Experiments by Foster and Dennison (Bact. Proc., page 124, 1950) indicated that zinc is essential for formation of pyruvic carboxylase in *Rhizopus nigricans* #45. Extracts from zinc-sufficient mycelium exhibited a very powerful carboxylase activity, whereas those from zinc-deficient cells had very small activity in decarboxylation of pyruvate.

Normal cells of this organism under aerobic conditions synthesize the C_4 dicarboxylic acids through a C_2 condensation reaction. Hence, under aerobic conditions C_2 moieties are made available from sugar metabolism most probably by means of the carboxylase reaction.

Under anaerobic conditions, this same organism synthesizes its C_4 acids by a $C_3 + CO_2$ reaction, i.e., not involving C_2 split products (Foster, Carson, Ruben, and Kamen, Proc. Natl. Acad. Sci., U.S., 27:590-596, 1941).

If zinc-deficient cells even in the presence of oxygen cannot produce C_2 moieties, or do so less efficiently, then the C_4 acid production probably proceeds for the most part in a manner similar to that observed in normal cells under anaerobic conditions, namely, through $C_3 + CO_2$.

In order to test this hypothesis, zinc-sufficient and zinc-deficient cells were prepared. The resting cells were allowed to produce fumaric acid from glucose under aerobic conditions. The gas phase contained high specific activity carbon dioxide. The results of two such experiments are as follows:

	Exp't. A		Exp't. B	
	Wt. of cells produced	Sp. act. of fumarate*	Wt. of cells produced	Sp. act. of fumarate*
Zn-	1.4 g	0.39	0.33 g	2.13
Zn+	2.1 g	0.29	1.9 g	0.86

*c/s/mg BaCO₃

It is quite clear that the zinc-deficient cells incorporated much more C* from C*O₂ into the fumarate than the zinc-sufficient cells. It is important to note from the weight of cell material produced *during growth* (i.e., before C*O₂ was introduced) that experiment B exhibited a much greater zinc deficiency, as shown by the difference in weights of cell material, as well as a much greater difference in C* incorporated into fumarate.

It appears as if a deficiency of zinc during growth caused changes in enzyme systems which forced the organism to exhibit a typical anaerobic metabolic pattern, even in the presence of ample oxygen.

RADIATION BACTERIOLOGY

E. H. Anderson (Leader)

Ruth W. Whittle

Effect of oxygen tension on induction of mutations in bacteria by X rays. (Anderson, Whittle) The work of Hollaender, Stapleton, and Martin in this Laboratory (ORNL-644 and ORNL-727) has shown that the radiosensitivity of *Escherichia coli* is materially influenced by the oxygen tension of the suspension during exposure to X rays. Other work previously reported from this Laboratory with higher organisms has shown that genetic effects induced by X rays are less in the absence of oxygen than in its presence (Giles and Riley, Proc

Nat. Acad. Sci., 35:640, 1949, for chromosome aberrations in *Tradescantia*; Baker and Sgourakis, *Ibid.*, 36:176, 1950, for lethal mutations in *Drosophila*; Kimball, Report ORNL-644 for mutations in *Paramecium*; and Gaulden, Report ORNL-727, for mitotic inhibition in grasshopper embryos). In view of these results and of the fact that techniques have been developed which make it possible to detect and assay certain biochemical mutations of bacteria, even when occurring at low frequencies, it was considered advisable to determine whether or not an increased oxygen tension during irradiation would alter the induction of bacterial mutations by X rays.

A streptomycin-dependent mutant and a purineless mutant of *E. coli* B/r were selected for use in these studies because of the ease with which back mutations of these organisms could be screened and assayed. Cells grown under aeration in appropriate media were washed and resuspended in phosphate buffer and samples placed in glass irradiation tubes through which the desired gas was bubbled for 15 or 20 minutes prior to irradiation. All irradiations were carried out at a constant rate of 40 kr per hour. Survival assays for the streptomycin-dependent strain were made on nutrient agar containing streptomycin. Assays for the back mutation to streptomycin nondependence were made on nutrient agar without supplement. Survival assays for the purineless strain were made on nutrient agar and the back mutation to purine nondependence was assayed for on a purine-free medium containing washed agar, salts, amino acids, and dextrose.

Survival ratios obtained in oxygen and in nitrogen plotted against exposure in kiloroentgens show both organisms to exhibit a much greater sensitivity to X rays in oxygen than in nitrogen. A dose reduction value of around 2.5 was obtained for the streptomycin-dependent strain and about 3.0 for the purineless strain.

The survival data indicate that a mechanism which is accelerated by the presence of oxygen is involved in the lethal effects of X rays on these two strains of coli. If this same mechanism acts on the nuclear components of the cells to produce mutations one would expect to find a pronounced increase in the number of mutations induced during exposure in oxygen over that induced during exposure in nitrogen. This was found to be the case for the purineless strain but not for the streptomycin-dependent strain.

Mutation assays for the streptomycin-dependent strain show that the mutation rate (mutants per 10^8 surviving cells) with respect to exposure in kiloroentgens increases about equally for the cells in oxygen and in nitrogen. Oxygen shows but slight additional effect on the rate of mutation induction at any given exposure over the range studied. At equal survival ratios in the two gases the rate of mutation induction in nitrogen is about 2.5 times as great as the rate in oxygen. This is the expected result of a direct action of X rays since the cells in nitrogen received approximately 2.5 times as much irradiation at any survival level as did the cells in oxygen.

On the other hand, the back-mutation rate of the purineless mutant is profoundly influenced by the presence of oxygen during irradiation. This is clearly shown by a comparison of the mutation rates, with respect to exposure in kiloroentgens, obtained in the two gases. At an exposure of 50 kr a mutation rate of 7200 mutations per 10^8 survivors was found in oxygen while the same exposure in nitrogen resulted in a mutation rate of only 300 mutations per 10^8 survivors. Mutation rates plotted against survival ratios for oxygen and for nitrogen give curves which coincide. The rate at any survival level is therefore equal with both gases, despite the fact that at equal survival levels the cells in nitrogen have received approximately three times the irradiation received by the cells in oxygen. This shows that oxygen plays essentially the same role in increasing both killing and mutations in the case of the purineless strain of coli.

These results indicate that, although the lethal effect of X rays is the result of the same process in these two strains of coli studied, the genetic effect examined for each strain is the result of different reactions. Present indications are that the back mutation to streptomycin nondependence in the streptomycin-dependent strain may be the result of a "direct action" of X rays. The small oxygen effect observed with this mutation may be due to a selective action of oxygen or of nitrogen on the newly formed mutants. On the contrary, the back mutation to purine nondependence in the purineless strain is induced by the same mechanism responsible for the lethal effects, since killing and mutation induction in this strain are increased to the same degree by the presence of oxygen during X irradiation.

BIOCHEMISTRY

BIOCHEMISTRY OF NUCLEOPROTEINS, AMINO ACIDS, AND ENZYMES

C. E. Carter (Leader)

W. E. Cohn
D. G. Doherty
F. Vaslow
E. Volkin
B. L. Strehler

M. Helen Jones
J. X. Khym
E. A. Lloyd
M. L. Morse
Ann R. Webster

J. R. Totter*

The distribution of isotopic formate in the nucleotides of ribo- and deoxyribonucleic acids of rats, and control and folic-acid-deficient chicks. (Totter, Carter, Volkin, Jones) Radioactive sodium formate was injected into normal rats and chicks and into folic-acid deficient chicks. After 16 hours the pooled viscera from each group were subjected to fractionation procedures for the isolation of the nucleic acids. Nucleotides obtained from the nucleic acids were isolated by anion-exchange chromatography and the specific activities determined. The data obtained are summarized in Table 6. Thymine was degraded and the activity found chiefly in the portion of the molecule associated with the methyl group.

TABLE 6

Relative activities of nucleotides from nucleic acids after injection of radioactive sodium formate

NUCLEOTIDE:	RAT		CONTROL CHICK		FOLIC-DEF. CHICK	
	RNA	DNA	RNA	DNA	RNA	DNA
Cytidylic acid	5	1	9	2.5	7	0.6
Adenylic acid	73	35	90	19	93	15
Uridylic acid	5	--	--	---	--	---
Guanylic acid	43	31	91	24	154	22
Thymidylic acid	--	15	--	14	--	11

The influence of folic-acid deficiency on the metabolism of isotopic formate, glycine and betaine by chick-liver slices. (Totter, Carter, Webster) Liver slices from control and folic-acid-deficient chicks were incubated with isotopic sodium formate, with $2, C^{14}$ glycine and with methyl-labeled radioactive betaine in separate experiments. Incubations were conducted for 1, 2 and 3 hours in each experiment. The liver slices were then subjected to fractionation by the Schmidt-Thannhauser procedure. Counting has been completed on the protein fraction and on serine isolated from the protein-free filtrate. The results indicate that folic-acid deficiency markedly impairs the ability of the liver slices to incorporate formate into protein. On the other hand free serine appears to be formed almost as rapidly in the deficient as in the control group.

The breakdown of glycine was much slower in the deficient group as compared with the controls.

The incorporation of the labeled methyl from betaine into serine and into protein was very low. The behavior was similar to that with formate. The remaining procedures to be done on the fractions will be conducted at the University of Arkansas.

Binding of acetyl-L-dibromotyrosine by chymotrypsin. (Doherty, Vaslow) Chymotrypsin will catalyze the formation of the anilide of acetyl-L-dibromotyrosine, as well as split various derivatives such as peptides, esters, and amides. On this basis and other evidence it is postulated that any anomalies in the thermodynamics of binding of a substance by an enzyme as compared with the binding of ions or molecules by nonenzyme proteins should also be shown by the binding of acetyl-L-dibromotyrosine with chymotrypsin. In fact it may be presumed that the amino acid undergoes an enzyme-catalyzed exchange of the carboxyl OH group with the solvent.

Acetyl-L-dibromotyrosine has been prepared by bromination and purification using an ion-exchange column of acetyl-L-tyrosine. The experiments have been made using the dialysis bag technique, measurements being made of the gamma radioactivity of the enzyme and buffer solutions with excess activity in the enzyme solution attributable to enzyme binding. An ionization chamber and vibrating reed electrometer have been used for the activity measurements.

The measurements have been made over a pH range of 5 to 8 at 5° and 20° C and at ionic strength of about 0.13 in phosphate-chloride buffer salt solu-

tion. The enzyme concentration was 1 per cent, and the amino acid concentration ranged from 5×10^{-7} to 10^{-3} molar.

Since the total number of enzyme binding sites could not be determined, only apparent values for ΔF and ΔS for 1:1 complexes can be given. However, ΔH can be calculated unambiguously. ΔH ranges from 0 at pH 5.5 to -4000 calories at pH 7.5, and above this the enzyme appears to decompose. Apparent values of ΔF range from -3000 calories at pH 5.5 to -2200 calories at pH 7.5 at 20°C. ΔS was +18 calories at pH 5.5 and -9 cal/mol deg at pH 7.5. There thus appears to be a correlation between the enzymatic activity and the thermodynamics of binding.

Studies on the structure of the isomeric nucleotides. (Doherty) Isolation of isomeric nucleotides from yeast nucleic acid by paper-chromatographic and ion-exchange techniques has reopened the question as to the structure of these natural products. The initial work was carried out on the two adenylic isomers, "A" and "B". It was found that these isomers were mutually interconvertible under acid conditions. Attempts to prepare derivatives substituted on the ribose moiety yielded identical compounds from both isomers.

A direct proof of the location of the phosphate residue was obtained by treatment of adenylic acid "B" with benzyl alcohol and dry hydrochloric acid to yield the benzyl phosphoriboside and adenine hydrochloric acid. Hydrogenation of this compound proceeded smoothly to yield an optically inactive phosphoribitol indicating that the phosphate residue was located on the 3' position of the ribose chain. Application of this reaction to the "A" isomer and to the guanylic acids yielded the same optically inactive phosphoribitol. Attempts are being made to apply this degradation to the pyrimidine nucleotides.

A comparison of such physical properties of the two adenylic acids "A" and "B" as melting point, solubility, rotation and infrared spectra seems to indicate an intramolecular structural isomerism. The interconvertibility of these compounds in acid medium compares favorably with the known lability of the nitrogen glycoside linkage towards mutarotation. These observations, plus the previous chemical evidence that the position of the phosphate residue is the same in both isomers, strongly suggest that these compounds "A" and "B" are α and β isomers.

Turnover rates of ribo- and desoxyribonucleic acid mononucleotides with the use of ^{32}P . (Volkin, Carter, Jones) Studies on the *in vivo* incorporation of ^{32}P in the mononucleotides of liver desoxyribonucleic acid (DNA) have been carried out, while previous data on ribonucleic acid (RNA) nucleotide turnover has been extended.

Injection of the isotope (inorganic ^{32}P phosphate) was administered intraplurally and the animals sacrificed after short periods of time (Table 7). Liver RNA was isolated by the guanidine salt method (Report ORNL-585) and liver DNA prepared according to the method of Mirsky and Pollister (J. Gen. Physiol., 30:117, 1946). The nucleic acids were purified to constant specific activity and were free of protein and cross contamination. Quantitative hydrolysis of RNA to mononucleotides was effected with mild alkali (Report ORNL-585) while DNA was converted to about 65 per cent mononucleotide by enzymatic digestion (Report ORNL-644). Separation and analysis of the resultant mononucleotides were carried out on an ion-exchange column as described by Cohn (J. Am. Chem. Soc., 71:2275, 1949) and the mononucleotide fractions so obtained were assayed for ^{32}P content.

The results of these analyses are given in Table 7. Although essentially uniform labeling occurs in the mononucleotides of mouse and rabbit RNA, an increased turnover rate of rat liver RNA adenylic acid is evident. This result is in accord with that found for rat liver RNA adenylic acid with the use of ^{14}C -labeled formate. The turnover of ^{32}P in DNA proceeds at a somewhat different rate for each mononucleotide, thymidylic acid having the highest specific activity in all cases studied.

TABLE 7

SOURCE	INJECTION DOSE	TIME	SPECIFIC ACTIVITY: COUNTS/MIN/ γ PHOSPHORUS								
			RNA				DNA				
			mc	Min	Cyt	Ade	Uri	Gau	Cyt	Ade	Thy
Rabbit	1.0	180	10.2	10.3	10.3	10.0					
Rat	1.0	20	4.3	6.4	4.4	4.1					
Rat	1.3	60						8.1	15.9	5.5	
Rat, regenerating liver	1.0	20	12.1	19.1	13.8	13.7	5.0	4.7	8.5	4.3	
Mouse	1.0	20	36.1	39.1	33.6	28.2	4.0	7.1	7.5	2.8	
Mouse hepatoma	1.0	20	36.0	38.6	33.6	33.4					

Firefly luminescence factors. (Strehler) Since the instigation of this problem on June 1, 100,000 fireflies, *Photinus pyralis*, have been collected for use in metabolic studies of phosphorylation and hydrogen transport and in the identification of chemical cofactors and enzymes involved in the luminescent reaction. These insects have been desiccated and stored for subsequent use. A fraction of them have been dissected for immediate use.

Various equipment for the isolation and purification of these factors and for use in enzymatic experiments has been built. This includes a large Soxhlet-type extractor, a ball mill, and a sensitive photomultiplier for measuring radiant emission. The last is far superior to earlier designs both in sensitivity and in its recording of light against time directly through a Brown recorder. In addition, a chromatotrain, or two-phase liquid-liquid chromatographic apparatus, of improved design is nearing completion which will afford a means of separating large amounts of the various diffusible factors involved in luminescence.

Identification of fluorescent firefly pigments. (Strehler, Totter) The work pursued here is a continuation of studies begun earlier elsewhere on the nature of luciferescence and luciferin, fluorescent compounds from fireflies. It seems likely that these compounds were pterins and since a number of properties were known from earlier work, the approach was from the synthetic direction. The absorption spectra, fluorescence emission spectra, and partition and solubility, as well as a rough guide as to possible structures from unpublished data on elementary analysis and qualitative spot tests were used as criteria for the synthesis of various model compounds. Numerous pterin derivatives of 2, 4, 5, 6-tetraminopyrimidine; 2, 5, 6-triamino-4-hydroxy-pyrimidine and 2, 4-dihydroxy-5,6-diaminopyrimidine were synthesized.

RADIOBIOCHEMISTRY

C. W. Sheppard (Leader)	Gertrude Beyl
W. T. Burnett, Jr.	E. B. Darden, Jr.
W. S. Wilde*	Leon Wish

Mary M. McNamee[‡]
W. G. Walker*

Research by Health Physics Trainees. (Sheppard, Sodaro, Emerson) Mr. Sodaro has completed an extensive investigation of the use of externally

* Research Participant

‡ Visiting assistant

applied Geiger-Müller counters in tracer studies with animals. This work has included the following studies:

1. Disappearance of ^{131}I from the stomach and its appearance in the thyroid of the dog.
2. Disappearance of intravenously injected gold colloids and gold-citrate complex from the circulation of the rabbit.
3. Disappearance of intravenously injected ^{54}Mn from the circulation of the dog and waves of circulatory mixing.
4. Appearance of radioactivity in the ear of the rabbit by externally applying a Geiger-Müller tube and injecting red cells labeled with ^{32}P in the opposite ear vein.
5. Vascular changes in the rabbit ear produced by various vasodilator and vasoconstrictor drugs, using the above method.

Reports on the observations by trainees are being prepared by them and will be completed in the near future.

Radiological physics: A. Maxitron 250 X-ray machine. (Sheppard, Darden) During the past quarter considerable time has been spent in supervising the installation of the new X-ray unit. Following its preliminary release to the Laboratory by Mr. Adams of the General Electric X-ray Corporation, the unit has had a stormy history, requiring the expenditure of considerable money and effort primarily by the vendor but also, to some extent, by our laboratory personnel. By making certain changes authorized by the company, including the installation of a new X-ray tube, and providing additional water cooling to the unit, successful operation has been achieved as shown by three periods of continuous steady operation at maximum output for 12 to 16 hours.

B. Effects of radiation on seed germination. (Darden; and Noggle, Bangson - Plant Physiology) Assistance was given the Plant Physiology Group in experiments concerning the effect of total dose of X and gamma radiation on the germination and early growth of peanuts; on gamma irradiation of Begonia seeds; and on beta irradiation of peanuts.

The Maxitron was used to give equal-sized monolayers of peanut exposures of 2500, 5000, 10,000, 20,000, and 40,000 r, at an average intensity of about 100 r per minute. A defect in the machine which developed during the longest run and culminated in complete failure of operation after completion of the run caused some unsteadiness of output and made necessary some reduction in intensity. However, the value of the total dose administered is believed not to have been seriously affected.

For gamma radiation the ^{60}Co source was used. The nuts were placed in a lucite exposure container with narrow curved walls so that the contents formed a vertical layer about 1.4 cm thick surrounding the source and equidistant from its axis. A hole was cut in the outside wall of the chamber to admit the standard thimble chamber. The intensity thus measured was about 900 r per hour, with the thimble chamber centered among the seeds. Although the seeds were only slightly more than a monolayer thick, the variation in intensity through this layer amounted to ± 150 r per hour with respect to the center. Vertical variation from the top to the bottom of the layer measured at two locations about 180 degrees apart as determined with the Victoreen was much less, amounting in each case to less than 2 per cent from the mean. These variations were compensated for to some extent by removing the peanuts about halfway through each run and reshuffling them.

The seeds have been planted and certain preliminary differences observed in the growth responses of the X- and gamma-rayed lots. Details of these results will be reported by others.

Begonia seeds have been subjected to gamma radiation. These seeds are minute, so that it was possible to mount a small monolayer about 0.3 mm thick between two small strips of polystyrene. Five of these units were mounted vertically and equidistant around the cobalt source and given doses of 2500, 5000, 10,000, 20,000, and 40,000 r, respectively, at an intensity of about 925 r per hour as measured with the standard thimble chamber. Effects on the germination of the seeds are not as yet in the data-taking stage.

Using a ^{32}P bakelite source in the exposure chamber developed by M. E. Gauden for irradiation of grasshopper neuroblasts, peanuts were exposed to beta radiation. The peanuts were mounted by G. R. Noggle in a lanolin medium in such a way that the embryo end of each seed was end on against the surface of the source, separated from it by a thin film of rubber hydrochloride. The control batch was mounted with the opposite end from the embryo in a similar position. The dose delivered to the embryo region, assuming a mean depth of 1 mm, was about 40 per cent of the surface dose according to the depth-dose curves of Raper, Zirkle, and Barnes for a tissue-like absorber. Since only a rather low-intensity source was available at the time, the total irradiation time was considerably longer than desirable, however a high-intensity source will shortly be available for further work.

C. Gamma-ray sources. (Darden) Calibration curves of the radial and vertical variation of intensity over limited regions inside the lead enclosure around the source have been taken using the standard thimble chamber for radial variation and the Victoreen for relative vertical variation in regions not available to the standard. It is hoped that this information will prove useful to prospective users of this facility.

D. Beta-ray facilities. (Sheppard) The large lucite handling box and associated equipment for measuring the surface exposure of phosphorus beta-plaques has been transferred to the Isotope Development Group at X-10. Henceforth individual users may obtain by request plaques of a known surface intensity activated according to specifications, and shipment will be made to the user in a standard, properly shielded shipping container.

Colloids and radioactive iron. (Wish) A more thorough study of the physical characteristics of the gold citrate sols was made. The light pink small particle colloids which were thought to be almost completely dispersed gave a positive orthotolidine test indicating a percentage of ionic gold probably complexed with citrate. Longer heating in their preparation gave negative ionic gold tests, the resulting colloid being orange-red in color and highly transparent. Electron microscope pictures of this type showed a fairly homogeneous particle with a radius of 3 to 5 $m\mu$ and the homogeneity was compared by ultracentrifuge pictures of the sedimenting sol. Electrophoresis indicated that the mobility of the colloidal particles paralleled that of the globulin fractions when mixed with rabbit plasma *in vitro*. Since the mobility of the sol itself was much faster, the globulins probably are adsorbed into the gold. Further animal experimentation is being carried out in cooperation with the Pathology Section.

Ten electrolytic cells have been set up for the electroplating of iron. These are now being used by the Pathology Section for the electroplating of ^{55}Fe and ^{59}Fe . Stock solutions of these isotopes as ferrous ammonium citrate were prepared and these were injected intravenously into donor rabbits. The special counting equipment necessary for these isotopes was set up.

Erythrocytes. (Sheppard, Beyl) Previous studies of sodium transport in irradiated human erythrocytes suggested that the simple two-compartment analysis developed to describe potassium exchange was inadequate for sodium.

To clarify this, sodium exchange was observed under varying experimental conditions:

1. at different shaking rates,
2. following removal of the buffy coat from the cells,
3. following accumulation of intracellular sodium,
4. reversal of usual tagging compartment, *i. e.*, by studying the outgo of radiosodium from tagged cells into an untagged plasma compartment.

Results indicate that the intracellular sodium consists of two fractions, only one of which exchanges rapidly. The second is probably bound and therefore unable to participate in the fast exchange.

Mineral metabolism. (Burnett, with surgical assistance of R. R. Bigelow, consultant) Radiomanganese was given to a third dog equipped with an artificial pancreatic fistula. The output of manganese by the pancreas of this dog appeared to parallel that of protein nitrogen. (Technical assistance in protein nitrogen determinations by Beyl and Wish; assistance in collection of pancreatic juice and in care of dog by Lloyd, of Biochemistry of Nucleoproteins group.) The experimental work in connection with our mineral metabolism program has essentially been concluded and the results are now being written up for publication under the tentative title "Radiomanganese Studies in the Pancreatic Fistula Dog."

The exchange rate of plasma and tissue potassium. (Wilde, Walker) Potassium is the most abundant metallic cation present in living protoplasm. It is essential for growth; it is a catalyzing coenzyme for biochemical reaction; it regulates excitability and determines the magnitude of electrical potential differences so characteristic across cell interfaces.

Though accumulated to such large extent in the protoplasm, potassium shows a rapid and often complete exchange between the cell and its bathing medium. Yet, at the same time, potassium is held in the cell to the exclusion of other cations, such as sodium which is so abundant in the medium. Attempts are being made to evaluate the exchange rate of potassium in resting muscle and liver and to test the effect upon it of hormonal and other agents. It is felt that such rates would clarify the role of potassium in biochemical processes and the manner of its binding.

Studies on potassium exchange rate in plasma and tissues in live rabbits begun at Tulane (Am. J. Physiol., 159:594, 1949) have been continued with Radiobiochemistry Group facilities in the Biology Division. The automatic counter and the automatic Brown recorder on the Group's flame photometer have accelerated the work tremendously. Specific activity of the short-lived ^{42}K , with ready access to the Oak Ridge pile, is four times that available by air freight at New Orleans. For the first time actual trace experiments with ^{42}K in circulating plasma are possible. Tagged potassium is available here on any day of the week. Fourteen stringers and pile rabbits have been handled. Mathematical aid is available from Dr. Sheppard and from Dr. L. J. Savage, consultant.

Experiments have shown that intravenous ^{42}K has apparently mixed completely with kidney potassium in 2 minutes, with liver potassium in 20 minutes, and with muscle potassium in 2 hours. But during 2 minutes the plasma specific activity has fallen 90 per cent of its initial value so that there is considerable question as to the state of mixing in the kidney. The plasma decline is nearly double the rate for heavy water and four times that for sodium. Attempts to explain this terrific rate seem to point more to an overall capillary phenomenon than to rapid extraction of ^{42}K by specific organs (kidney, liver), which exhibit more rapid uptake. Total evisceration of the rabbit, including kidney removal, does not reduce the initial plasma disappearance rate to the order of magnitude shown by sodium, considering the slower mobility of sodium ion.

PLANT PHYSIOLOGY

G. R. Noggle (Leader)

J. S. Bangson* M. Eleanor Schumacher
L. P. Zill J. H. Taylor†

*The estimation of nucleic-acid content of anthers of *Lilium longiflorum*.* (Taylor, Noggle, Schumacher) Since the interpretation of data on nucleic acids requires an exact knowledge of the growth and development of anthers, considerable time was spent in collecting this necessary information. Fig. 13 summarizes a large part of these data. Daily increase in length of about

* Research Participant

† Consultant

GROWTH CURVE FOR LILIUM ANTERS

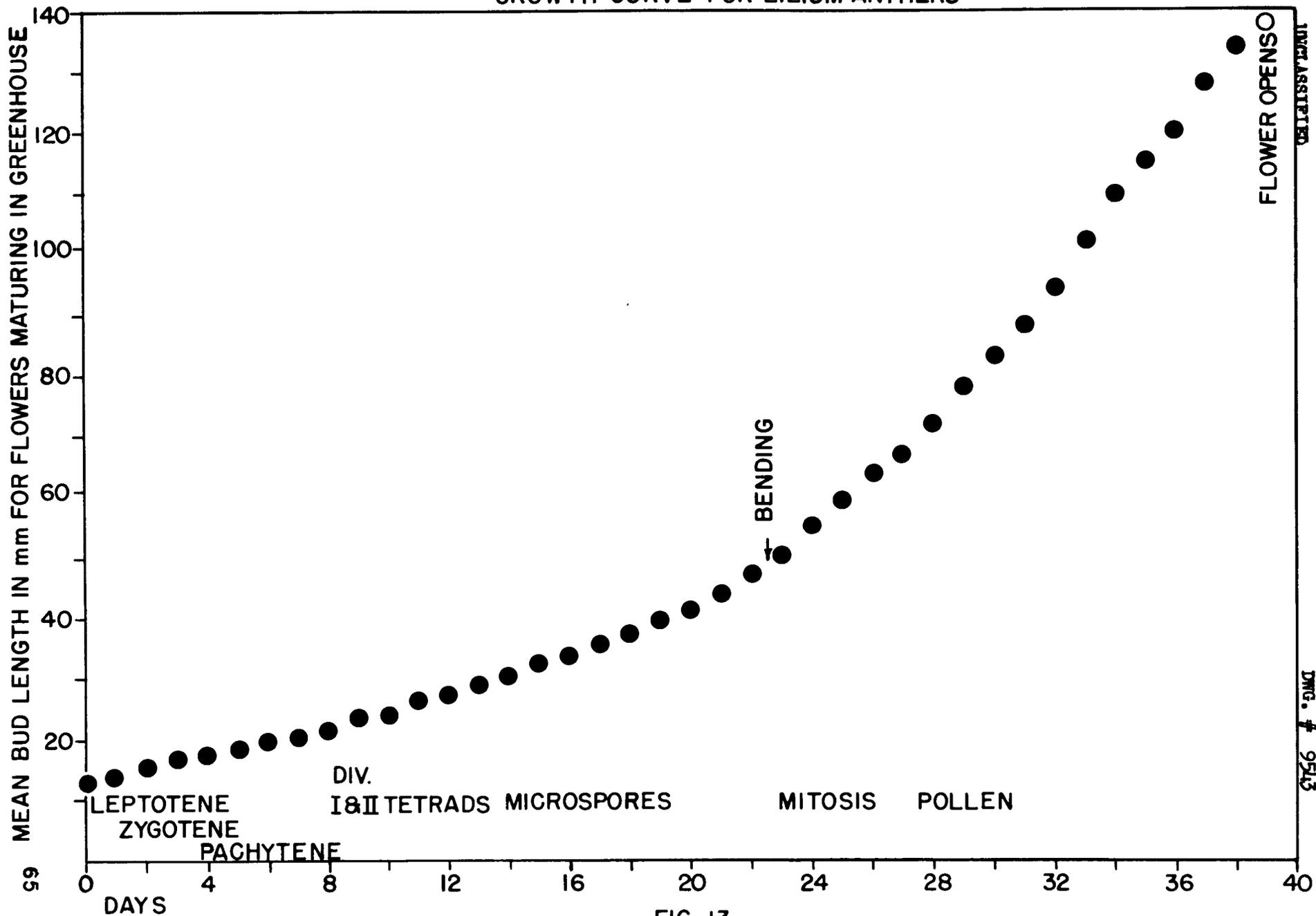


FIG. 13

forty buds was measured from the time buds could be reached among the terminal leaves until opening of the flowers. Other buds were taken from the plant at various stages in development and measurements made of these as well as the individual anthers. One anther was examined cytologically to determine the stage of development. The remaining five anthers were analyzed for nucleic acid content immediately, or frozen for later analysis. Previous examination of all six anthers of buds had shown a high degree of synchronization among the individual anthers during development; therefore, the examination of one is a good measure of the stage of development of the remainder. Fig. 13 gives the growth curves for buds with the sequence and duration of the various stages shown as determined by the correlation of these data. It is worthy of note that the increase in length is almost linear from the early stages of meiosis until mitosis occurs in the microspores. There is an increase at this point which is also correlated with the bending of the buds from an upright to a horizontal position. The elongation soon becomes nearly constant again, but proceeds at a faster rate than in early development. When the data are plotted on semilogarithmic paper (Fig. 14) it is noted that the actual inflection in the growth curve of the bud occurs several days before bending of the pedicel.

Nucleic acids were extracted by the method of Ogur and Rosen (Arch. Biochem., 25:262, 1950). This procedure involves the extraction of the ribonucleic acid (RNA) in cold (4° C) 1.0 N perchloric acid after most of the soluble substances that would interfere with estimation of the nucleic acid by ultraviolet absorption has been removed. After the RNA is removed the deoxyribonucleic acid (DNA) is extracted with hot (70° C) 0.5 N perchloric acid. That such extracts do not contain more substances absorbing in the range measured than nucleic acids prepared by other methods is indicated by a comparison of the ultraviolet absorption of these extracts and certain other samples of DNA and RNA. Figs. 15 and 16 show such a comparison for one sample of each.

The first extracts were removed with cold 10 per cent perchloric acid for RNA and hot 5 per cent perchloric acid for DNA. Previous to the extraction of the RNA the acid-soluble compounds were removed by a rapid extraction with cold 2 per cent perchloric acid. It was found that this extract showed an absorption peak at 260 $m\mu$, which indicates that some RNA was probably being

GROWTH CURVE FOR LILIUM ANTERS

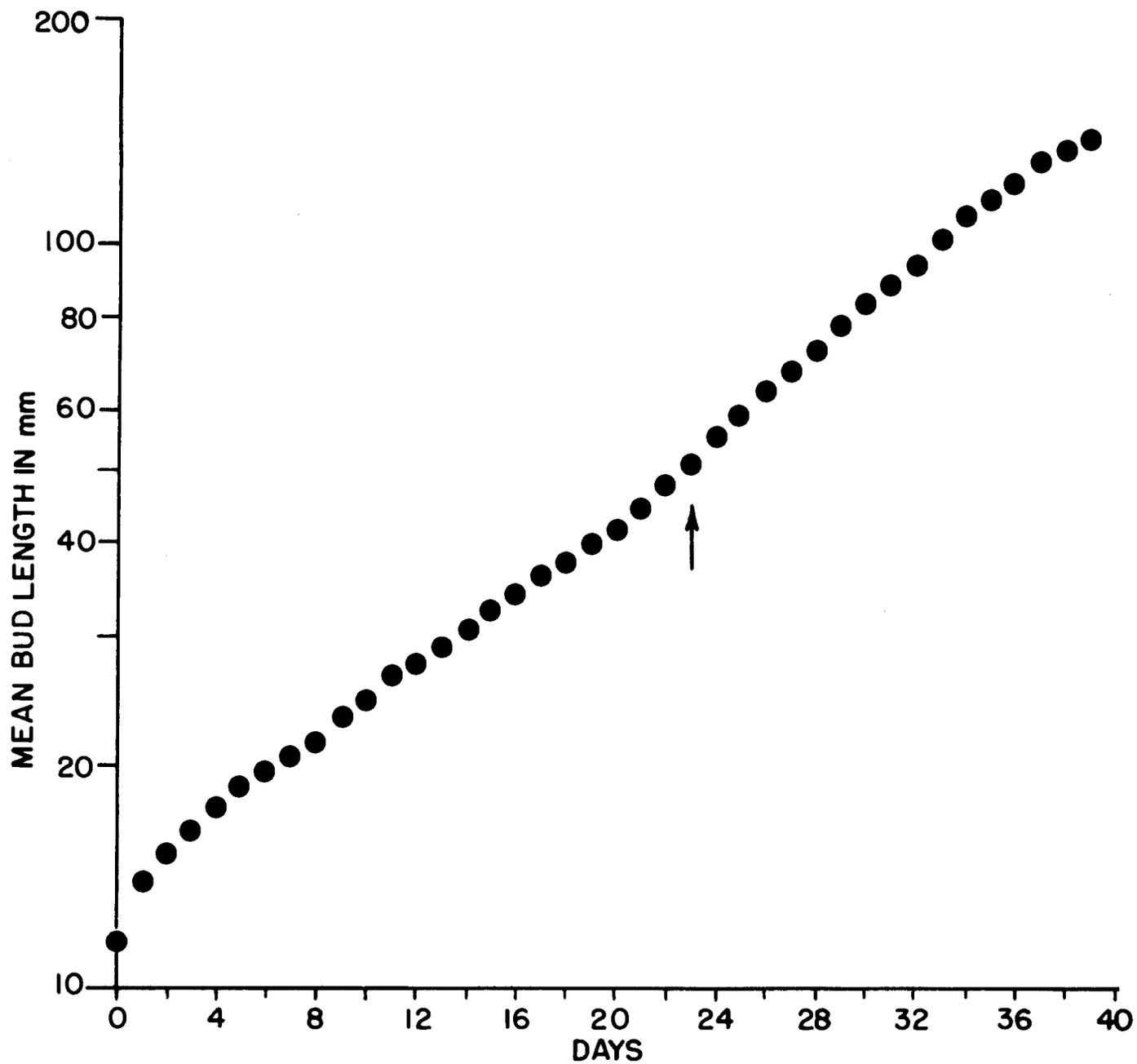


FIG. 14

UNCLASSIFIED
 COMPARISON OF ABSORPTION CURVES OF APPROXIMATELY EQUAL
 SAMPLES OF DNA

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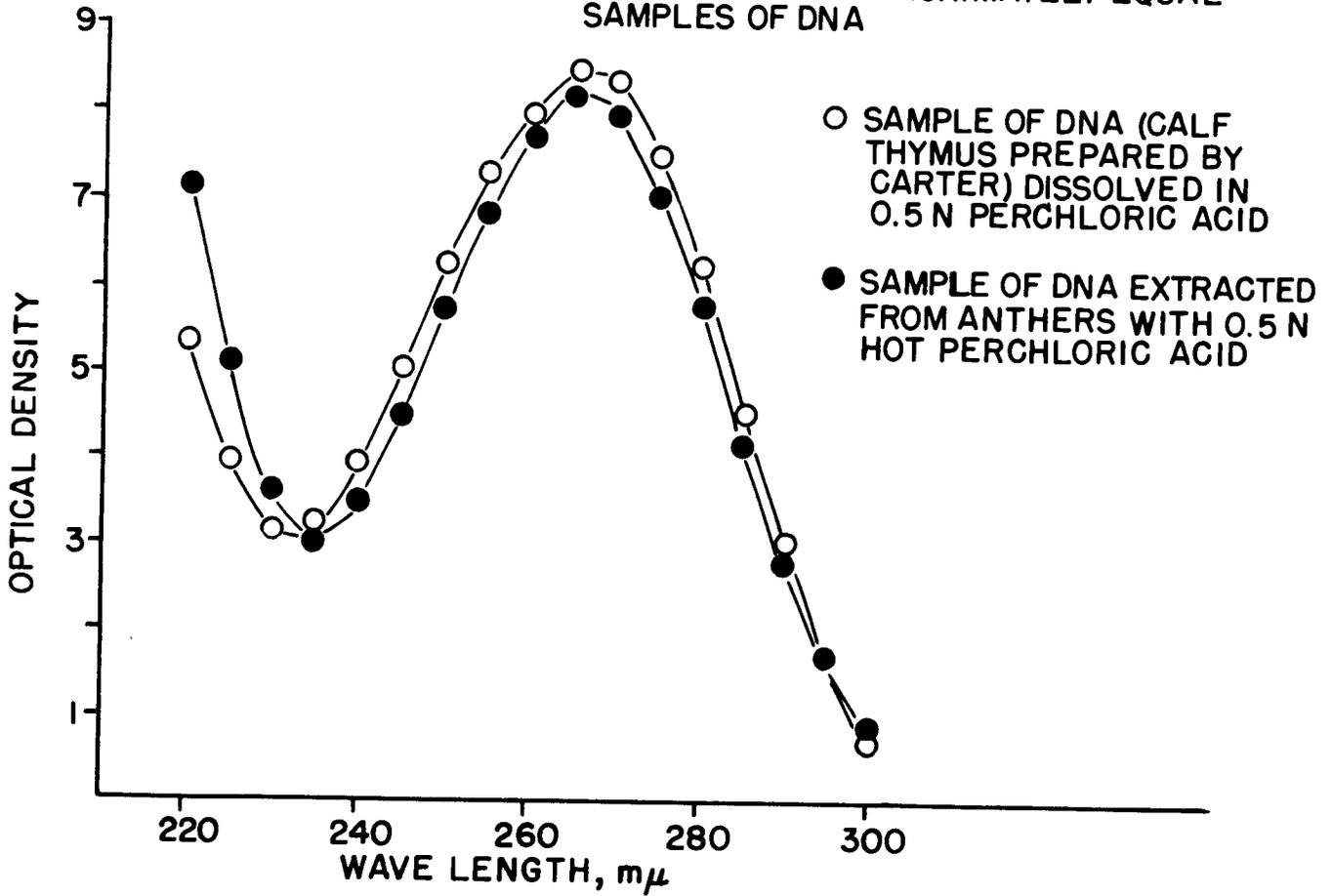


FIG. 15

COMPARISON OF ABSORPTION CURVES OF APPROXIMATELY EQUAL
 SAMPLES OF RNA

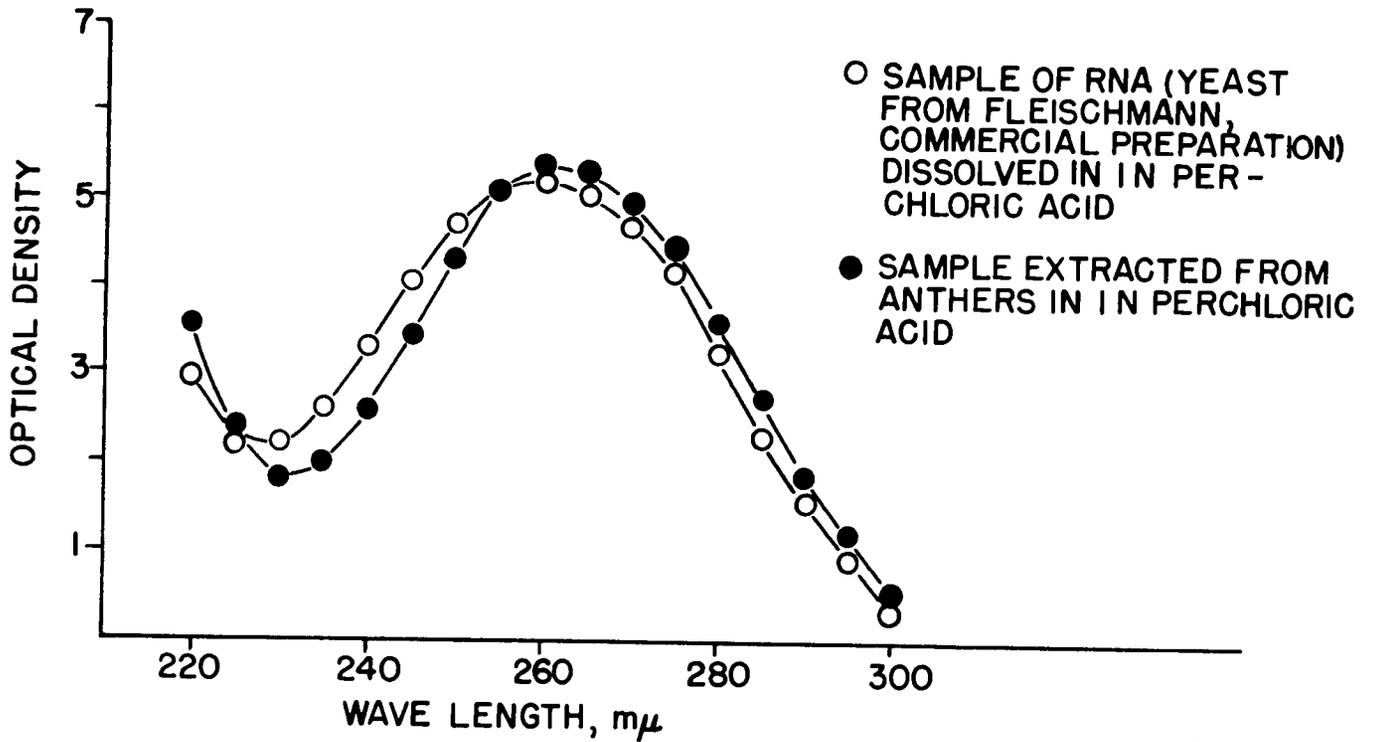


FIG. 16

lost. Later extractions were made using 0.1 N (0.59 per cent) perchloric acid for the acid soluble compounds and concentrations of 1.0 N and 0.5 N for extraction of RNA and DNA, respectively. No peak at 260 $m\mu$ appears in the first extract and higher yields of RNA were obtained. Fig. 17 shows the DNA and RNA content of anthers based on the extraction with 5 and 10 per cent perchloric acid. Fig. 18 shows the results of extraction with the lower concentrations. It will be noted that the curves are similar for the two methods even if the absolute amounts are lower by the first procedure. The absolute amount of DNA is based on a standard calf thymus DNA (obtained from C. E. Carter) which has been highly purified. The standard used for RNA was a commercial sample of RNA from yeast prepared by the Fleischmann Laboratories. Fig. 19 shows the estimation of the desoxyribose content of the DNA extracts by the diphenylamine reaction of Dishe (Mikrochemie, 8:4, 1930) as modified by Seibert (J. Biol. Chem., 133:593, 1940). It will be noted that rather close agreement is obtained by the two methods. However, the absolute amount indicated in the extracts was 20 per cent higher by the diphenylamine test than by ultraviolet absorption measurements with the standard DNA sample used. Corrections for this were made before plotting the data in Fig. 19.

The data thus far obtained is rather surprising and interesting. The increase in nucleic-acid content does not follow the growth curve, although it must be admitted that the increase in length (or volume) is not the same as the increase in dry weight. Insufficient data are now available for a complete comparison with increase in dry weight of the anthers. There is a rapid rise in DNA content during meiotic prophase up to diplotene. This rise falls off during the divisions of the microspore mother cells. There may be a slight peak at metaphase I, but this is still questionable. Perhaps one of the difficulties in determining this is the low frequency of cases in which high proportions of metaphases are obtained in several anthers. The small drop following meiosis and tetrad formation may not be significant, but due only to experimental error. The rise that follows during early stages of microspore development is striking. Following this period a sharp decline is indicated. This may be correlated with the disappearance of the tapetal tissue which occurs about this time. Another rise is shown about the time of mitosis in the microspores, with a sharp decline as the pollen matures.

The change in RNA follows that of DNA but the peaks of the curve are less sharp. A few tests on cultured anthers indicate a decrease in DNA synthesis

NUCLEIC ACID CONTENT OF LILIUM ANTHERS DURING DEVELOPMENT

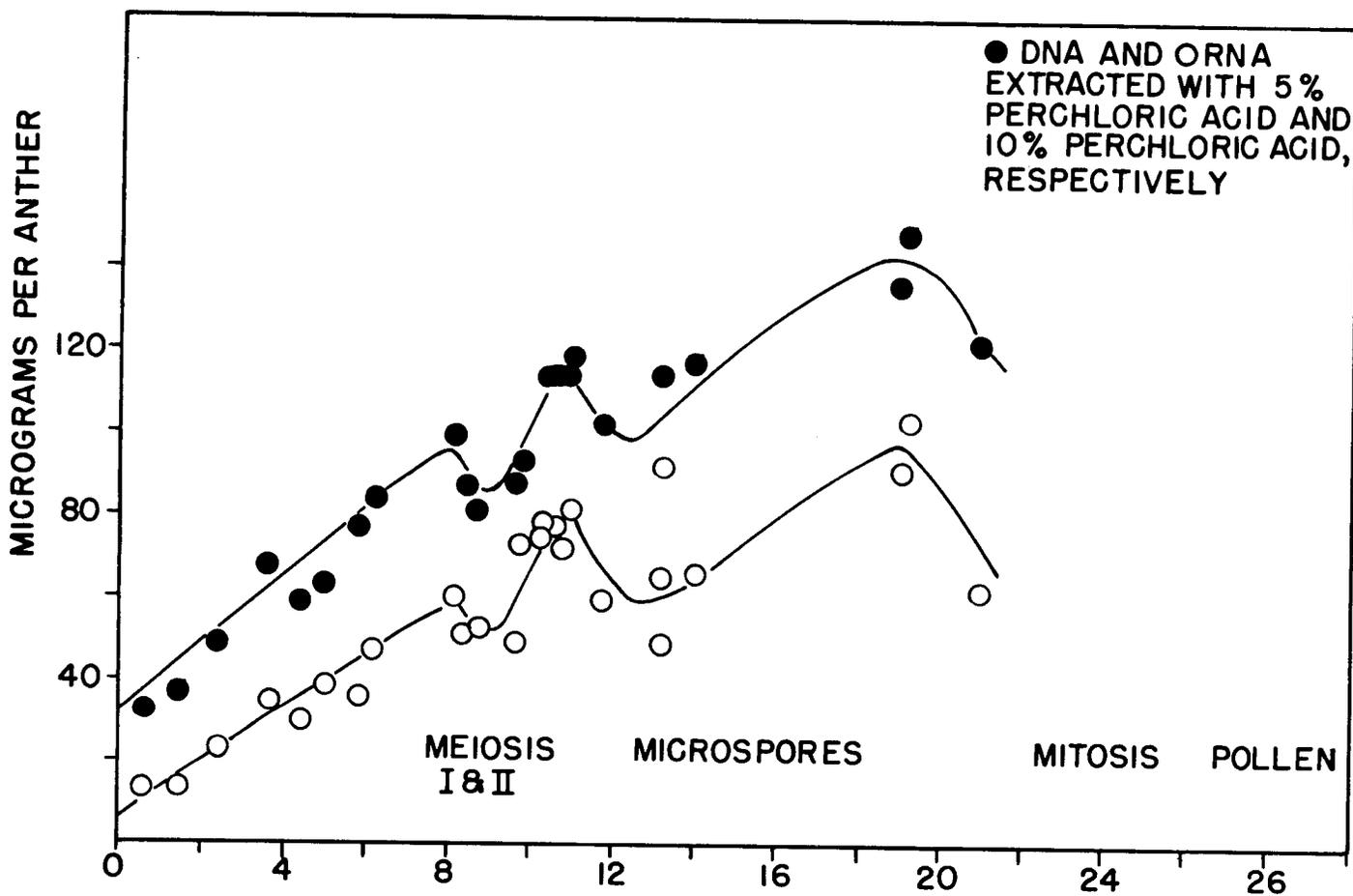


FIG. 17

NUCLEIC ACID CONTENT OF LILIUM ANTERS DURING DEVELOPMENT

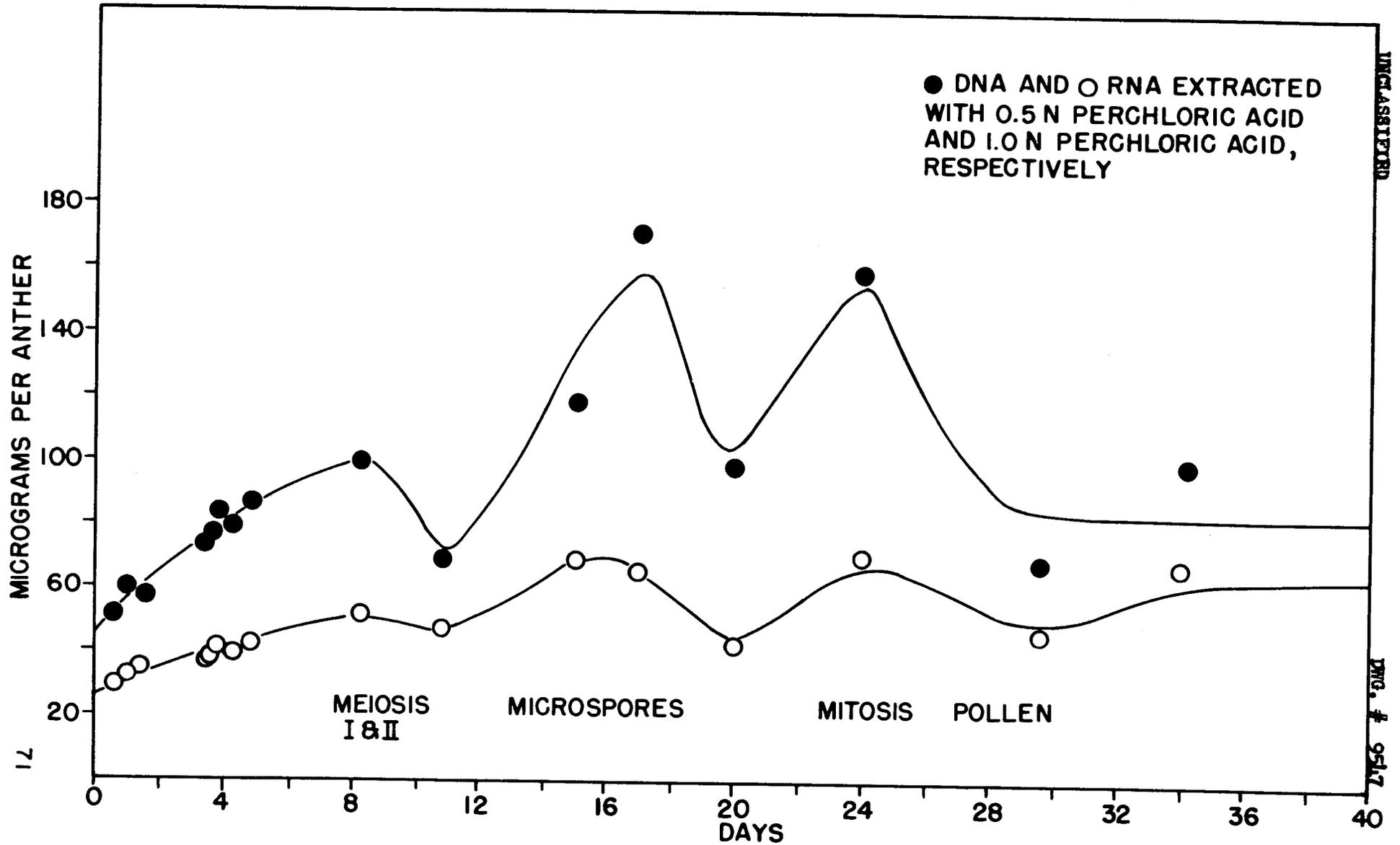


FIG. 18

NUCLEIC ACID CONTENT OF LILIUM ANTERS DURING DEVELOPMENT

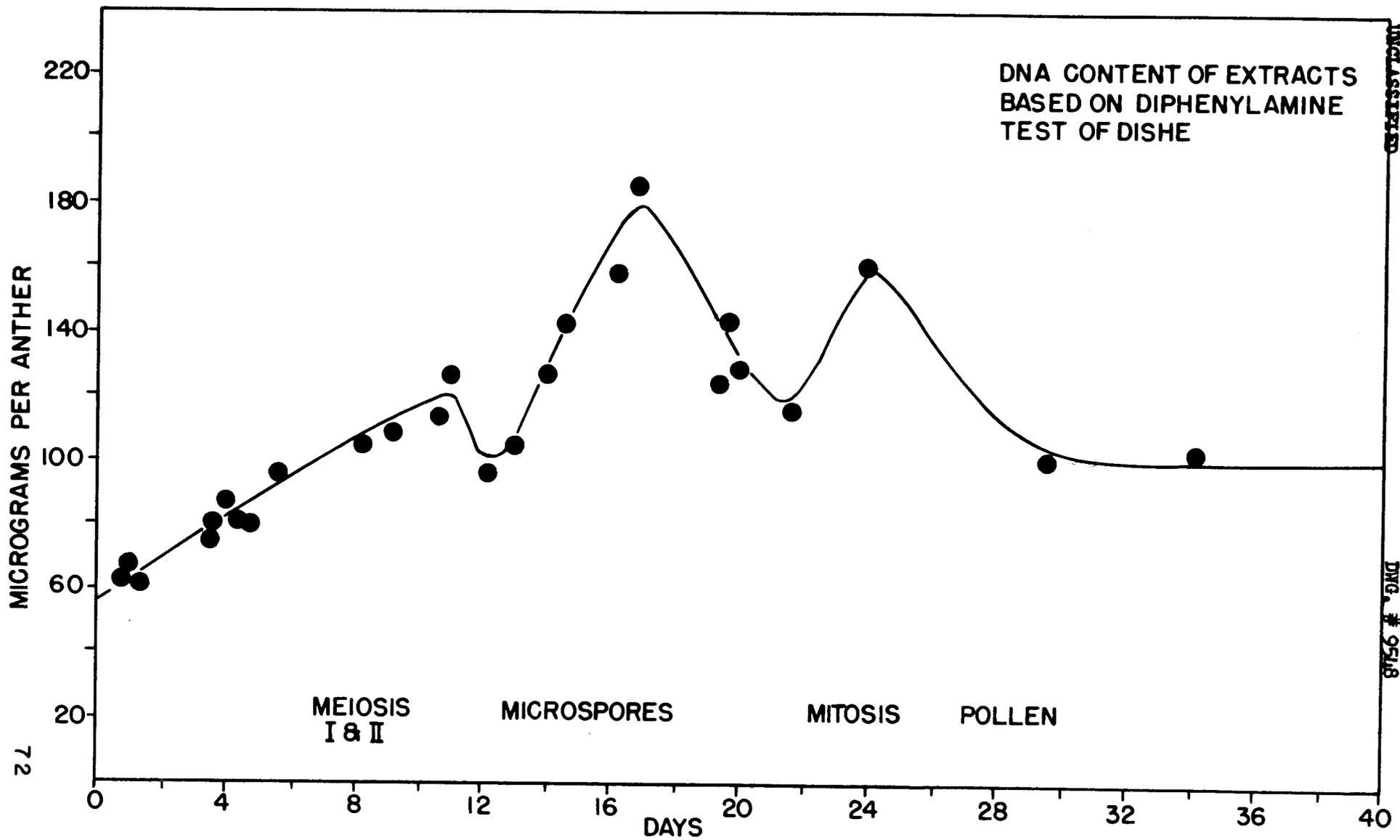


FIG. 19

72

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in culture. RNA had almost completely disappeared after 7 days in culture. This apparently occurs even when meiotic divisions continue. The relation of the lower DNA content to the changes in chromosome coiling and elongation in cultured material is a subject that should be investigated.

Lilium provides excellent material for the study of DNA content, synthesis, and turnover, since so many synchronized divisions and cells with such a high DNA content are rarely found. It should be possible to find out whether there is an increase in DNA content of a chromosome or nucleus during the division cycle by additional determinations such as have been made, and by paper-chromatographic separation of the nucleotides from the various extracts as a further check. When fresh anthers are available the cell number per anther should be determined for the purpose of estimating the DNA and RNA per cell. Comparison of the diploid and tetraploid forms of *Lilium*, should answer the questions of the quantitative relationship between chromosome number and DNA content, and whether or not there is a definite amount of DNA characteristic of a chromosome or a nucleus.

Studies are being set up on the rate of turnover of nucleic acids by the use of tracers, perhaps with phosphorus. The use of anther cultures may prove helpful in such studies.

The growth of peanuts following irradiation with X and gamma rays. (Noggle, Bangson; and Darden of Radiobiochemistry) During the 1949 season a series of peanut seeds were irradiated with X and gamma rays in this laboratory. The seeds were treated with the following levels of radiation: 2500, 5000, 10,000, 20,000, and 40,000 r. These seeds were sent to the Agronomy Department of the North Carolina Agricultural Experiment Station, Raleigh, North Carolina, where they were grown in the greenhouse. Their data indicated that the gamma rays were about twice as effective as X rays in decreasing the growth of the plants. Observations on this experiment were considered interesting enough to warrant further investigation during the 1950 season.

Seeds of the same variety (NC4) used last year were obtained from the Agronomy Department of North Carolina State. Dose levels were carefully worked out on the new X-ray machine and on the gamma source. After irradiation, the seeds were planted in the greenhouse in a split-block design with eight replications.

Fig. 20 shows a portion of the plants growing in sand in the greenhouse. The picture was taken 21 days after planting. Four rows are shown for each treatment — the two left-hand rows in each treatment were irradiated with gamma rays while the other two rows were X-irradiated. There appears to be no striking difference between the two treatments. Fig. 21 gives the data for the percentage of germination of the seeds 17 days after planting. At the higher dose levels there is a difference in the effectiveness of the two treatments. This difference may be related to the dose-intensity factor since a longer time was required for a comparable dose of gamma rays than of X rays. Table 8 gives the time of irradiation of the various seed treatments. This aspect of the problem is to undergo further investigation.

TABLE 8

DOSE	TREATMENT	TIME
2500 r	X rays	26.5 min
	γ rays	2 hr, 44 min
5000 r	X rays	50 min
	γ rays	5 hr, 18 min
10,000 r	X rays	100 min
	γ rays	10 hr, 32 min
20,000 r	X rays	3 hr, 2 min
	γ rays	20 hr, 52 min
40,000 r	X rays	6 hr, 2 min
	γ rays	41 hr, 54 min

A separate series of treated seeds were planted for investigation of the number of lateral roots. These roots were counted on the plants and the data are shown in Fig. 22.



FIG. 20
75

PERCENTAGE OF GERMINATION SEVENTEEN DAYS AFTER PLANTING

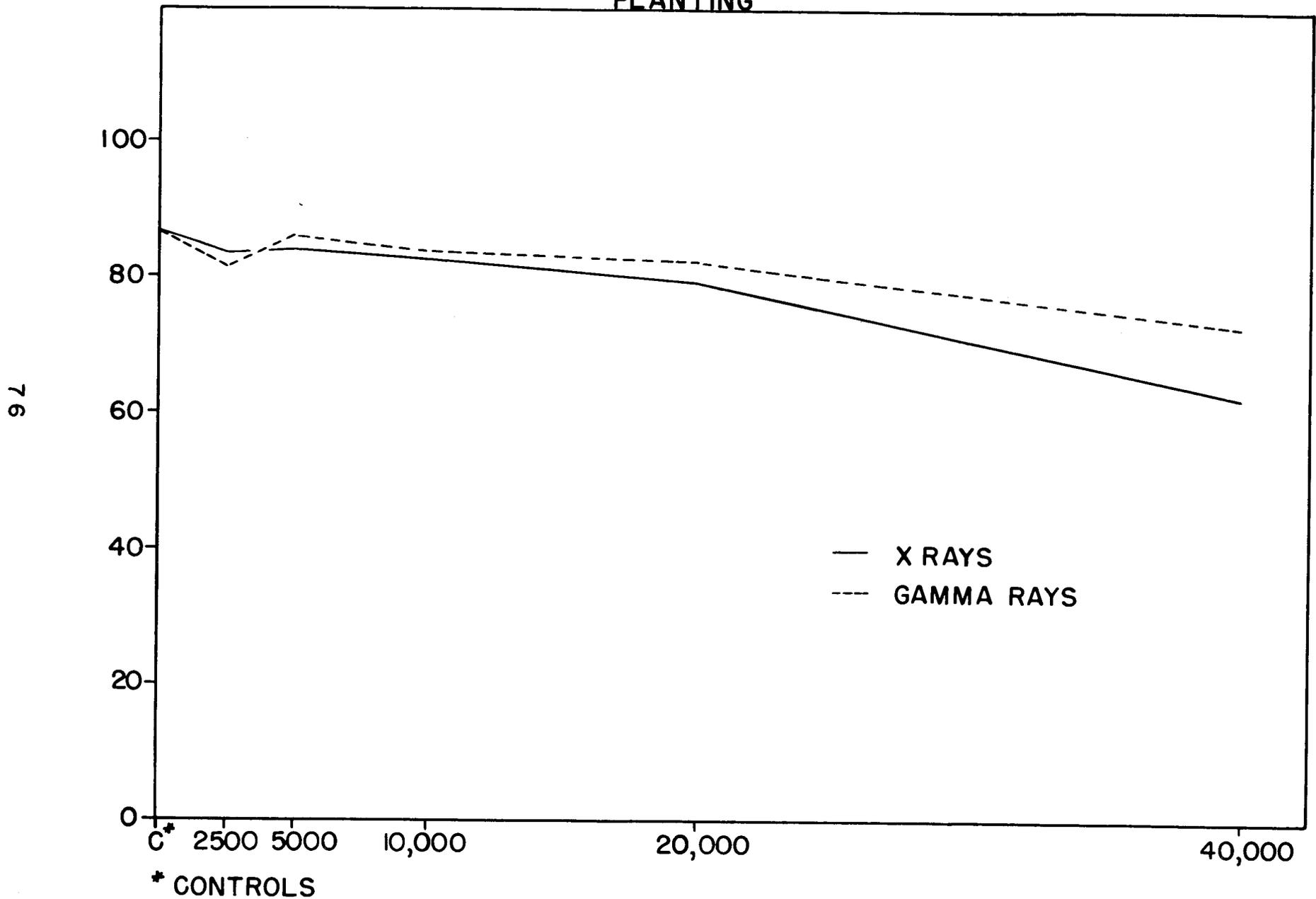


FIG. 21

NUMBER OF LATERAL ROOTS
SEVEN DAYS AFTER PLANTING

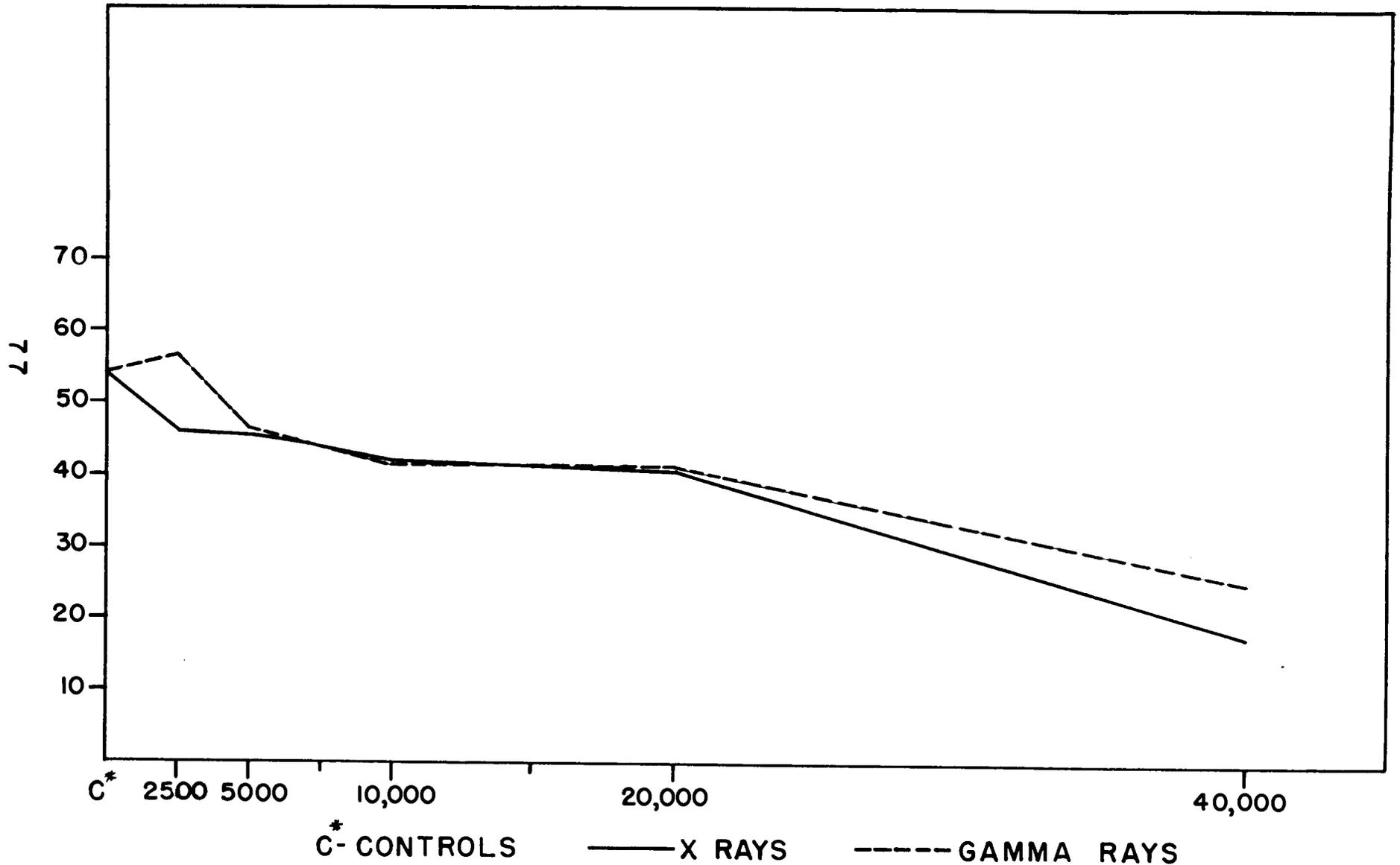


FIG. 22

Additional data have been taken on the major peanut experiment. The length of the main stem, the number of expanded leaves on the main axis and on the cotyledonary axis have been determined. The data are now being analyzed and will be reported later. A record is also being kept of the dates of appearance of flowers on the variously treated plants.

BIOPHYSICS

W. A. Arnold (Leader) Jane T. Thompson

Photosynthesis. (Arnold, Thompson) In collaboration with E. W. Burdette and J. B. Davidson of the Instrument Department we have constructed and tested an automatic Warburg apparatus. The device uses a Statham "pressure transmitter" to measure the pressure difference between the experimental vessel and an empty one-liter bottle immersed in the thermostat. The pressure differences are recorded graphically once each second by a Brown potentiometer. The details of construction and the circuits used will be given in a report that is now being written on this instrument.

This automatic Warburg apparatus has been used to investigate the question of whether or not there is any photoreactivation of the ultraviolet killing of photosynthesis. Previous work has shown that when a suspension of *Chlorella* cells is exposed to ultraviolet light, both the rate of photosynthesis and the rate of respiration are reduced approximately exponentially with the ultraviolet dose, but that the reduction of photosynthesis is a number of times faster than the reduction of respiration.

The curve (Fig. 23) shown gives the results for an experiment made in the following way: 10 cmm of *Chlorella* cells was suspended in 2 cc of carbonate buffer in one of the small quartz vessels. The apparatus was started and the cells kept in darkness until a constant rate of respiration had been attained. Then the cells were illuminated with neon light filtered through red cellophane. After a constant rate of oxygen production had been maintained for some time the ultraviolet lamp was started, both the neon and the ultraviolet light remaining on for the rest of the experiment. As can be seen from the curve, the rate of gas production begins to slacken, then stops and reverses. Thus after some time only respiration remains, all the photosynthesis having been killed by the ultraviolet light.

We interpret the experiment in the following way:

Let

P_0 = the rate of photosynthesis before the ultraviolet light treatment

P = the rate of photosynthesis after we have given t minutes of ultraviolet light.

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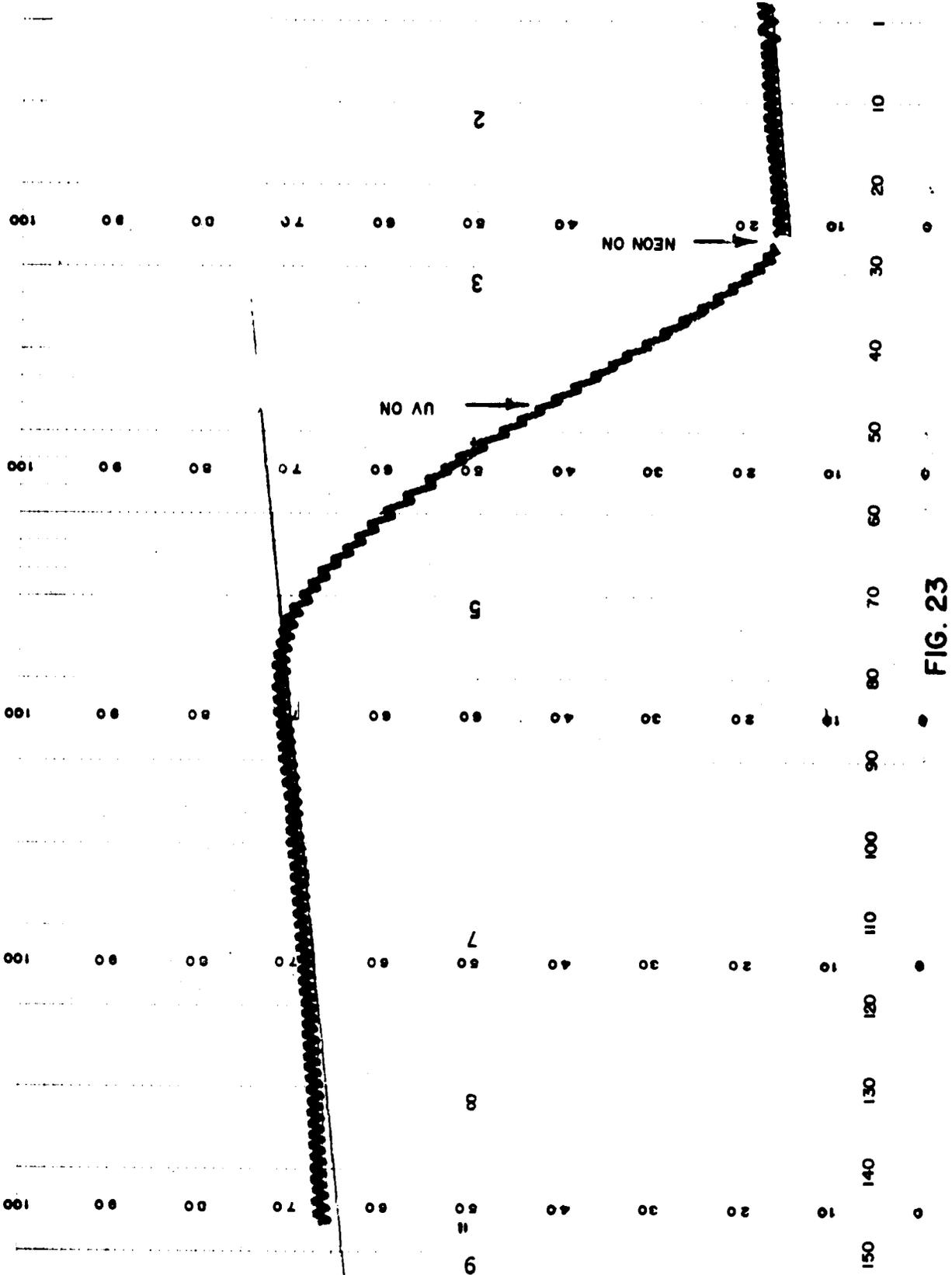


FIG. 23

We have from previous work the relation

$$P = P_0 e^{-\alpha t}$$

where α is a measure of the ultraviolet damage done to the cells.

Let

R = the rate of respiration.

While it is true that the rate of respiration is also changed by the ultraviolet light and probably by the photosynthesis itself we will consider it constant for this simple interpretation of these experiments.

Let

Q = the rate of oxygen production at any time.

Thus

$$Q = P_0 e^{-\alpha t} - R.$$

Let

G = the total gas production after the ultraviolet light has been started.

$$G = \int_0^t Q dt = \int_0^t (P_0 e^{-\alpha t} - R) dt$$

$$G = \frac{P_0}{\alpha} (1 - e^{-\alpha t}) - Rt$$

For times long enough to essentially stop photosynthesis, we have

$$G = \frac{P_0}{\alpha} - Rt.$$

If we now extrapolate back to the time $t = 0$, that is, to the time at which the ultraviolet light was started, we have a measure of $\frac{P_0}{\alpha}$. (This extrapolation is shown by the line drawn on the curve.) Since we have a measure of P_0 from the part of the curve made before the ultraviolet treatment, we have only to divide to obtain α .

The same experiment was repeated with a high-intensity projection lantern illuminating the cells in addition to the red neon light (this is actually the experiment shown in the graph). Tables 9 and 10 give the values of α for two different intensities of ultraviolet light, with or without this possible photoreactivating white light. As can be seen from the table, the white light seems to have no effect on the ultraviolet inactivation of photosynthesis.

TABLE 9

	NEON WITH RED FILTER	NEON WITH PROJECTION LAMP
$\frac{P_0}{\alpha}$ Taken from curve by extrapolation	31.5 Div	33.3 Div
P_0 Rate of photosynthesis corrected for respiration	1.45 $\frac{\text{Div}}{\text{Min}}$	1.55 $\frac{\text{Div}}{\text{Min}}$
$\alpha = \frac{P_0}{P_0/\alpha}$.046 Min^{-1}	.0465 Min^{-1}

TABLE 10

	NEON WITH RED FILTER	NEON WITH PROJECTION LAMP
$\frac{P_0}{\alpha}$ Taken from curve by extrapolation	65.2	69.5
P_0 Rate of photosynthesis corrected for respiration	1.85	2.10
$\alpha = \frac{P_0}{P_0/\alpha}$.0284	.030

PATHOLOGY AND PHYSIOLOGY

Jacob Furth (Leader)	Emma Jane Beale
J. B. Kahn	A. W. Burke
R. H. Storey	Mary M. Knoohuizen
R. V. Talmage*	Peggy Ledford
J. J. Lane	Virginia Benefiel †
W. D. Gude	Germaine Click

Exposure of mice to slow neutrons. (Ophthalmology by Dr. Christenberry, hematology in association with Dr. Andrews, assisted by Lane and Gude) This long-term project, outlined in an earlier report, is being continued. Animals of one set are being killed at various intervals following exposure to the LD-50 or fractions thereof, while animals of another set are observed until natural death. The building up of this series is slow, for several reasons. Only four animals can be exposed at one time in the biological tunnel of the reactor, and 80 minutes are required for the animal in the most exposed position to receive an LD-50.

Of the significant findings thus far, that of the leukemia incidence is worth mentioning. Table 11 lists the number and types of leukemias thus far observed in relation to irradiation dose. The literature stresses the induction of thymic lymphomas and neglects the induction of other types of leukemias. It deserves emphasis that several of the leukemias found were myeloid, as indicated in Table 11, and several of the lymphoid leukemias were not thymic.

TABLE 11

*Preliminary survey of mice exposed to slow neutrons
and X rays before January 1, 1950*

	THERMAL NEUTRONS				X RAYS			CONTROLS
	(Minutes Exposure)				(Minutes Exposure)			
	80 ^a	20-40	5-10	2-5	80 ^b	20-40	5-10	
No. in group	98	92	63	16	19	46	26	110
Leukemias:								
lymphoid	11	6	2	0	5	2	1	1
myeloid ^c	3	5	1	0	1	3	1	0
all	14	11	3	0	6	5	2	1
Per cent	14.3	11.9	4.8	0	38	10.9	7.7	0.9

^a Thermal neutron flux $.9 \times 10^9$ n/cm²/sec with high energy gamma contaminants 6-8 r per minute.

^b 6.4 r per minute.

^c Gross diagnosis in most cases; being checked in sections.

* Research Participant

† Visiting assistant

The number of mice in these groups is small and as most mice of these series are alive the final values will be higher. However, the trend is evident and conforms with earlier observations indicating a direct relation between irradiation dose and leukemia incidence.

Only a few cases of other neoplasms have been observed thus far; it is known from previous studies that the induction of lung, ovarian, and other tumors has a much longer latency period.

A systematic ophthalmological examination of the eyes of many mice is being continued. It seems that opacities of the lens set in sooner after exposure and after much smaller doses than has hitherto been supposed. In normal mice, 9-12 months old, the nucleus and posterior capsule frequently show some increased density but opacities (cataracts) are seldom seen. Those occasionally seen are in the center of nucleus. In mice of the same age exposed to neutrons or X rays, postsubcapsular opacities of the coralliform type (posterior subcapsular opacities with radiations) are common. Frequently there are vacuoles at the anterior capsule.

Changes in cell and plasma volumes produced by total body X irradiation. (Kahn, Storey, Beale; and Wish of Radiobiochemistry) In extension of the work recently published (Proc. Soc. Exp. Biol. & Med., 74:242-244, 1950) the drop in red-cell volume following irradiation is being further analyzed. It seemed essential to "build up" mice and rabbits with erythrocytes tagged with radio-iron. The results thus far suggest that erythrocytes are not made fragile by irradiation *in vivo* with the LD-50 doses and that loss of erythrocytes in irradiated hosts is negligible. Since erythropoiesis ceases for about 15 days after irradiation in rabbits and 10 days in mice, the drop in red cells during this period is due mainly to aging of erythrocytes. Whether this alone accounts for the radiation anemia (and red-cell volume drop) cannot be answered until data become available on the longevity of red cells of rabbits and mice. To our knowledge no such data are recorded for either rabbits or mice. The permeability problem is being quantitatively studied by Dr. Bigelow.

Effect of X rays on plasma proteins. (Storey) Muntz, Barron and Prosser (Arch. Biochem., 23:434-445, 1949) reported on changes in the electrophoretic pattern of the plasma of dogs after exposure to lethal doses of X rays.

Our work with rabbits (Proc. Soc. Exp. Biol. & Med., 74:242-244, 1950) has shown a plasma-volume drop until about the tenth day after irradiation when the plasma volume begins to rise to above normal levels. It seemed desirable to determine the behavior of various protein components in relation to time after exposure, dose of X rays, and changes in plasma volumes.

The electrophoretic pattern of the plasma of rabbits exposed to an approximate LD-50 dose (800 - 1000 r) is being studied at various times after exposure using a Perkin-Elmer Model 38 Tiselius apparatus with a 2-cc capacity cell and a veronal-citrate buffer, pH 8.6.

The + 1-, 5- and 7-day plasma patterns seem to show increases of the globulin components, the most notable being the appearance of the α globulin component.

The + 11-day plasma had a profound rise in globulin fractions and reduction in albumin fraction similar to the terminal changes observed by Muntz, et al. in dogs. These studies are being continued.

Determination of plasma, cell and organ-blood volumes with isotope technics in normal and tumor-bearing mice. (Knoohuizen, Storey, Kahn) Marked hypervolemia with congestive changes in liver, spleen, and adrenal are encountered only in mice with granulosa tumors. A slight hypervolemia, with slight or no congestive changes, has been encountered with breast tumors. Other neoplasms studied had neither of these changes. The purpose of the study of organ-blood volumes was to determine, if possible, the primary site of the albumin production and that of blood storage. The most interesting (unexpected) finding in the course of this work is the low blood volume of the tumors examined. Since sections of these tumors frequently show cavernous vessels the working hypothesis is made that the actual volume of the blood in tumors may be greater than the values obtained after the usual 6-minute mixing time would indicate. In any event the tumors appear anoxic - an interesting observation in view of the well known predominance of relatively anaerobic metabolism of tumors.

On the nature of hypervolemia - effect of injection of large quantities of homologous albumin. (Storey, Beal) Since the postirradiation state is characterized by hypoalbuminemia and the endocrine hypervolemia studied in this laboratory is characterized by an albumin rise, it seemed desirable to

determine the effect of administration of very large quantities of homologous albumin on the blood volume.

Rabbit albumin (Cohn Fraction V — prepared for us by Armour and Company, through the courtesy of Drs. Lesh and Seidel) was injected intravenously into a rabbit in quantities calculated to triple the plasma volume. Two sets of injections of 4.5 g albumin each were given over a period of 7 days with a 3-day interval between the two series.

After completion of the first series of injections the plasma volume showed an 11.5 per cent increase. One day after completion of the second series of albumin injections the plasma volume was 58 per cent above, and 5 days later it was still 20 per cent above the preinjection value.

The changes in the globulin concentration are worthy of special note. On the tenth day of the experiment, the total circulating globulin was 82 per cent (in grams per 100 cc), 40 per cent above the preinjection value.

The albumin concentration, expressed as grams per 100 cc, decreased throughout the experiment. Its lowest value was reached on the fifth day when it was 23 per cent below the preinjection value and the total circulating albumin was 16 per cent. On the eighth day the total circulating albumin was 25 per cent above the preinjection value.

Electrophoretic patterns were studied during the course of the experiment. There was a globulin rise with appearance of an α globulin peak and, in addition, an increase in the other globulins. On the thirteenth day of the experiment the electrophoretic picture bears a remarkable resemblance to that of an irradiated animal.

The hematocrit and cell volume (estimated on the basis of T-1824 measurements) dropped by 60 per cent on the eighth day after which they began to rise, not reaching the preinjection value by the thirteenth day.

This experiment is reported in detail because of the remarkable finding of a rise in globulin following injection of large quantities of homologous albumin. Since rabbit albumin is not available, repetition of this experiment will be delayed.

Relation of hypervolemia to sex hormones. (Kahn, Burke, Knoohuizen, Bigelow, consultant) The presence of a marked cavernous dilatation of blood

vessels of viscera of mice with nonestrogen-secreting carcinomas confirm earlier observations that estrogen and plethorin production do not go hand in hand. Furthermore, testosterone was administered to nine ovariectomized granulosa tumor-bearing mice. Up to 5 mg of testosterone per day failed to abolish the continued estrus except in one mouse, but even in this case a hypervolemic change did not seem to have been effected (15-20 μ g of testosterone was found to neutralize the daily estrus-maintaining dose of 0.05 μ g of estradiol in the ovariectomized nontumor-bearing mouse).

Conversely, large quantities of estradiol were given to five mice bearing luteomas. About 1,000 rat units of estradiol administered daily would bring a mouse bearing a medium sized luteoma into continued estrus. This dose failed, however, to produce the characteristic congestive changes.

It is noteworthy that the growth of luteomas and granulosa tumors was not arrested by the administration of large quantities of the antagonistic hormone.

Adrenalectomy failed to prevent the development of hypervolemia in the two tumor-bearing mice tested.

These observations indicate that congestive changes are not specific to granulosa tumors. They are seldom encountered and, if present, they are so only to a mild degree with other tumors; with granulosa tumors, congestive changes are marked and constant. The nature of plethorin, its site of production, and its mode of action remain to be determined.

The hypervolemia of arteriovenous aneurysm seems to differ from endocrine hypervolemia in that, unlike the latter, it goes with enlargement of the heart, heart failure, and globulin rise.

Transmissible splenomegaly with anemia and leukopenia. (Knoohuizen, Gude) In the normal stock used for the neutron studies one mouse developed a splenomegaly (without enlargement of lymph nodes) with marked erythroblastic anemia and leukopenia. After injection of splenic tissue into a group of normal mice of this strain, several developed a similar disease after a latency period of 4 months. Splenic fragments from these mice were again injected into normal mice. Several of the recipients again developed splenomegaly after a latency period of about 4 months. A few animals of the second subtransfer generation are being currently studied. The section of these large

spleens show marked erythroblastic metaplasia, and the bone marrow thus far studied shows erythroblastic hyperplasia and erythrophagocytosis. This puzzling disease is either one of erythroblastosis or an infection of unknown etiology causing splenomegaly with "hypersplenism." Attempts are being made to keep the strain going since (1) it would give us an opportunity to study the hitherto poorly understood "hypersplenism" and (2) because of the recently discovered role of the spleen in irradiation anemia.