

ORNL 889
Series A 9
Progress Report

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES



3 4456 0352695 9

BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT FOR
PERIOD ENDING NOVEMBER 10, 1950

Classification Cancelled

Or Changed To _____

By Authority Of A.E. 9.13.71

By C. Goldberg Date 11.5.71



OAK RIDGE NATIONAL LABORATORY

CENTRAL RESEARCH LIBRARY

CIRCULATION SECTION

4500N ROOM 175

LIBRARY LOAN COPY

DO NOT TRANSFER TO ANOTHER PERSON

If you wish someone else to see this report, send in name with report and the library will arrange a loan.

UCN-7969 (3 9-77)

OAK RIDGE NATIONAL LABORATORY
OPERATED BY
CARBIDE AND CARBON CHEMICALS DIVISION
UNION CARBIDE AND CARBON CORPORATION



POST OFFICE BOX P
OAK RIDGE, TENNESSEE

CONFIRMED UNCLASSIFIED

David Hamrin ORNL CO 7/17/2013
Date

ORNL-889
Progress Report

This document consists of 64 pages.
Copy 9 of 116 Series A.

Contract No. W-7405-Eng-26

BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT
for Period Ending November 10, 1950

Edited

by

Alexander Hollaender

Date Issued: **JAN 15 1951**

OAK RIDGE NATIONAL LABORATORY
operated by
CARBIDE AND CARBON CHEMICALS DIVISION
Union Carbide and Carbon Corporation
Post Office Box P
Oak Ridge, Tennessee



3 4456 0352695 9

Internal Distribution

1	G. T. Felbeck (C&CCD)	25	J. S. Felton	43	W. L. Russell
2-3	706-A Library	26	E. H. Taylor	44	R. F. Kimball
4	706 B Library	27	A. H. Snell	45	G. R. Noggle
5-6	Biology Library	28	K. Z. Morgan	46	Seymour Pomper
7	Health Physics Library	29	F. L. Steahly	47	M. E. Gaulden
8	Metallurgy Library	30	M. T. Kelley	48	C. J. Collins
9-10	Training School Library	31	D. W. Cardwell	49	E. H. Anderson
11-14	Central File	32	W. H. Pennington	50	A. H. Doerman
15	C. E. Center	33	A. S. Householder	51	Jacob Furth
16	C. E. Larson	34	R. S. Livingston	52	Forrest Western
17	W. B. Humes (K-25)	35	C. P. Keim	53	W. E. Cohn
18	W. D. Lavers (Y-2)	36	G. H. Clowett	54	A. W. Kimball
19	A. M. Weinberg	37	C. D. Supano	55	J. Moshman
20	J. A. Swartout	38	W. A. Arnold	56	E. J. Slaughter
21	E. D. Shipley	39	S. F. Carson	57	J. S. Kirby-Smith
22	E. J. Murphy	40	C. E. Carter	58	S. Warren (AEC, Washington)
23	F. C. VonderLage	41	N. H. Giles, Jr.	59	P. B. Pearson (AEC, Washington)
24	A. Hollaender	42	C. W. Sheppard	60	A. G. Kammer (Univ. of Pittsburgh)
				61	Max Zelle (AEC, Washington)

External Distribution

62-69 Argonne National Laboratory
70-72 Atomic Energy Commission, Washington
73-76 Brookhaven National Laboratory
77-78 Du Pont Company
79-82 General Electric Company, Richmond
83-84 General Electric Company, Schenectady
85-87 Los Alamos
88-89 Naval Radiological Defense Laboratory
90-91 New York Operations Office
92 Patent Branch, Washington
93-97 University of California, Radiation Laboratory
98-112 Technical Information Branch, ORE
113 Mound Laboratory
114 UCLA Medical Research Laboratory (Warren)
115 University of Chicago Toxicity Laboratory
116 University of Rochester

TABLE OF CONTENTS

INTRODUCTION	4
PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC	6
Publications	6
Papers Presented at Scientific Societies	10
Visiting Lecturers for Biology Seminars	11
Traveling Seminar Program	11
CYTOGENETICS	12
Cytogenetic Effects of Radiation	12
Effects of Radiation on Rate of Mitosis	19
Effects of Radiation on Microorganisms	25
MAMMALIAN GENETICS	27
Genetic and Developmental Effects of Radiation in Mice	27
PATHOLOGY AND PHYSIOLOGY	29
MICROBIOLOGY	42
Tracer Studies on Metabolism	42
BIOCHEMISTRY	49
Studies on Nucleic Acids, Enzymes, and Energy-Transfer Systems	49
Radiobiochemistry	56
Plant Physiology	61
BIOPHYSICS	64
Photosynthesis	64

INTRODUCTION

Alexander Hollaender

Review of research progress. Coincident with the change of emphasis in some projects in the Division, as mentioned last quarter, a new group has been organized whose efforts are directed toward the evaluation of chemical compounds for protection against radiation effects. This study is already showing promise of early results which will be discussed in the next quarterly report.

Basic studies on oxygen effects have been continued with stress on verification of some previously obtained results, and on the question of whether some of the phenomena observed are general or are specific for certain organisms only. Much of the material developed in this study is now being assembled in report form. From the work with paramecia, we are able to state that there is an oxygen effect in this organism, just as in all living cells tested.

Yeast maintains its excellence as material for genetic evaluation of certain biochemical mutations. It has been possible to photoreactivate the mutations which have been produced in diploid yeast with almost no killing effect on irradiated cells.

No significant modification by hydrogen sulfide of the X-ray sensitivity of genetic material in *Drosophila* has been found. It has been suggested that the effects of X rays express themselves in the production of hydrogen peroxide, which in turn causes changes in genetic material. To investigate this point, female flies were irradiated and mated immediately to nonirradiated males, on the presumption that the hydrogen peroxide would be found in the reproductive tract of the female, where the sperm remains for considerable time. Any residual effect should be recognizable in the offspring of these females. As such an effect could not be found, we may draw the conclusion that, at least under these conditions, no hydrogen peroxide is formed in sufficient concentration to produce an effect.

Interesting data have been obtained through the irradiation of mice during prenatal life which were characteristic of the embryonic stage treated. Irradiation during preimplantation stages caused an elimination of abnormal types before birth.

The project on the effects of slow neutrons and X rays on cataract formation in mice continues to give noteworthy results. The present status is summarized here and data in detail are being prepared for publication. Important information in regard to radiation effects is being derived from the analysis of the pathogenesis of postirradiation anemia. The fall of erythropoiesis immediately after massive irradiation shows that the fall is due to at least three conditions: (a) death of normally aging red cells with cessation of new cell formation, (b) loss of red cells caused by heightened capillary permeability and secondary damage and (c) possibly other factors hitherto not recognized. The hypothesis is advanced that continuous leakage of erythrocytes through damaged endothelium to tissue spaces and lymph channels is a major factor in the causation of postirradiation shock and anemia.

Several projects in metabolism studies have now reached the publishable stage. Of continued interest are the results obtained through the ion-exchange methods developed in this laboratory. Chymotrypsin appears to be much more resistant to gamma radiation if irradiation is done in dry ice than when it is done in water solution at room temperature.

Great difficulty is still encountered with gamma contamination in the slow neutron source.

Significant dividends are beginning to be realized from the work on biosynthesis of chemical compounds by yeast. In a cooperative study, two groups in the Division have found that poke weed chloroplasts produce effectively measurable luminescence under high light intensities. Such a phenomenon had been suspected by other investigators, but this is the first time that its actual existence has been shown. The intensity of the luminescence is approximately a thousand times that of fluorescent light.

Dr. John S. Kirby Smith, formerly of K-25, has joined the Radiobiochemistry group and will concentrate his efforts on certain aspects of infrared studies and irradiation of certain individual parts of the cell. Mr. Leighton A. Nutting, the first Oak Ridge Institute of Nuclear Studies graduate fellow assigned to the Oak Ridge National Laboratory, has completed his Ph.D. work and will receive his degree from Virginia Polytechnic Institute. Two more graduate fellows have begun thesis research in the Division this quarter -- Mr. R. W. Rogers, who seeks the Ph.D. in cytogenetics from the University of Tennessee, and Mr. F. W. Denison, Jr., who is working toward the doctorate in bacteriology from the University of Texas.

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications. A total of forty-three papers by the Biology Division members have been published in open or project literature, or in both, during the past quarter. Two additional papers have been accepted for open publication but have not yet appeared. All these titles are listed. Many of our people are engaged in writing or reviewing material for publication. A number of papers representing the work of the summer Research Participants are in preparation.

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
Rene A. Bolomey and Leon Wish	Thenoyl Trifluoroacetone as a Complexing Agent for the Isolation and Purification of Carrier-Free Radioberyllium	J. Am. Chem. Soc., 72:4483, 1950	
C. E. Carter	Partial Purification of a Nonphosphorylytic Uridine Nucleosidase from Yeast	J. Am. Chem. Soc., In Press	ORNL-757
C. E. Carter	Enzymatic Evidence for the Structure of Desoxyribose Nucleotides	J. Am. Chem. Soc., In Press	ORNL-758
W. E. Cohn and C. E. Carter	The Separation of Adenosine Polyphosphates by Ion Exchange and Paper Chromatography	J. Am. Chem. Soc., 72:4273, 1950	
W. E. Cohn	The Isolation and Identification of Desoxy-5-Methyl-Cytidylic Acid from Thymus Nucleic Acid	J. Am. Chem. Soc., In Press	ORNL-845
W. E. Cohn	The Separation of Nucleic Acid Derivatives by Ion Exchange (Abstract)	Abstracts of Papers of the 118th Meeting Am. Chem. Soc., page 9M, 1950	
W. E. Cohn	The Application of Ion Exchange to the Chemistry of Nucleic Acids (Abstract)	Abstracts of Papers of the 118th Meeting Am. Chem. Soc., p. 5G, 1950	

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
David G. Doherty and Fred Vaslow	Binding of Acetyl-L-Dibromotyrosine by Chymotrypsin (Abstract)	Abstracts of Papers of the 118th Meeting Am. Chem. Soc. p. 60C, 1950	
David G. Doherty	Studies on the Structure of the Isomeric Nucleotides (Abstract)	Abstracts of Papers of the 118th Meeting Am. Chem. Soc. p. 56C, 1950	
Mary E. Gaulden and J. G. Carlson	Studies <i>in Vitro</i> of the Effects of Colchicine on Spindle, Chromosomes, and Cleavage in the Grasshopper Neuroblast, with Special Reference to the Origin of the Spindle		ORNL-852
Mary E. Gaulden and Marjorie Nix	Effect of Oxygen Tension on X-Ray Induced Mitotic Inhibition (Abstract)	Records of the Genetic Soc. of Am., p. 99, 1950	
Mary E. Gaulden and Marjorie Nix	Effect of Oxygen Tension on X-Ray Induced Mitotic Inhibition (Abstract)	J. Tenn. Acad. Sci., XXV:222, 1950	
N. H. Giles, Jr. and A. V. Beatty	The Effect of X Irradiation in Oxygen Under Pressure on Chromosome Aberration Frequency in <i>Tradescantia</i> Microspores (Abstract)	Records of the Genetic Soc. of Am., p. 100, 1950	
N. H. Giles, Jr.	Recent Evidence on the Mechanism of Chromosome Aberration Production by Ionizing Radiations		ORNL-811
A. S. Holt, I. A. Brooks, and W. A. Arnold	Some Effects of 2537 A on Green Algae and Chloroplast Preparations		ORNL-771
A. S. Holt	The Photochemical Reduction and Dark Reoxidation of Cytochrome C by Chloroplast Preparations		ORNL-752
A. S. Holt, R. F. Smith, and C. S. French	Dye Reduction by Illuminated Chloroplast Fragments		ORNL-753
Alexander Hollaender	Physical and Chemical Factors Modifying the Sensitivity of Cells to High Energy and Ultraviolet Radiation		ORNL-844

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
Alexander Hollaender, G. E. Stapleton, and F. L. Martin	X-Ray Sensitivity of <i>E. coli</i> as Modified by Oxygen	Nature, In Press	ORNL-832
R. F. Kimball	The Relation Between Induced Mutation and Retardation of Cell Division Brought about by Ultraviolet Irradiation of <i>Paramecium aurelia</i> (Abstract)	Records of the Genetics Soc. of Am., p. 107, 1950	
R. F. Kimball and Nenita Gaither	The Influence of Light upon the Action of Ultraviolet on <i>Paramecium aurelia</i>	J. Cell. & Comp. Physiol., In Press	ORNL-806
J. C. Kile, Jr.	An Improved Method for the Artificial Insemination of Mice	Anat. Rec., In Press	ORNL-808
H. I. Kohn	On the Modification of the Toxicity of X Rays by Immunization		ORNL-386
H. I. Kohn	The Effect of Immaturity, Hypophysectomy and Adrenalectomy upon the Changes in the Blood Plasma During the Acute Radiation Syndrome in the Rat		ORNL-656
H. I. Kohn	Changes in the Composition of the Blood Plasma of the Guinea Pig During the Acute Radiation Syndrome	Am. J. Physiol., 162:703, 1950	ORNL-464
G. R. Noggle and René A. Bolomey	The Biosynthesis of Carbon-14-Labeled Compounds. I. The Chromatographic Separation of Glucose and Fructose		ORNL-787
G. R. Noggle	The Use of Isotopes in Soil Research		ORNL-863
Seymour Pomper	Yeast Mutants Requiring Purines and Pyrimidines		ORNL-812
W. L. Russell	The Incidence of Sterility and Partial Sterility in the Descendants of X-Irradiated Mice (Abstract)	Records of the Genetics Soc. of Am., p. 123, 1950	
Liane B. Russell	X-Ray Induced Developmental Abnormalities in the Mouse and Their Uses in the Analysis of Embryological Patterns. I. External and Gross Visceral Changes	J. Exper. Zool., 114:545, 1950	ORNL-595

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
Liane B. Russell and W. L. Russell	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 (Abstract)	Records of the Genetics Soc. of Am., p. 123, 1950	
C. W. Sheppard and W. R. Martin	Cation Exchange Between Cells and Plasma of Mammalian Blood: II. Sodium and Potassium Exchange in the Sheep, Dog, Cow, and Man and the Effect of Varying the Plasma Potassium Concentration	J. Gen. Physiol., In Press	ORNL-617
C. W. Sheppard, P. F. Hahn, and G. Jordan	The Disappearance of Isotopically Labeled Gold Colloids from the Circulation of the Dog	Am. J. Physiol., In Press	ORNL-689
S. R. Suskind	Resuscitation of Heat-Inactivated Seeds by X-Radiation	J. Hered., XLI:97, 1950	ORNL-579
P. C. Tompkins, Oscar M. Bizzell, and Clyde Watson	Radioactive Decontamination of Laboratory Surfaces: II. Paints, Plastics, and Floor Materials	Indust. & Engin. Chem., 42:1475, 1950	
P. C. Tompkins and Oscar M. Bizzell	Radioactive Decontamination Properties of Laboratory Surfaces: I. Glass, Stainless Steel, and Lead	Indust. & Engin. Chem., 42:1469, 1950	
J. R. Totter, E. Volkin, and C. E. Carter	The Distribution of Isotopically Labeled Formate in the Nucleotides of Ribo- and Desoxyribonucleic Acids (Abstract)	Abstracts of Papers of the 118th Meeting Am. Chem. Soc., p. 55C, 1950	
J. R. Totter, Elliot Volkin, and C. E. Carter	Incorporation of Isotopic Formate into the Nucleotides of Ribo- and Desoxyribonucleic Acids		ORNL-866
Elliot Volkin and C. E. Carter	The Incorporation of Isotopic Phosphate in the Mononucleotides of Liver Nucleic Acids		ORNL-851
Elliot Volkin and H. I. Kohn	A Factor in the Plasma of the Irradiated Rat which Changes the A/G Ratio	Arch. Biochem., In Press	ORNL-734
Elliot Volkin and C. E. Carter	The Preparation and Properties of Mammalian Ribonucleic Acids	J. Am. Chem. Soc., In Press	ORNL-803
Elliot Volkin, J. X. Khym, and W. E. Cohn	The Preparation of Desoxynucleotides	J. Am. Chem. Soc., In Press	ORNL-943

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION	PROJECT PUBLICATION
Leon Wish, J. Furth, and R. H. Storey	Direct Plasma and Cell Volume Determinations with Isotope Techniques: Organ-Blood and Hematocrit Values in Normal and Hypervolemic Mice	Proc. Soc. Exper. Biol & Med., 74:644, 1950	ORNL-749
Leon Wish and Rene A. Bolomey	Spectrophotometric Studies of Beryllium Thenoyl Trifluoroacetone	J. Am. Chem. Soc., 72:4486, 1950	

Papers presented at scientific societies. The Division has been well represented on the speakers' platforms of national and international societies during the quarter. Following is a list of presentations.

W. T. Burnett, Jr. (Bigelow, Sheppard)	Am. Physiol. Soc., Columbus, Ohio	Radiomanganese Studies in the Pancreatic Fistula Dog
W. E. Cohn	Am. Chem. Soc., Chicago, Ill.	The Application of Ion Exchange to the Chemistry of Nucleic Acids
W. E. Cohn	Am. Chem. Soc., Chicago, Ill.	The Separation of Nucleic-Acid Derivatives by Ion Exchange
D. G. Doherty (F. Vaslow)	Am. Chem. Soc., Chicago, Ill.	Binding of Acetyl-L-Dibromotyrosine by Chymotrypsin
D. G. Doherty	Am. Chem. Soc., Chicago, Ill.	Studies on the Structure of the Isomeric Nucleotides
D. G. Doherty	S. E. Branch of Soc. Exp. Biol. & Med., Winston-Salem, N. C.	Effect of Gamma Radiation on Alpha- Chymotrypsin
M. E. Gaulden (Marjorie Nix)	Gen. Soc. of Am., Columbus, Ohio	Effects of Oxygen Tension on X-Ray Induced Mitotic Inhibition
A. V. Beatty (N. H. Giles, Jr.)	Gen. Soc. of Am., Columbus, Ohio	The Effect of X Irradiation in Oxygen under Pressure on Chromosome Aberration Frequency in <i>Tradescantia</i> Microspores
R. F. Kimball	Gen. Soc. of Am., Columbus, Ohio	The Relation Between Induced Mutation and Retardation of Cell Division Brought about by Ultraviolet Irradiation of <i>P.</i> <i>aurelia</i>

G. R. Noggle	Am. Soc. of Plant Physiologists, Columbus, Ohio	Sugar Metabolism in Detached Sugar Beet Leaves
Liane B. Russell	Gen. Soc. of Am., Columbus, Ohio	Changes in the Relative Proportions of of Different Axial Skeletal Types within Inbred Strains of Mice Brought about by X Irradiation at Critical Stages in Embryonic Development
W. L. Russell	Gen. Soc. of Am., Columbus, Ohio	The Incidence of Sterility and Partial Sterility in the Descendents of X- Irradiated Mice
J. R. Totter (E. Volkin & C. E. Carter)	Am. Chem. Soc., Chicago, Ill.	The Distribution of Isotopically Labeled Formate in the Nucleotides of Ribo- and Desoxyribonucleic Acids
W. G. Walker (W. S. Wilde)	Am. Physiol. Soc., Columbus, Ohio	Rate of Exchange of Potassium in Plasma, Liver, and Muscle

Participants in approaching society meetings are as follows: Dr. Jacob Furth, Southern Medical Association, St. Louis, November; Dr. Alexander Hollaender, Dr. W. L. Russell, Dr. L. B. Russell, and Dr. J. C. Kile, Jr., American Association for the Advancement of Science, Cleveland, December.

Visiting lecturers for Biology seminars. The Division has been fortunate in securing the services of Dr. J. N. Dent, of the Miller School of Biology-University of Virginia, and of Dr. E. Newton Harvey, of Princeton University for lectures in the seminar program. Dr. Dent spoke on "Some Aspects of Limb Regeneration in the Amphibia" and Dr. Harvey's subject was "Light Production in Animals." In addition to these, seminars were heard by the group of Research Participants from thirteen Southern Universities assembled here for the summer.

Traveling seminar program. The Biology Division is again participating in the Oak Ridge Institute-Oak Ridge National Laboratory program of Traveling Seminars. Seventeen senior members of the Division have been listed to serve during this seminar season. To date, fifty requests have been received from schools in the Southern College and University group.

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

	R. F. Kimball (Leader)
R. P. Geckler*	Nenita Gaither
A. D. Conger	Mary Kathryn King
Seymour Pomper	D. S. Daniels
A. H. Doermann	Lucille M. Fairchild
Betty B. Hill	Kathryn Meyer

The determination and inheritance of reduced vigor in Paramecium aurelia. (Kimball, Geckler, Gaither, King). It has been shown by several investigators that *Paramecium aurelia* exposed to radiation or to nitrogen mustard produces many clones at the next autogamy which multiply slowly or die. The production of such clones of reduced vigor has been attributed by Kimball (Genetics 34: 412, 1949) to gene mutations induced by the radiation and made homozygous by autogamy. However, Geckler (Genetics 35:253-277, 1950) has shown that similar reduced vigor brought about by nitrogen mustard treatment shows certain features which do not fit the gene mutation interpretation although other features are in agreement with it.

Spontaneous reduced vigor very similar to that induced by radiation is known to occur in the autogamous progeny of certain stock crosses. We have recently found such a case which is especially favorable for investigation. Of the two stocks involved, both from variety 4, one is a killer, *i.e.*, it produces a substance which kills other paramecia; the other is a nonkiller, sensitive to this killing substance. The two stocks are known to differ from each other in a single gene and are supposed to be otherwise isogenic. Following conjugation between these stocks, the killer member remains a killer and the sensitive, a sensitive, thus making possible a determination of the cytoplasmic origin of the two members of the pair after conjugation. The known gene difference allows determination that the occurrence is true conjugation rather than cytogamy. All pairs upon which the conclusions of this section are based are true conjugants.

The exconjugants from crosses between these stocks are quite viable; but, when the exconjugant clones undergo the self-fertilization process of autogamy, many exautogamous clones of reduced vigor (*ca.* 40 per cent) are produced.

* Research Participant.

This behavior, now found repeatedly in crosses between the stocks, was not characteristic when they were first made isogenic. It may be that gene differences between them have arisen in the meanwhile. If, in fact, the stocks differ from each other in one or more genes and the segregation of these in unfavorable combinations is responsible for the reduced vigor after autogamy, one would expect that the two members of a pair would be alike in respect to the per cent of reduced vigor at the following autogamy. This was not the case. A corresponding dissimilarity between members of pairs, one member of which had been treated with nitrogen mustard, was one of the major pieces of evidence which Geckler (1950) found against the gene mutation hypothesis for the effect of this agent. Therefore, it seemed desirable to investigate in some detail the present spontaneous case of the same type of behavior.

The outline of the major experiment is shown in Fig. 1. A number of conjugant pairs were obtained between the two stocks, the two members of each pair were kept separately and allowed to divide, and one product of the first division from each was discarded. The other product of each was allowed to divide again and these two products were kept separately for several days as daily isolation lines. Three days before autogamy was to be obtained two products of the division of the single animal, isolated the day before, were put into separate containers and allowed to multiply to form small mass cultures. From each such culture, 25 autogamous animals were isolated and checked for survival and amount of growth in 4 days. Thus from each original pair, eight groups of autogamous animals were obtained as shown in the figure. In all, 28 pairs were carried through the procedure.

The statistical analysis of the data demonstrates the following: (1) The per cents of reduced vigor from the two cultures obtained late in the history of each line of descent were not different from each other within the limits of sampling error. (2) The per cents of reduced vigor in the progeny of the two products of the second division were significantly different from each other in the material taken as a whole. (3) Despite this difference, the per cents from the two products were correlated with each other. (4) The two members of a pair showed a similar correlation with each other. (5) No difference was found in the behavior of the killer members and sensitive members taken as groups.

These findings demonstrate that the simple hypothesis that the reduced vigor arising in such a cross is due to unfavorable combinations of genes from

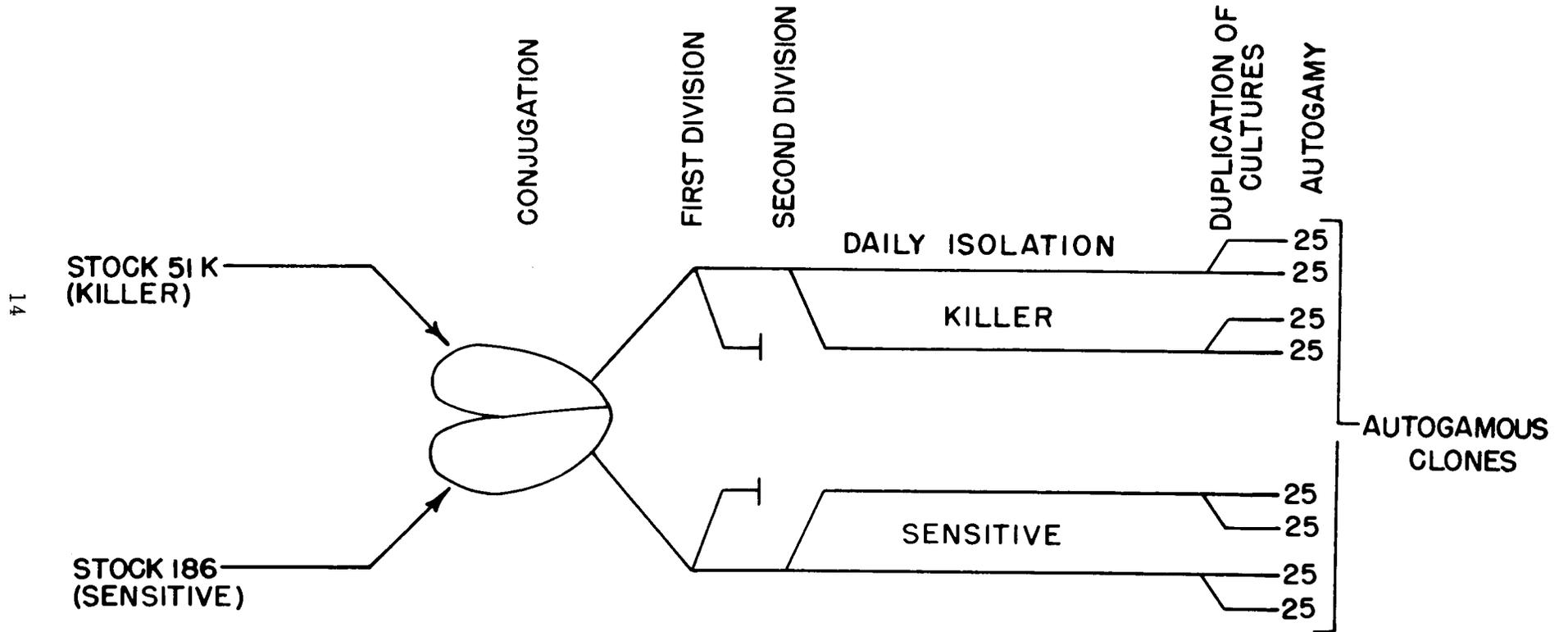


FIG. 1

the two parent stocks is not tenable without modification. Significant differences can arise between the two members of the pair and even during the vegetative multiplication of the exconjugant clones. These differences must involve some sort of transmission since the two products of the second division are correlated. Likewise, the correlation of the two members of a pair shows that something happens during conjugation which leads to similarity between the two members of the pair. The fact that the killer members as a group are not different from the sensitive members as a group demonstrates that the causative factor in bringing about the differences from simple genic expectation cannot be cytoplasmic materials inherited from only one of the parent stocks. It is to be noted that events occurring long before autogamy play an important role in determining the amount of reduced vigor, while possible environmental differences between the cultures in which autogamy takes place appear to have no effect.

Two other experiments have confirmed certain of these conclusions and have given no contradictory evidence. A complete interpretation of the results is not possible as yet, but there is clear demonstration that the per cent of reduced vigor after autogamy is subject to other hereditary influences than gene segregation.

The effect of oxygen on the induction by X rays of reduced vigor after autogamy. (Kimball, Gaither, King). Previous attempts were made to demonstrate an effect of oxygen upon the induction by X rays of reduced vigor after autogamy in stock 90 of variety 1 of *P. aurelia*. These attempts were made by evacuation and replacement of the air with nitrogen, helium, or oxygen. Some effects were found but on the whole the experiments were negative. It appeared possible that the method was failing to bring about adequate gas exchange with the suspension fluid in which the animals were irradiated and so a technique of bubbling the desired gas through the medium was adopted. With this technique, clear-cut evidence for an oxygen effect was obtained as shown in Table 1. In each experiment, 40 animals for each gas were exposed to the X rays. From each exposed animal, 25 autogamous progeny were derived and the per cent of these which were of reduced vigor is shown in the table. A statistical analysis has been completed only on the data for the first experiment. The difference between the two groups in this case was clearly significant. The marked difference between the two experiments in which nearly the same dose was given is unexplained. It can be concluded that X irradiation in the presence of oxygen is more effective in producing reduced vigor in *Paramecium* than it is in the absence of this gas.

TABLE 1

Effect of oxygen upon the per cent of reduced vigor after autogamy

DOSE OF X RAYS	OXYGEN (per cent)	NITROGEN (per cent)
1300	44.0	24.5
5400	59.4	31.5
5450	82.7	59.2

Yeast genetics. (Pomper and Meyer). Further experiments have been carried out with the system which was tentatively reported (ORNL-807) as showing the accumulation of a precursor in purine synthesis and the response thereto by another purineless mutant. It has been found that the assay organism, i.e., the responding mutant, is affected in its growth requirements by the pH of the medium. Thus, using this organism for assay is risky, and the question of whether indeed there is an active precursor being accumulated cannot be answered with the present test material. A detailed report of studies to date on purine- and pyrimidine requiring mutants has been prepared (ORNL-812).

The pH-sensitive mutant has been analyzed in detail and with some success. This mutant, which was isolated after a single ultraviolet irradiation, required five accessory factors for growth in a synthetic medium: adenine, histidine, methionine, pantothenic acid (Pa), and *p*-aminobenzoic acid (PAB). The synthetic medium employed in all growth experiments had phosphate buffer, and a pH, after autoclaving, very slightly on the acid side, viz., 6-6.5. When the pH was dropped below 6, for example to 4.5, the organism no longer required external supplies of adenine and histidine. This mutant is the reverse of the *Neurospora* mutant which requires pyridoxine at low pH's but not at higher values (ca. 6-6.5). The *Neurospora* mutant has been thought to show this response because at the low pH values there was an insufficient supply of free ammonia. There is no ammonia effect with the yeast mutant -- the requirement and its abolition follow the same pattern in experiments whether ammonium salts are present or absent.

A series of experiments were then performed in which high concentrations of the five growth factors were tested for their ability to replace any of the

other requirements. It was found that increased concentrations of PAB were able to replace the adenine and histidine requirements simultaneously at pH values well over 6, viz., 6.6. Thus, by increasing the PAB concentration the requirements for adenine and histidine could be abolished at all pH levels. It was further observed that the methionine requirement could be eliminated by increased concentrations of PAB at lower pH's (below 6). It is not yet certain whether PAB can also replace the Pa requirement. Preliminary data suggest that it may, but check experiments are not yet complete. Summary data are presented in Table 2 illustrating the PAB replacement effect. From the

TABLE 2

The replacement by p-aminobenzoic acid of the adenine, histidine, and methionine requirements of a multiple yeast mutant (62-28)

ADENINE (γ /ml)	HISTIDINE (γ /ml)	METHIONINE (γ /ml)	PAB* (γ /ml)	pH	PER CENT ABSORPTION** (4 days, 30° C)
0	0	0	0.5	6.7	5
10	10	10	0.5	6.7	30
10	10	10	50.0	6.7	38
0	10	10	0.5	6.7	5
0	10	10	50.0	6.7	35
10	0	10	0.5	6.7	8
10	0	0	50.0	6.7	35
0	0	10	0.5	6.7	6
0	0	10	50.0	6.7	34
10	10	0	0.5	6.7	4
10	10	0	50.0	6.7	7
10	10	0	0.5	5.0	5
10	10	0	50.0	5.0	59

* Depending on the pH, different concentrations of PAB will suffice for replacement. 50 γ /ml is well in excess of actual needs.

** Turbidity (growth) measurements in the Lumetron 400 A colorimeter.

presently available data, it seems reasonable to conclude that the key requirement in this mutant is for PAB. Further, it seems logical to interpret the replacement effects in terms of PAB acting as the coenzyme in the syntheses of adenine, histidine, and methionine. This view is in harmony with data

in the biochemical literature on the function of PAB. Two additional points might also be noted: (1) high concentrations of the other factors do not appear to replace the PAB requirement, although they do lower the quantitative need, and (2) lysine, shikimic, and pimelic acids were inactive in replacing the PAB requirement; folic acid at high concentrations (100 γ /ml) replaces the PAB requirement if the other factors are present, but not if any of them are absent.

Ultraviolet irradiation experiments have been continued with haploid and diploid yeasts, as first reported in Quarterly Report ORNL-807. It seems desirable to delay a detailed report of these experiments until they are entirely complete. However, certain points may be noted at this time. As previously reported, reversions to adenine and uracil independence can be induced by ultraviolet irradiation, independently of killing, in the diploid yeast. It has now been found possible to photoreactivate these mutations, *i.e.*, to reverse the induced reversions. Work on the haploid yeast is still hampered by cell aggregation, but some progress in removing this hindrance is being made. The available data with the haploid indicate that it is more susceptible to ultraviolet than the diploid, in terms of killing, *i.e.*, ability to form colonies on agar. The induced mutation frequencies are comparable for most of the dosage range. At dosages causing fairly considerable killing of the haploid, the mutation frequency falls off markedly (as compared with the diploid for the same exposure). It is possible to photoreactivate both killing and mutational effects in the haploid. The quantitative aspects of this work will be treated in the more detailed presentation at its completion.

Genetics of bacteriophage T4. (Doermann and Hill). In previous quarterly reports (ORNL-644 and -727) experiments have been discussed which are intended to extend our knowledge and control of genetic material available in the bacteriophage T4. These experiments have been continued with the immediate purpose of isolating in pure stocks the various genetic factors responsible for the T4_{r₄₈} types #1, #2, #5, and #6. When completed they should prove or disprove the working hypothesis previously proposed (ORNL-727) for describing their genotypes by making all possible crosses between them and predicting the recombination quantitatively. The data are still incomplete and will not be presented in detail here, but it may be said that the results thus far obtained are in agreement with the hypothesis proposed.

Another aspect of this problem concerns the nature of the genetic factors of bacteriophage. Hershey and Rotman (Proc. Nat. Acad. Sc. 34:89, 1948),

isolating some 20 independently arising spontaneous r^- mutants of T2 found no two to be genetically identical. This implies that a very large number of loci control the r^+ phenotype. The T4 material described provides a good opportunity to test the generality of this situation with mutants of the m type (ORNL-644) and the single mutant type #2 (ORNL-727), since it is comparatively simple to isolate independent mutants in these categories. Isolation of the #2 type from the nonmutant #1 type has been started. Five pure stocks are now available and are being tested.

EFFECTS OF RADIATION ON RATE OF MITOSIS

Mary Esther Gaulden (Leader)
J. Gordon Carlson* Elizabeth Sgourakis
W. K. Baker* Nyra Harrington
R. W. Rogers** Marjorie Nix

Effects of oxygen tension on X-ray-induced mitotic inhibition. (Gaulden and Nix). This work has been completed and a full report is being prepared for publication. A summary of results is presented.

Fourteen-day-old embryos of the grasshopper *Chortophaga viridifasciata* were given 64 r of X rays (32 r/minute) while at different tensions of oxygen: 0 (nitrogen, carbon dioxide, or vacuum), 2, 5, 10***, 21 (air), and 100 per cent oxygen. At least 12 embryos (1500-2000 neuroblasts) were examined for effects of irradiation on mitotic rate at each oxygen tension. Mitotic rate was determined as described in Quarterly Report ORNL-727. Biometrical analysis of the data was made by Mr. Jack Moshman of the Mathematics Panel.

Exposure of mitotic cells to X radiation causes a period of complete mitotic inhibition (no cells in mid-mitosis), which is followed by a compensatory period during which the number of cells in mid-mitosis exceeds that in the untreated embryo. By increasing the oxygen tension around the eggs at the time of irradiation, both the duration of the inhibition period and the duration of the interval between irradiation and the occurrence of the maximum number of mid-mitotic cells can be lengthened. Fig. 2 shows the increase in the interval between irradiation and the peak of mid-mitotic cells cooperant

* Consultant.

** ORINS Fellow.

*** Helium was used to make the mixtures of oxygen.

Unclassified

DWG. 9995

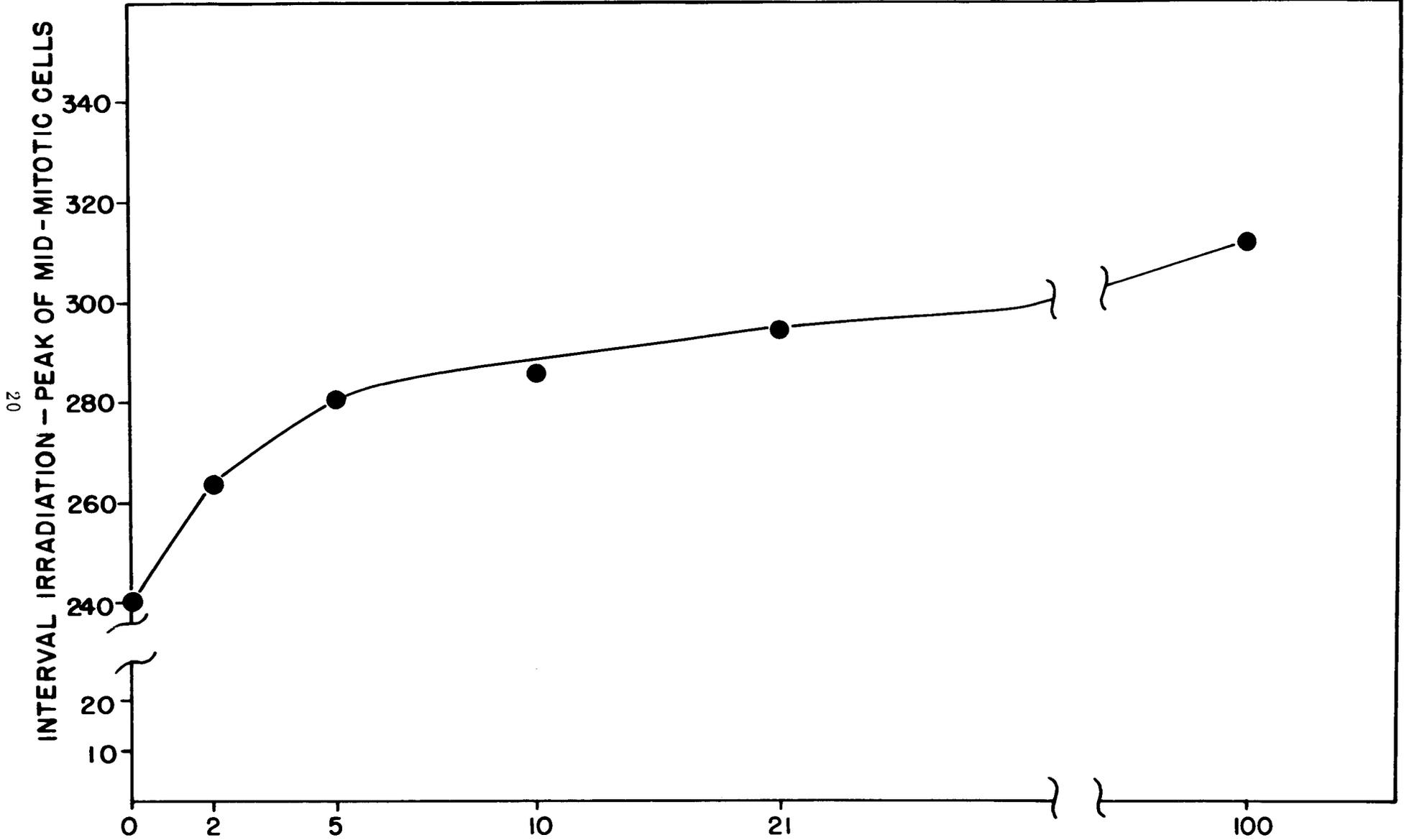


FIG. 2

with the increase in oxygen tension. In other words, the sensitivity of mitosis to X radiation is greater at the higher oxygen tensions. It is of interest that the shape of the curve in Fig. 2 is quite similar to the curves obtained by Giles and Riley (Proc. Nat. Acad. Sci., 36:337-342, 1950) when the effect of oxygen tension on the frequency of X-ray-induced chromosome breakage was determined.

Cells exposed to the various gas mixtures, but not to irradiation, showed no aberrations in mitotic rate.

Effects of X rays on the mitotic cell. (Gaulden and Nix). An extensive series of experiments has been initiated to investigate in some detail the effects of X rays on the mitotic process and on the chromosomes. As in much of the previous work, a dose of 64 r of X rays has been chosen for this study. The study seeks determination of (1) the effects of X rays on over-all mitotic rate during the 6-hour period following irradiation, (2) the relative sensitivities of different stages of mitosis to the retardation effects of X rays and their relationship to the curve obtained in part 1, and (3) the relative sensitivities of the different stages of mitosis with respect to breakage of the chromosomes.

Part 1 of this study has been completed. Grasshopper eggs were irradiated and, immediately following irradiation, the embryos were removed and made into culture preparations. Mitotic rate was determined as described in Quarterly Report ORNL-727, p. 41. Fig. 3 represents the results of analysis of 59 embryos or a total of 4602 neuroblasts. Mitotic progress is completely blocked about 1 hour after irradiation, i.e., the number of cells in mid-mitosis drops to zero. The cells begin to recover after about 1 hour of inhibition. Approximately 6 hours after irradiation mitotic rate has returned to normal.

Effects of radiation on Drosophila genetics. (Baker, Sgourakis). Experiments of a preliminary nature were conducted in the past (see previous quarterly reports) to study the effect of respiratory enzyme poisons on the X-ray-induced mutation rate in *Drosophila melanogaster*. These initial experiments gave indication of a slightly lower than expected induced mutation rate in flies maintained in sublethal concentrations of hydrogen sulfide prior to and during X irradiation; whereas, flies maintained in hydrogen cyanide for a like period showed no alteration or modification of the induced mutation rate. Data of the experiments conducted with hydrogen sulfide are reported here.

Unclassified

DWG. 9996

64 r x RAYS (IN AIR) 59 EMBRYOS, 4602 CELLS

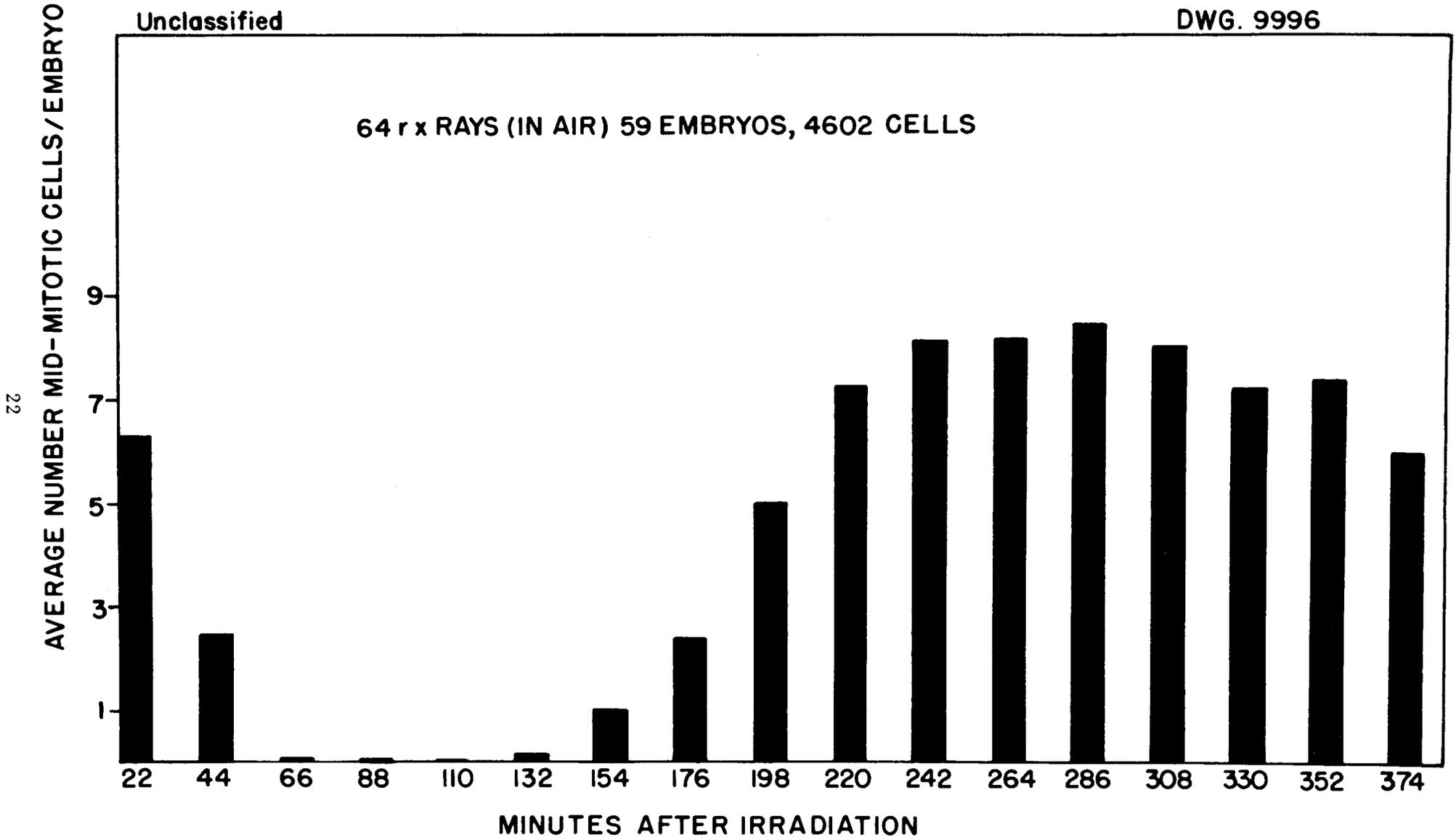


FIG. 3

Flies were exposed to four different gaseous environments: (1) 1 per cent hydrogen sulfide and 99 per cent nitrogen, a mixture which was prepared and tested by the Ohio Chemical Company; (2) pure nitrogen, from a Linde cylinder; (3) air from an Ohio Chemical Company cylinder; and (4) a mixture of 0.04 per cent hydrogen sulfide balance air which was prepared by mixing known amounts of hydrogen sulfide with known amounts of air in a spirometer. Exposure of flies to these gases was accomplished by placing males of the Oregon-R strain of *D. melanogaster* in lucite chambers, after which the chambers were alternately evacuated and flushed with the desired gas or gas mixture five times. The gas or gas mixture was then trapped in the exposure chamber, the chamber placed under the X-ray tube, and the flies exposed to 3000 r. After X-ray treatment, the flies were removed from the chambers, exposed to the air, and then mated with Muller-5 females. The Muller-5 method for detecting sex-linked recessive lethal mutations was followed in these experiments. Table 3 summarizes the results obtained. Statistical analysis of these data shows that hydrogen sulfide does not significantly modify X-ray sensitivity of the genetic material of *Drosophila melanogaster*.

TABLE 3

TYPE OF EXPOSURE	NO. CHROMOSOMES TESTED	NO. OF LETHALS	PER CENT LETHALS
H ₂ S plus N ₂ control	840	1	0.12
H ₂ S plus air control	624	0	0.00
H ₂ S plus N ₂ plus 3000 r	828	40	4.83
N ₂ plus 3000 r	823	48	5.83
H ₂ S plus air plus 3000 r	615	72	11.71
air plus 3000 r	606	58	9.57

Previous work indicates that, among the sperm of *D. melanogaster* fertilizing the eggs laid the first 2 or 3 days after X irradiation, there is no difference in the percentage of sex-linked lethal mutations induced in males irradiated in an atmosphere of oxygen or of nitrogen. This is in marked contrast to the pronounced difference noted in sperm which fertilize eggs laid from the third to the seventh day after treatment. Experiments were undertaken to determine if this similarity is due to the fact that the radiosensitivity of mature sperm is not altered by the oxygen concentration. One group

of previously fertilized females was exposed to 3000 r of X rays while in an atmosphere of pure oxygen, and another group exposed to the same dosage while in nitrogen. The percentage of sex-linked lethal mutations induced in the sperm was determined in each group. Of the mature sperm irradiated in nitrogen, 9.0 per cent carried X chromosomes with lethals; while, in the oxygen group, 21.0 per cent carried lethal mutations. This indicates that the maturity of the sperm *per se* is not a factor in the effect described above. Incidental to these results it was found that the percentage of mutations induced in the X chromosome of the egg and the amount of nondisjunction of this chromosome were higher in the females irradiated in oxygen as compared to those treated in nitrogen.

One of the theories postulated to explain oxygen alteration of the X-ray sensitivity of biological material is based on the formation, when water is irradiated, of the compound hydrogen peroxide and the radicals HO_2 and OH . Because of the relatively long life of the hydrogen peroxide, it would seem possible to isolate its effect, if any, from that of the free radicals. Indirect evidence on this point was obtained by determining whether the products formed by X irradiation within the female reproductive tract were effective in inducing mutations in unirradiated sperm introduced into the tract. Prior to and during irradiation, virgin females of *D. melanogaster* were placed in a hydrogen cyanide-air mixture of a concentration which totally anesthetized the flies but still allowed almost total recovery. The cyanide was used to poison any catalase in the reproductive tract which could break down the hydrogen peroxide. Immediately after exposure to 3000 or 4000 r of X rays these females were allowed to mate with males for no longer than 1 hour. The percentage of sex-linked mutations induced in the untreated sperm was determined and compared with the spontaneous mutation rate. Of 1233 sperm tested, only two contained X chromosomes with lethal mutations. This is not significantly different from the control rate. We conclude, therefore, that the products of radiation produced in the female reproductive tract have no mutagenic action on sperm introduced into the tract within 1 hour after the end of irradiation. This gives strong indication that hydrogen peroxide is not the agent involved in the oxygen effect.

EFFECTS OF RADIATION ON MICROORGANISMS

A. Hollaender (Leader)
G. E. Stapleton
E. H. Anderson

R. B. Grayson
Frances L. Martin
Ruth W. Whittle

The effect of temperature on inactivation of E. coli (B/r). (Hollaender, Stapleton, Martin). In regard to the mechanism of lethal action of X radiation on bacterial cells, the effect of temperature on inactivation of *E. coli* (B/r) has been investigated in the presence and absence of oxygen. Using a constant-temperature exposure system it was possible to irradiate suspensions of this organism over a wide range of temperatures. Buffered suspensions of 24-hour cells were irradiated with 25 kv of X rays at 1200 r/minute, at 50 temperature intervals.

Over the temperature range from 0-40° C, the cells show an increased sensitivity with increasing temperatures in both the presence and absence of oxygen. The Q_{10} in nitrogen-saturated suspensions is quite low (about 1.2), being still lower in the presence of oxygen (about 1.08).

X-ray sensitivity of Serratia marcescens. For many experiments it is desirable to have an extremely sensitive organism. The sensitivity of *Serratia marcescens* has been studied recently and preliminary data indicate that this organism is several times more sensitive than either strain of *E. coli* previously used. Fig. 4 shows the comparative sensitivity of *S. marcescens* in oxygen- and nitrogen-saturated suspensions. A plot of *E. coli* (B/r) sensitivity in nitrogen is given for comparison. The relative sensitivity of dose in nitrogen to dose in oxygen is 3.5.

Attempts have been made to determine if cultural conditions alter the sensitivity of this organism as they do for *E. coli*. Some difficulties were encountered in culturing *S. marcescens* in glucose broth; however, the organism does grow slowly under anaerobic conditions. Under these growth conditions, it displays an increased resistance similar to that of *E. coli*(B/r).

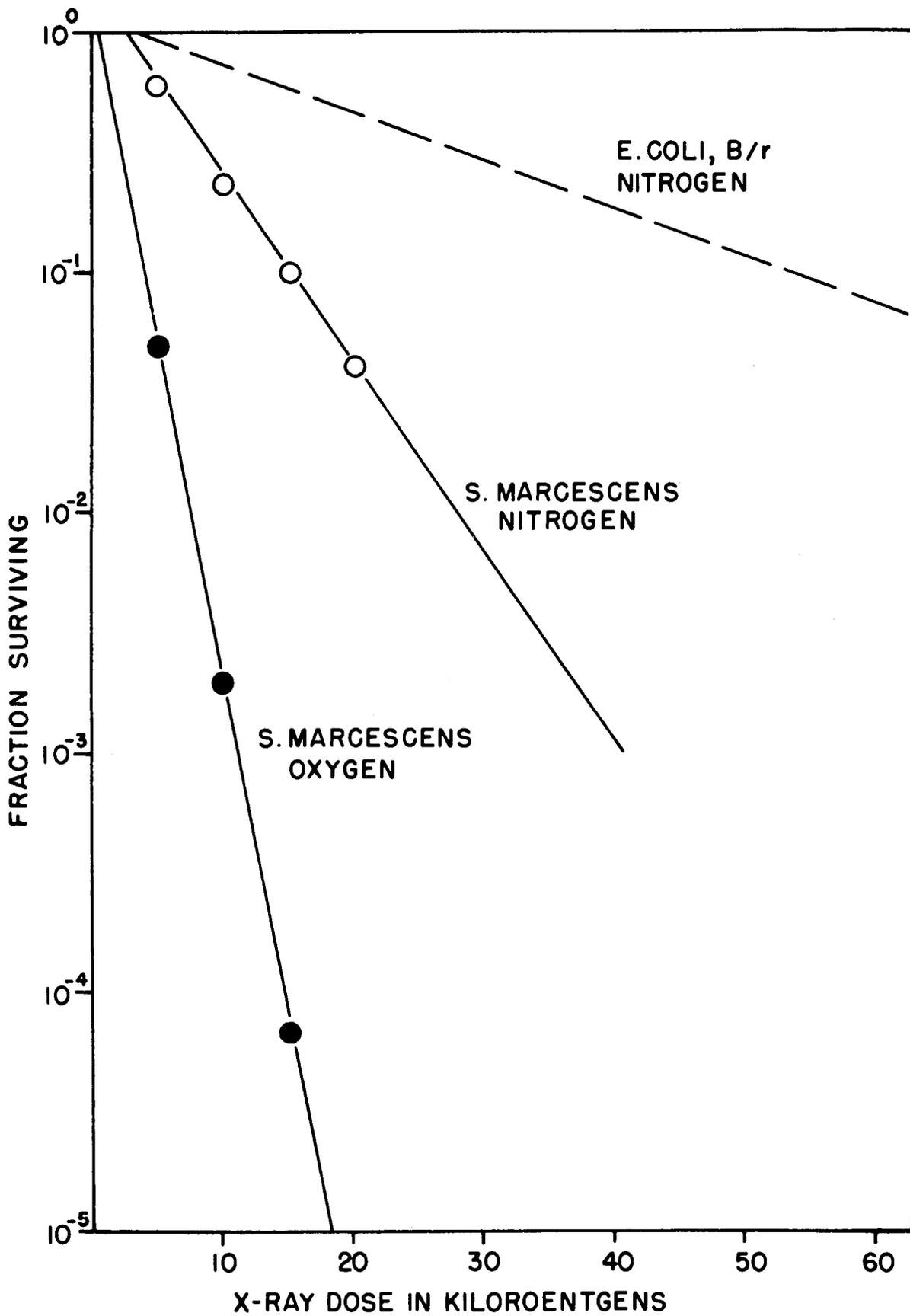


FIG. 4

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell (Leader)	Jane E. Crowell
Liane B. Russell	Mary Henderson
Josephine S. Gower	Gloria J. Jones
J. C. Kile, Jr.	Elizabeth M. Kelly
Louis Wickham	Patricia A. Sarvella

The effects of radiation on the preimplantation stages of the mouse embryo. (Russell, Russell, Henderson) In a large-scale experiment completed earlier (Russell, J. Exper. Zool., 114:545-602, 1950), it was found that irradiation during prenatal life produced effects which were characteristic of the embryonic stage treated. Following irradiation during postimplantation stages (later than day 5½) there was little or no prenatal death, but a high incidence of abnormalities at birth making possible the accurate mapping of critical periods in the development of certain characters. Irradiation during preimplantation stages, however, appeared to cause an elimination of abnormal types before birth; the yield in young per treated female was reduced but those animals which were born were normal in almost all cases. Since the preimplantation stages in the mouse are also pregastrulation and since certain classical vertebrate monsters were to be expected from interference with development during that time, an experiment was undertaken to determine the time of death of the irradiated embryos and, if possible, their morphology.

Method. C57 black females, previously tested for fertility, were mated to NB strain males and irradiated on day ½, 1½, 2½, 3½, or 4½ following the observation of the vaginal plug. Treatment consisted of 200 r total body irradiation with 250 kvp X rays. Each irradiated female was paired with a control handled not only in an identical fashion (except for the actual irradiation) but, as far as possible, simultaneously. Two subseries were run within each group; one dissected 13½ days after mating, the other 10½ days. All embryos and resorbing bodies, a total of 831, were carefully examined and measured.

Results. In comparison with controls, it is assumed that any reduction in the total number of implantation sites (live embryos plus resorbing bodies) is due to death before implantation, whereas a relative increase in resorbing bodies is accounted for by death after implantation.

In over-all effect, the earliest stages are most sensitive: the average number of living embryos per treated female is approximately 20 per cent of the controls in groups irradiated on day $\frac{1}{2}$, $1\frac{1}{2}$, or $2\frac{1}{2}$, but 31 and 57 per cent following treatment on days $3\frac{1}{2}$ and $4\frac{1}{2}$ respectively. In groups irradiated on days $2\frac{1}{2}$ and $3\frac{1}{2}$, the major part (81 and 53 per cent respectively) of the reduction in live embryo count is accounted for by death after implantation; whereas, in groups irradiated on days $\frac{1}{2}$, $1\frac{1}{2}$, and $4\frac{1}{2}$, the majority of the deaths (71, 71, and 75 per cent, respectively) occurs before implantation. Postimplantation death probably occurs considerably before day $10\frac{1}{2}$. The main or sole expression of preimplantation death is on entire litters in groups other than those irradiated on day $\frac{1}{2}$ or $1\frac{1}{2}$ where 39 and 22 per cent respectively of the preimplantation death is due to selective mortality within litters. Total-litter death may be due to radiation effect on the mother rather than direct lethal action on embryos.

PATHOLOGY AND PHYSIOLOGY

Jacob Furth (Leader)	Emma Jane Beale
M. C. Woods	Mary M. Knoohuizen
R. H. Storey	J. J. Lane
J. B. Kahn	Peggy Ledford
W. D. Gude	Germaine Click

R. R. Bigelow*
K. W. Christenberry*

Exposure of mice to slow neutrons. (Christenberry, Lane, Gude, Ledford). The set-up of this long-term project on the effect of slow neutrons on longevity, neoplasia and cataract incidence, and type of neoplastic changes begun in August 1949 has been completed on a reduced scale. This series now consists of 970 neutron-exposed mice, 825 X-rayed mice with 417 controls, a total of 2,212 mice. No further slow-neutron exposures will be made until the high gamma contamination (estimated at 320 to 640 r at the LD-50 level) has been cut down.

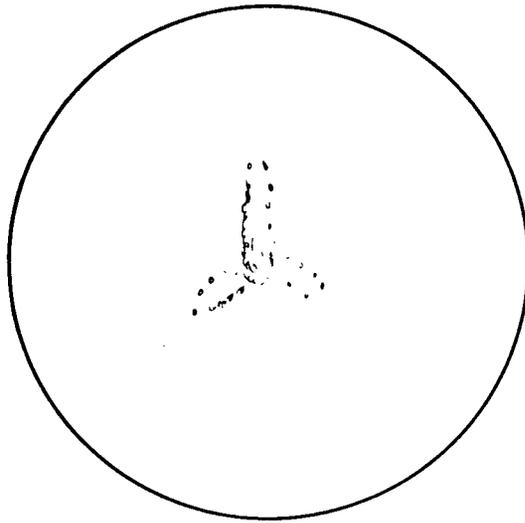
The high leukemia incidence in the neutron-exposed and X-irradiated animals, as tabulated in our last report, is being maintained.

The opacities (cataracts) are first noticeable after a latency period of 2½ to 3 months in the posterior pole of the lens. The earliest sign is a small group of dot-like opacities arranged around the posterior pole. Later they form a pattern of an inverted Y, as illustrated in Fig. 5a. Further advance occurs in the subcapsular region of the same area in the form of linear radiant extensions into the posterior cortex of the lens (Fig. 5b). Vacuoles frequently appear beneath the anterior pole of the capsule. The amount of this change is dependent upon the radiation dose and is more severe with neutrons than with X rays. The maximum changes seen at 14 months (X 80 and n 80 doses) consist of large opaque sunburst-like lesions covering the posterior pole (Figs. 5c and 5d) and extending two-thirds the distance to the equator of the lens. In addition, numerous opacities are seen extending to the equator. Associated with these lesions there is almost consistently a dense anterior polar opacity. Nuclear density is noted in all normal animals after the age of 10 months and is considered to be due to factors of age. Siblings of X-rayed and neutron-exposed animals exhibit no change other than this increased

*Consultant

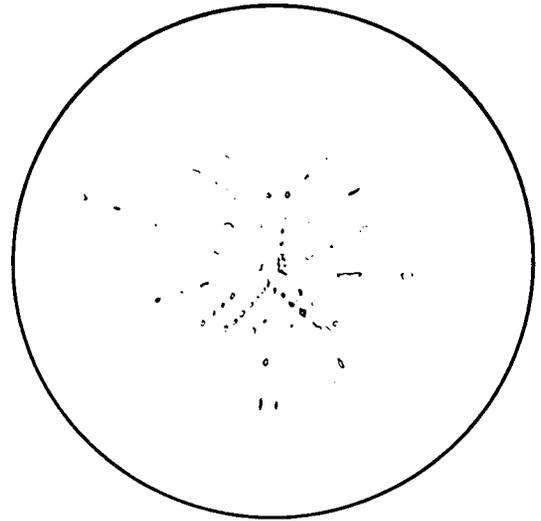
Fig. 5

- a. Pattern of early subcapsular opacity.
- b. Radiating stria in relation to subcapsular opacities.
- c. Frontal view of advanced cataract seen at 14 months after irradiation.
- d. Lateral view of advanced cataract seen at 14 months after irradiation.



A

EARLY SUBCAPSULAR PATTERN



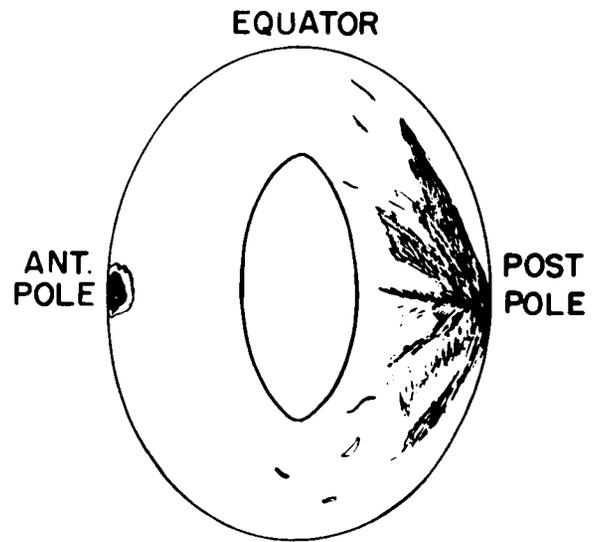
B

RADIATING STRIA IN RELATION TO
SUBCAPSULAR OPACITY



C

FRONTAL VIEW



D

LATERAL VIEW

MOST ADVANCED CHANGES AT
14 MO. X 80 N 80

FIG. 5

nuclear density (Figs. 6a and 6b).

Table 4 is a summary of the cataract incidence as of November 8, 1950. The trend shown in Table 4 indicates that (a) at 7 to 10 months of age specific lesions appear in the lens at all dose levels in all experimental animals; (b) at 4 to 6 months one half or more of the mice exposed to either slow neutrons or X rays develop opacities -- the greater the dose, the higher the per cent of these lesions; and (c) the preferential effect of neutrons as concerns cataract induction is clearly indicated in the last two groups (findings at 1 to 6 months). Neutrons induce cataracts sooner and in a higher per cent of mice.

The effect of X irradiation on erythropoiesis, plasma and cell volumes. (Storey, Wish of Radiobiochemistry, Andrews,* Bigelow, Knoohuizen, Beale, Click). A paper summarizing the results of experiments thus far performed will be presented at the coming meeting of the Southern Medical Association and will appear in its Journal. These studies suggest that the pathogenesis of postirradiation anemia is as follows.

After massive irradiation, erythropoiesis ceases almost immediately and is not resumed until after 7 to 14 days (as indicated by histological studies of the bone marrow and reticulocyte counts). During this time there is a fall in total red-cell mass as indicated by direct isotopic determinations. This fall is due, (a) to death of normally aging red cells with cessation of new cell formation, (b) to loss of red cells caused by heightened capillary permeability and secondary damage, and (c) possibly to other factors hitherto not recognized.

Simultaneous changes in plasma volume mask the magnitude of drop in erythrocyte mass. Consequently red cell counts, hematocrits, and hemoglobin values do not indicate the exact degree of postirradiation anemia.

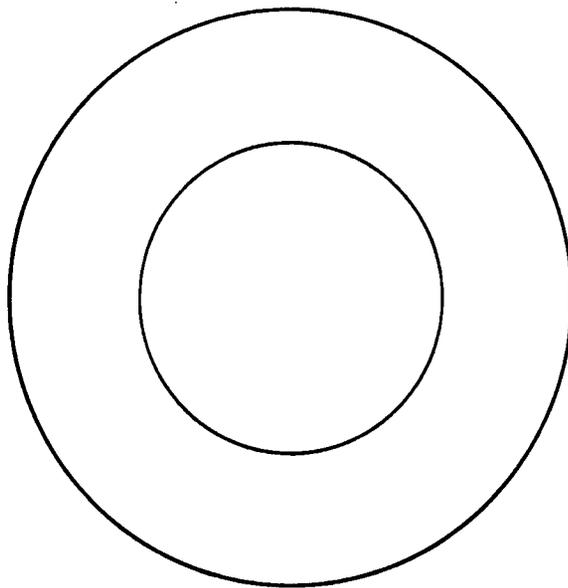
Experimentally, the heightened permeability of capillaries following irradiation is indicated by rapid disappearance of various substances introduced into the circulation. Phagocytic reticulo-endothelial function is not markedly altered by irradiation.

The hypothesis is advanced that continuous leakage of erythrocytes through damaged endothelium to tissue spaces and lymph channels is a major

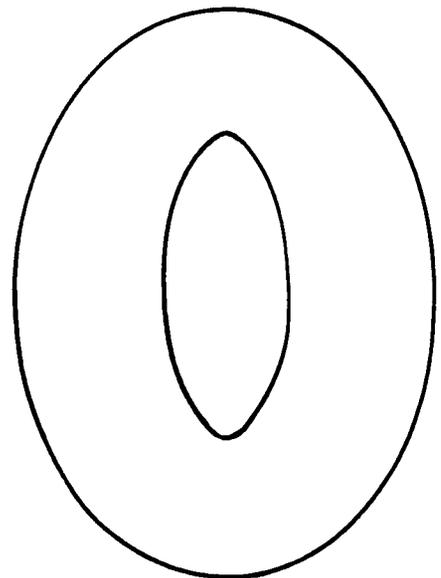
*Hematologist, ORINS.

Fig. 6

- a. Frontal view of the lens of 10- to 14-month-old normal mice showing a slight nuclear density.
- b. Lateral view of the lens of 10- to 14-month-old normal mice showing a slight nuclear density.



A
FRONTAL VIEW
NORMAL NUCLEAR DENSITY
10 MO. AFTER



B
LATERAL VIEW
NUCLEAR DENSITY
10 MO. AND AFTER

FIG. 6

TABLE 4

Incidence of opacities (cataracts) in mice exposed to slow neutrons and X rays

TIME AFTER EXPOSURE	EXPOSURE																	
	n 80*			X 80** (512 r)			n 20			X 20 (128 r)			n 5			X 5 (32 r)		
	No. of mice			No. of mice			No. of mice			No. of mice			No. of mice			No. of mice		
	Exam.	+	%+	Exam.	+	%+	Exam.	+	%+	Exam.	+	%+	Exam.	+	%+	Exam.	+	%+
11-14 months	15	15	100	9	9	100	9	9	100	9	9	100						
7-10 months	51	51	100	3	3	100	19	19	100	17	17	100	7	7	100	12	12	100
4-6 months	42	40	95	27	25	93	11	10	91	8	5	63	22	11	50	18	9	50
1-3 months	39	33	85	19	8	42	16	5	31	21	2	11	19	4	21	8	0	0

Controls: 42 controls ranging from 2-9 months of age were free from nuclear opacities.

*80 minutes exposure at a slow neutron flux of 9×10^9 per cm^2 per second with an estimated 4 to 8 r of high energy gamma per minute.

**80 minutes exposure to X rays at a dose rate of 6.4 r per minute. The factors of irradiation were as follows: 250 kvp, 12.5 ma, 3.0 mm Al inherent filtration, 3.5 mm Cu added, T.S.D. 74.8 cm.

factor in the causation of postirradiation shock and anemia.

Current research of this problem is proceeding in two directions.

1. (Woods, Storey) The degree of capillary permeability is studied in dogs through samplings of thoracic duct lymph. In a manner recently described by Brown and associates, but independently from them, Bigelow prepared in dogs lymphatic-venous anastomoses by introducing a cannula into the thoracic duct of dogs, another cannula into a branch of the jugular vein and exteriorizing the connecting loop for purposes of sampling. On such animals a comparative study is made of the normal chemical and cellular constituents of lymph and blood and of the rate of entry into the lymph of varied substances introduced into the blood stream.

2. (Kahn) The iron metabolism and the pathogenesis of radiation anemia in X-rayed and normal animals are being investigated. The experimental set-up is as follows. Homologous erythrocytes tagged with radioiron are injected intravenously in the same amount into (a) normal animals, (b) animals massively irradiated immediately before injection, and (c) animals receiving the same dose of X rays immediately after injection. Thus in groups b and c the animals receive the same dose of X rays but only in group c are the introduced cells irradiated simultaneously. The results thus far suggest that the increased red-cell loss from the blood of X-rayed animals is not due to a direct effect of X rays on erythrocytes but to some hitherto unknown effect on the host. Fig. 7 shows the result of such an experiment. It indicates there is a greater disappearance of ^{55}Fe activity from the blood of X-rayed animals than from that of normal rabbits. This is in conformity with numerous experiments already described, in which the disappearance rate of diverse substances was studied in parallel series in normal and X-rayed mice and rabbits.

The greatest loss of activity in the X-rayed rabbits was on about the thirteenth to seventeenth days. The recovery which follows may be explained by reutilization of the iron stored during the period of cessation of erythropoiesis. The fact that the final values (35 days) in X-rayed rabbits did not reach the level of the normals may be explained by assuming that some iron was lost during the period of depressed red cell production and/or, less likely, that erythropoiesis was still in a state of depression, or that some radioiron was still concentrated in iron depots.

Fig. 7

Fig. 7 shows the loss of ^{55}Fe activity from the blood of rabbits that had been injected with 4 cc of whole blood of the same donor whose erythrocytes were labeled by repeated injection of ^{55}Fe ammonium citrate. The base line is the average activity of the 10- and 30-minute samples. Two rabbits were X-rayed (850 r) immediately before, and one immediately after injection. The values of the latter are indicated by unconnected triangles.

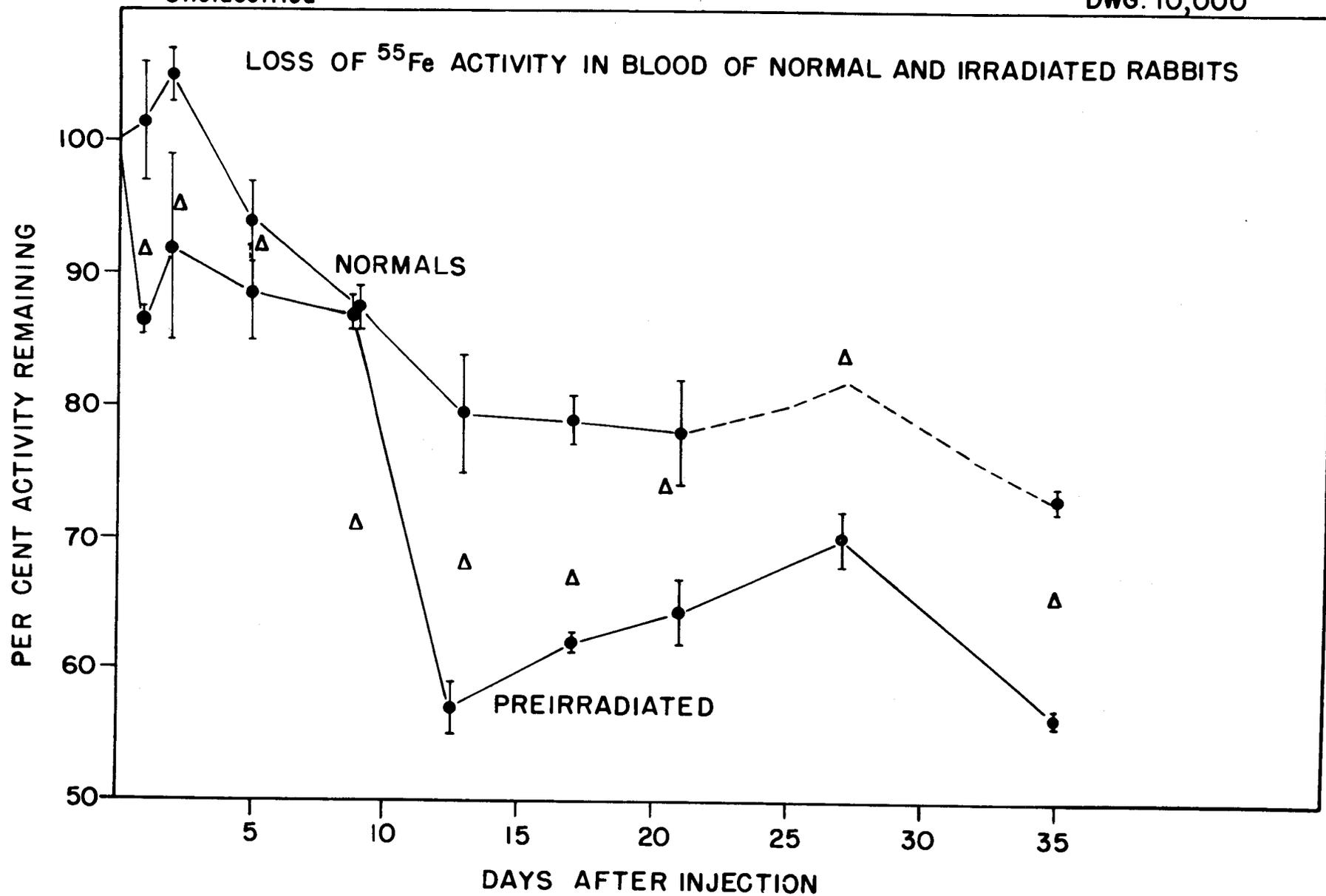


FIG. 7

Studies of neoplasms related to irradiation. (Furth) An extensive study on "Polycythemia with Features of Cushing's Syndrome Produced by Luteomas" made in association with R. G. Gottschalk in Dallas has been completed and will appear in *Acta Hematologica*. In this study the progressive changes of plasma and red-cell volumes of numerous mice bearing masculinizing luteomas and feminizing granulosa tumors were studied in relation to alterations of white- and red-cell counts and hematocrits. The observations were correlated with data from the literature on the effect of hormones on hemopoiesis and on blood volume.

It was found that masculinizing and feminizing ovarian tumors of the mouse have different hematological effects. Transplants of a luteoma produce features of Cushing's syndrome with polycythemia and masculinization. There is an absolute increase of the red-cell volume resulting in polycythemic hypervolemia. As testicular androgens, progestin or cortical hormones, all promote erythropoiesis, only actual isolation can identify the causative hormone.

Transplants of feminizing granulosa tumors, on the contrary, produce an increase of plasma volume and relative anemia. Erythropoiesis is not depressed. The hypervolemia is simple or oligocytic. The polycythemia of luteomas goes with stimulation of erythropoiesis, and marked rise of reticulocytes in the blood and extramedullary erythropoiesis, but with no excessive red-cell destruction.

Hormones appear to play a role not only in hemopoiesis but also in blood volume maintenance and the two are interrelated.

On the specificity of hypervolemia and congestive changes in tumor-bearing mice. (Moshman of the Mathematics Panel, Knoohuizen, Beale) The specificity of blood-volume changes in association with various neoplasms has interested us during the past few years. The salient data on the subject available thus far have been drafted in an article which is about to be submitted for publication.

Table 5 gives the significance of differences in blood volume and some supplementary hematological data between tumor-bearing and normal mice. A significant increase in blood volume is found in mice bearing transplantable

TABLE 5

Significance of differences between tumor-bearing and control mice by sex

MICE		CELL VOLUME		PLASMA VOLUME		TOTAL BLOOD VOLUME	PERIPHERAL HEMATOCRIT
		Direct	Indirect	Direct	Indirect		
Male	Ovarian cancer XIX	2.23	2.36	7.90	7.91	10.20	25.9
	Normal control	3.48	2.84	4.83	5.42	8.01	42.8
	Difference	-1.25	-.48	3.07	2.49	2.19	-16.9
	Probability	<.01	.02	<.01	<.01	<.01	<.01
Female	Ovarian cancer XIX	1.16	1.69	5.75	7.52	7.61	25.7
	Normal control	3.65	2.92	4.99	5.49	8.32	43.0
	Difference	-2.49	-1.23	.76	2.03	-.71	-17.3
	Probability	*	.12	.42	*	.48	<.01
Male & Female	Breast cancer M	2.43	1.59	11.19	17.07	13.39	13.75
	Normal control	2.45	2.29	4.64	5.10	6.57	36.51
	Difference	-.02	-.70	6.55	11.97	6.82	22.76
	Probability	.97	<.01	<.01	<.01	<.01	<.01
Male & Female	Breast cancer R		1.94	7.58		9.49	22.5
	Normal control		2.29	4.64		6.57	36.5
	Difference		-.35	2.94		2.92	-14.0
	Probability		.31	.04		<.01	<.01
Male	Granulosa tumor V	3.62	3.00	9.09	9.15	11.90	30.43
	Normal control	3.48	2.84	4.83	5.42	8.01	42.84
	Difference	.14	.16	4.16	3.73	3.89	-12.41
	Probability	.46	.48	<.01	<.01	<.01	<.01
Female	Granulosa tumor V	3.78	3.29	10.63	11.09	14.23	26.20
	Normal control	3.65	2.92	5.99	5.49	8.32	42.98
	Difference	.13	.37	5.64	5.60	5.91	-16.78
	Probability	.73	.20	<.01	<.01	<.01	<.01

*Probability unassessable due to single female reading.

ovarian carcinomas (Strain XIX), breast tumors (Strains M and R), and granulosa tumors (Strain V).

Common to all strains with hypervolemia is the drop in hematocrits and rise in plasma volumes, but there is this difference between the granulosa and the other tumors causing hypervolemia — the cell volumes are below normal with all but the granulosa strains. With the latter the mean cell volume is above normal, though the increase is not statistically significant. The conclusion is however, justified, that with granulosa tumors the plasma-volume rise is not secondary or compensatory to a drop in erythrocyte mass as might be the case with some of the other tumors.

All neoplasms thus far known to be associated with hypervolemia are either histogenetically related to "sex" hormone secreting cells or are tumors of the mammary gland — a target organ of sex hormones. The available data indicate that the hypothetical agent raising the blood volume (plethorin) is not a sex hormone, although it may be related to it or dependent on it.

MICROBIOLOGY

TRACER STUDIES ON METABOLISM

S. F. Carson (Leader)

E. H. Mosbach
E. F. Phares

Mary V. Long
Betty Ann Gwin

L. A. Nutting*
F. W. Denison, Jr.*

Anaerobic biosynthesis of lactate by C₂ + C₁ reaction. (Nutting, Carson). Thesis research culminating in the doctoral dissertation has been completed by Leighton A. Nutting, ORINS graduate fellow. Two publications based upon this work will be submitted shortly. A resume of the problem and some of the significant results follow:

The ubiquitous distribution of the pentose molecule in nature and particularly its presence in certain enzymes and in nucleic acids emphasizes the metabolic significance of these carbohydrates. In living systems the pentoses are undergoing continuous metabolic changes. It thus appeared that investigations concerning the metabolic decomposition of the pentose molecule would be important from a comparative biochemical point of view. The advantages of a microbial system as a working model for biochemical investigations are well known. The present investigations were, therefore, carried out with washed bacterial cell suspensions utilizing xylose as the sole substrate.

Previous investigators have obtained good evidence that one of the first reactions in the fermentation of pentoses was a carbon bond cleavage resulting in the production of a C₃ and a C₂ fragment.

The importance of the C₂ fragment in enzymatic systems is well recognized and it thus seemed possible that investigations on bacterial pentose fermentations would be of significant value to the field of intermediary metabolism. Preliminary investigations revealed that cells of *Escherichia coli* K-12 grown in the presence of pentose possessed the ability to ferment pentoses in the nonproliferating state.

* ORINS Fellow.

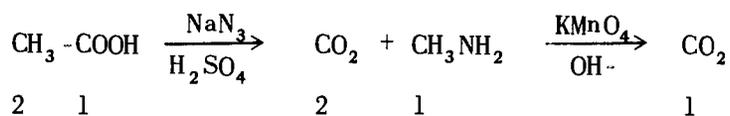
The results of the present investigations have re-emphasized the metabolic importance of the C_2 fragment. In fermentations conducted at low pH, lactic acid was produced in a ratio of approximately 1.3 moles per mole of xylose fermented. This was taken as a priori evidence that the C_2 portion of the C_5 molecule was involved in the formation of lactic acid. Furthermore, at low pH, there was a net fixation of carbon dioxide which indicated that a direct participation of carbon dioxide was involved in the production of lactate. There were a number of pathways by which lactate could have been formed from C_2 and carbon tracer experiments were conducted in order to determine the main mechanism of $C_2 \longrightarrow C_3$ in this system. These experiments demonstrated that C_2 tracers ($C^{14}H_3COOH$ and $C^{14}H_3CH_2OH$) were converted to the $CH_3-CHOH-$ portion of lactate whereas C_1 tracers ($C^{13}O_2$ and $HC^{14}OOH$) appeared in the lactate carboxyl. This latter piece of evidence was a further indication that lactate was formed through a $C_2 + C_1$ condensation. This condensation functioned at pH 7.4 as well as at pH 5.3. With $C^{14}H_3COOH$ as tracer (pH 7.4) succinate was labeled almost exclusively in the methylene carbons; as the lactate was labeled in the β carbon only, and not equally labeled in both the α and β carbons, it was concluded that the lactate was not in close equilibrium with succinate.

The production of lactate by $C_2 + C_1$ condensation further emphasizes the general role of this reaction in intermediary metabolism. The fact that C_2 produced from pentoses apparently can be converted to C_3 also provides a mechanism for the conversion of pentoses into hexoses and vice versa.

Kojic acid fermentation. (Denison, Carson). Kojic acid is one of the few heterocyclic compounds produced in excellent yield by the action of microorganisms. It is produced by *Aspergillus* species from C_2 to C_7 compounds; the fact that excellent yields of kojic acid can be obtained from a variety of substances (C_2 to C_7) makes this an excellent model system for studies concerning the mechanism of biosynthesis of a heterocyclic compound.

Three main lines of work have to be pursued: (1) production of kojic acid by resting cells, (2) methods for isolation and purification of the acid, and (3) chemical synthesis and degradation of labeled kojic acid. Excellent progress has been made on the first two, and discussions have been held concerning possible methods for the third part.

Kojic acid was isolated from culture filtrates on a celite column. Dilute sulfuric acid was the inside phase and CB-10 (chloroform, 90-butanol, 10)



Step 1 Reduction-amination of α -ketoglutaric acid to D,L-glutamic acid.
(Kögl *et al.*, Rec. Trav. Chim. 67:391, 1949)

An 0.11-g quantity of palladium-on-carbon catalyst (5 per cent palladium), was reduced with hydrogen in the presence of 7 ml of 5.4 per cent ammonia solution in a 50-ml flat-bottom flask fitted with a magnetic stirrer. After the prereduction was complete (about 1 hour) 0.44 g (3 mM) of α -ketoglutaric acid was added to the flask and the system refilled with hydrogen. No more hydrogen was taken up after 20 hours, and contents of the flask were filtered, washed, and made to 10 ml. A 2-ml aliquot of this was taken for assay with the ninhydrin reaction. The yield of glutamic acid was 1.2 mM or 57 per cent.

Step 2 Conversion of glutamic acid to butan-4-al-oic acid.
(Ber., 42:2360, 1909)

The remaining portion of the glutamic acid (1.41 mM) was evaporated to dryness under reduced pressure at 60-70° C. The residue was dissolved in 15 ml of water and cooled to 10° C. A 1.5-ml volume of sodium hypochlorite solution, 5 per cent available chlorine, was added, and the solution, now at pH 6-7, was dripped from an ice-jacketed funnel into a stream of steam. The aldehyde acid, which is nonvolatile due to immediate dimerization, was not assayed.

Step 3 Reduction of butan-4-al-oic acid to butyric acid: The aldehyde from the steam hydrolysis was treated by a modified Wolff-Kishner reduction.
(Huang-Minlon, J. Am. Chem. Soc., 68:2487, 1946)

One gram of potassium hydroxide pellets, 5 ml of 65 per cent hydrazine hydrate solution, and 25 ml of redistilled triethylene glycol were added to the solution containing the aldehyde acid. The mixture was refluxed for ½ hour, after which time the water was distilled off and the temperature allowed to rise to 190-200° C. The refluxing was resumed at this temperature for 1 hour, 10 ml of water was added, and the mixture was filtered. The filtrate was adjusted to pH 1.0 with 10 N sulphuric acid. The solution was subjected to a steam distillation, and all the volatile acid was collected in about 100 ml of distillate. The yield of butyric

acid was 0.52 mM, or 36.7 per cent based on glutamate and 17.3 per cent from α -ketoglutarate. The identity of the butyric acid was confirmed by Duclaux distillation, by partition chromatography and by the melting point of the *p*-bromophenacyl ester (62.0-63.5° C; theoretical 63.2° C).

Step 4 Degradation of butyric to propionic acid.

One-half millimole of sodium butyrate was evaporated to dryness and decarboxylated by the Schmidt reaction, using conditions similar to those described previously (Quarterly Report, ORNL-537) for degradation of propionic acid and acetic acid. The carbon dioxide was collected and determined as barium carbonate. The propylamine collected in 5 ml of dilute acid was transferred to a 50-ml flask, 5 ml of 5 per cent potassium permanganate added, and enough sodium hydroxide was added to make the pH about 12. The flask was stoppered and allowed to stand at room temperature for about 1 hour. The resulting mixture of acid was resolved on a partition column to 0.35 mM propionic acid and 0.04 mM acetic acid.

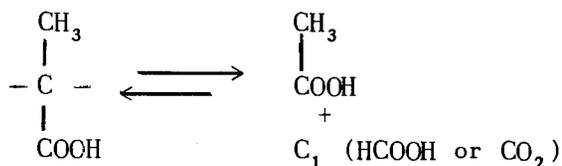
Step 5 The propionic acid was converted (Quarterly Report, ORNL-537) through ethylamine to acetic acid, and the latter in turn degraded to methylamine. The carbon dioxide from the carboxyl groups in each case were determined as barium carbonate. The methylamine was oxidized to carbon dioxide with potassium permanganate and likewise determined. Final yield of individual carbons from acetic acid was about 40 mg of barium carbonate, an ideal sample for radioactivity determinations as performed in this laboratory. This amounts to a yield from butyric acid to methylamine of about 5 per cent, and from α -ketoglutaric a yield of about 10 per cent.

It appears that yields of several steps of the degradation can be improved, allowing a smaller amount of starting material.

It is more feasible to determine the carboxyl next to the keto group by using a separate sample of about 0.2 mM α -ketoglutarate and decarboxylating with permanganate rather than collecting the carbon dioxide after the sodium hypochlorite treatment in *Step 1*.

Synthesis of C¹⁴-labeled α -ketoglutaric acid is in progress and the above degradation will be checked for clean separation and proper specific activities of each carbon atom.

Function of one-carbon intermediates in aerobic lactate formation by fungi. (Foster, Carson, Jefferson, Phares, Long). In all microbial systems thus far studied with respect to the following reversible reaction:



the C₁ component has been found to be in equilibrium with the carboxyl group only of the C₃ acid.

A rather different and interesting state of affairs was found during aerobic lactate formation by *Rhizopus* MX. Fasting cells were allowed to form lactate from glucose under vigorously aerobic conditions (for details of cell preparation and other conditions, Quarterly Report, ORNL-807, pp. 48-51). Both formate and carbon dioxide were used as tracers, the former labeled with C¹⁴ and the latter with C¹³.

The lactate was isolated from celite solvent chromatograms and degraded to individual carbon fragments, which were measured for both C¹³ and C¹⁴ specific activities. Following are the results obtained:

	C ¹⁴ c/s/mg BaCO ₃	C ¹³ Atom per cent
CH ₃ -CHOH-COOH	0.55	
CH ₃ -	1.38	1.073 ± 0.004
-CHOH-	0.07	1.108 ± 0.004
-COOH	0.10	1.238 ± 0.007

Fumarate was also isolated from the column, and degradation yielded the following results:

	C ¹⁴ c/s/mg BaCO ₃	C ¹³ atom per cent
COCH-CH=CH-COOH	0.23	1.25 ± 0.01
=CH-	0.34	1.104 ± 0.003
-COOH	0.11	1.40 ± 0.01

These results demonstrate that, in this case, formate donates to the methyl group of lactate, which is in contrast to previous results with all other microbial systems. The C¹³ data indicate that carbon dioxide is in equilibrium with the carboxyl group of lactate only. The experiments are being repeated.

Biosynthesis of acetic acid from carbon dioxide and hydrogen. (Carson, Gwin). It has been decided to use Wieringa's little-known, but nevertheless remarkable, *Clostridium acetium* as a model system to study the intermediates in biosynthesis of C₂, C₃ and C₄ compounds from C₁.

This organism is the only known biological system which almost quantitatively converts a C₁ compound (carbon dioxide) into a C₂ compound (acetic acid). The equation follows:



Yield of acetic acid = 92 - 97 per cent

Clostridium acetium is a very strict anaerobe; it grows and metabolizes only at an unusual pH (9.0) and has complex and difficult growth requirements (i.e. substances). It has been possible to find a substitute medium which serves quite as well as Wieringa's special extracts of Dutch canal mud. The medium now consists of 0.5 per cent dextrose, 0.1 per cent yeast extract, 0.1 per cent malt extract, minerals, 1 per cent sodium bicarbonate and 0.1 per cent sodium sulfide.

Resting-cell suspensions can now be prepared and will be used for the tracer experiments.

BIOCHEMISTRY

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

W. E. Cohn (Leader)
D. G. Doherty
E. Volkin
B. L. Strehler

M. Helen Jones
J. X. Khym
E. A. Lloyd
F. Vaslow

Composition and structure of nucleic acids. (Volkin, Cohn, Khym, Jones). This problem requires quantitative isolation, identification, and assay techniques for each of the degradation products of nucleic acids. The (ion-exchange) isolation techniques for mononucleotides are good but most of the products of ribonuclease digestion are polynucleotide in nature, requiring the development of new isolation techniques or the improvement of older ones. Work on this phase is in progress. With regard to identification and assay procedures, the characteristic spectrophotometric absorption ratios and extinction coefficients for the nucleotides, nucleosides, and bases have been carefully determined (Cohn). This work, which will soon be prepared for publication, is partially summarized in the Table 6. A third phase is the preparation of fairly large quantities of purified and characterized nucleic acids from various sources; this is under way (Volkin).

The products of enzymatic hydrolysis of ribonucleic acids. (Volkin). Investigations are in progress which attempt to isolate and characterize the products of enzymatic digestion of ribonucleic acids (RNA). It is anticipated that such an approach will lead to more complete knowledge of the structure of the intact nucleic acid. Though the work is still in the preliminary stages, the data described serve as an example of one such experiment.

Purified RNA, prepared from dried, viable yeast by a mild technique avoiding the use of strong alkali or acid, was hydrolyzed with crystalline ribonuclease to the completion of the reaction. The digest was dialyzed in the cold against 20 volumes of distilled water for 72 hours. It was found that 6 per cent (in terms of spectrophotometric units) of the nuclease-hydrolyzed RNA was dialyzable.

The nondialyzable material was adjusted to pH 3.5 with acetic acid at which point a precipitate formed. The supernatant solution was fractionated with ethanol, precipitates developing at alcohol concentrations of 35 and 80 per cent.

TABLE 6

Spectrophotometric extinction coefficients and ratios for nucleic-acid derivatives

pH →	MICROMOLECULAR EX- TINCTION COEFFI- CIENT AT 260 m μ			RATIOS OF OPTICAL DENSITIES								
	2	7	12	250/260			280/260			290/260		
				2	7	12	2	7	12	2	7	12
Adenosine	14.2	14.8	14.8	.83	.78	.78	.215	.144	.144	.03	.002	.002
Adenylic (a and b)	14.2	15.0	15.0	.85	.80	.80	.225	.15	.15	.038	.009	.009
Desoxyadenylic	14.5	15.3	15.3	.83	.78	.78	.235	.15	.15	.038	.009	.009
Adenine	12.7	13.3	10.2	.76	.76	.57	.375	.125	.60	.035	.005	.025
Hypoxanthine	7.7*	8.1	11.0	1.40*	1.32	.78*	.07*	.092	.14*	.005	.010	.015
at pH 10.5			11.0			.84			.124			
Inosine	7.4	7.4	12.1	1.68	1.68	1.05	.24	.25	.18	.025	.025	?
Guanine	8.2	7.4	6.65	1.37	1.42	.93*	.84	1.04	1.15	.495	.54	.59
Guanylic (a and b)	11.8	11.8	11.8	1.02*	1.15	.89	.68	.68	.60	.41*	.285	.11
Desoxyguanylic	11.8	11.8	11.8	1.04*	1.15	.89	.70	.68	.60	.47*	.285	.11
Guanosine	11.8	11.8	11.8	1.02*	1.15	.89	.67	.67	.61	.40*	.275	.13
Xanthine	8.15	7.5*	4.4*	.58	.68*	1.20*	.68*	.75*	1.95*	.08	.20*	1.40*
at pH 10						1.29			1.71			.92*
Xanthosine	8.7	7.9	7.9	.75	1.29*	1.30	.28	1.10*	1.13	.03	.58*	.61
at pH 8						1.30			1.13			.61
Thymine	7.4	7.4	3.7	.67	.67	.65	.53	.53	1.31	.09	.09	1.41
Thymidylic	8.4	8.4	6.7	.64	.64	.74	.72	.72	.67	.23	.23	.165
Thymidine	8.4	8.4	6.7	.65	.65	.75	.715	.715	.655	.235	.235	.16
Uridine	9.9	9.9	7.3	.74	.74	.83	.35	.35	.29	.03	.03	.02

TABLE 6 (Cont'd)

pH →	MICROMOLECULAR EX-TINCTION COEFFI-CIENT AT 260 mμ			RATIOS OF OPTICAL DENSITIES								
				250/260			280/260			290/260		
	2	7	12	2	7	12	2	7	12	2	7	12
Uridylic { a } { b }	10.0	10.0	7.4	{ .80	.78	.85	.28	.30	.25	.03	.03	.02
				{ .75								
Uracil	8.2	8.2	4.1	.84	.84	.71	.175	.175	1.40	.01	.01	1.27
Cytosine	6.2	5.7	4.95*	.48	.78	.76*	1.53	.58	.80*	.78	.08	.32*
Cytidine	6.2	7.4	7.4	.45	.86	.86	2.10	.93	.95*	1.55	.28	.31*
Cytidylic { a } { b }	6.8	7.6	7.6	{ .48	.90	.90	1.80	.85	.85	1.22	.26	.26
				{ .45								
Desoxycytidylic	~6.5	~7.6	~7.6	.43	.82	.82	2.12	.99	.99	1.55	.30	.30
5-Methyl-cytidylic	~4.8	~7.6	~7.6	.36	.96	.96	3.15	1.52	1.52	3.4	1.02	1.02

51

* Rapid shift in values with pH; unreliable without exact pH control.

The dialyzable fraction of the digest was partially resolved by ion-exchange chromatography. Large amounts of the RNA cytidylic and uridylic acids were found to be enzymatically hydrolyzed to free mononucleotides; whereas, of the purine nucleotides, only a slight amount of free guanylic was present. After removal of the mononucleotides from the column, a well-defined elution peak of a polynucleotide was eluted with 0.01 *N* hydrochloric acid. A polynucleotide with similar elution characteristics has been observed from nuclease hydrolyzed mammalian ribonucleic acids. The polynucleotide fraction remaining on the column was eluted with 2 *M* sodium chloride, 0.01 *N* hydrochloric acid. Under these conditions, two large, ill-defined polynucleotide peaks were removed. The material in the first peak was fractionated with alcohol, yielding precipitates at alcohol concentrations, 40 and 85 per cent. The polynucleotide contained in the second peak was precipitated only with 85 per cent alcohol. Some loss of polynucleotide soluble in 85 per cent alcohol was observed with both fractions.

All the isolated polynucleotide fractions (dialyzable and nondialyzable) were then hydrolyzed to mononucleotides by digestion with 0.5 *N* sodium hydroxide at 38° C for 17 hours. The nucleotide compositions of these fractions were then determined by ion-exchange analysis.

Table 7 summarizes the results of such an analysis. If it is assumed that increasing molecular size of the polynucleotides is reflected by (a) nondialyzability, (b) strength of binding to the exchange resin, and (c) ease of precipitability, then the following conclusions are evident from the data shown in the table: (1) Ribonuclease releases as free mononucleotides a large percentage of the RNA pyrimidines, (2) with increasing molecular size of the polynucleotide degradation products, the purine/pyrimidine ratio increases, and (3) the polynucleotide of largest size (nondialyzable, pH 3.5 insoluble) consists almost entirely of purine nucleotides.

Effect of gamma radiations on chymotrypsin. (Doherty). Previous studies carried out on dilute solutions of α -chymotrypsin showed this enzyme to be very resistant to radiation. A maximum inactivation of about 30 per cent was obtained at a dosage of 80,000 r. In order to try higher dosage levels dry samples of chymotrypsin were irradiated. The samples were sealed in polyethylene bags, cooled in dry ice and irradiated at the rate of 0.14×10^6 r/sec. The dosages administered were 1×10^5 , 5×10^5 , 1×10^6 , 5×10^6 , 1×10^7 r. The activity of the enzyme was determined as previously described.

TABLE 7

Products of hydrolysis of yeast RNA by crystalline ribonuclease

FRACTION (In order of increasing size)	MOLAR RATIOS					PER CENT OF TOTAL SPECT. UNITS	
	MONONUCLEOTIDE				PURINE/PYRIMIDINE		
	CYTIDYLIC	ADENYLIC	URIDYLIC	GUANYLIC			
Whole yeast RNA	1.0	1.16	1.43	1.50	1.09		
Dialyzable						67	
Free mononucleotides	66%*	0	80%*	6%*	0.05	23	
Polynucleotides eluted with 0.01 N HCl	2.71	1.05	1	2.55	0.97	7	
Eluted with 2 M NaCl, 0.01 N HCl	Peak 1						
	Pptd with 85% alcohol	0	2.26	2.16	1	1.51	9
	Pptd with 40% alcohol	1	3.75	6.01	9.87	1.94	8
Peak 2							
Pptd with 85% alcohol	0	1	1.26	1.85	2.26	12	
Loss						8	
Nondialyzable						33	
Pptd with 80% alcohol	1	3.68	1.37	3.10	2.86	10	
Pptd with 35% alcohol	1.66	6.98	1	7.21	5.33	8	
Isoelectric, pH 3.5 pptd	1	4.65	0	7.15	11.80	15	

*% of total corresponding mononucleotide in RNA.

The results obtained were partly similar to those obtained in solution although the dosage level was much higher (Fig. 8). The inactivation up to 200,000 r was similar to the results obtained with solutions in that the activity of the enzyme was reduced at a very slow rate. Above this value the activity fell off rapidly to about 35 per cent of the original at 1×10^7 r. The K_m of the enzyme remained unchanged throughout, indicating a complete destruction of the activity rather than a small alteration of the active center. Physically, one could judge the dosage by the color of the samples, the 1×10^7 r being definitely cream colored compared to the controls.

Enzyme-substrate equilibria. (Vaslow) The binding of acetyl-L-dibromotyrosine by chymotrypsin that had received 10^7 r of electron radiation was measured. The binding based on the total weight of irradiated material was about one-fourth that of the nonirradiated material, however on the basis of nondialyzable protein material, the binding was about equal to that of un-irradiated chymotrypsin.

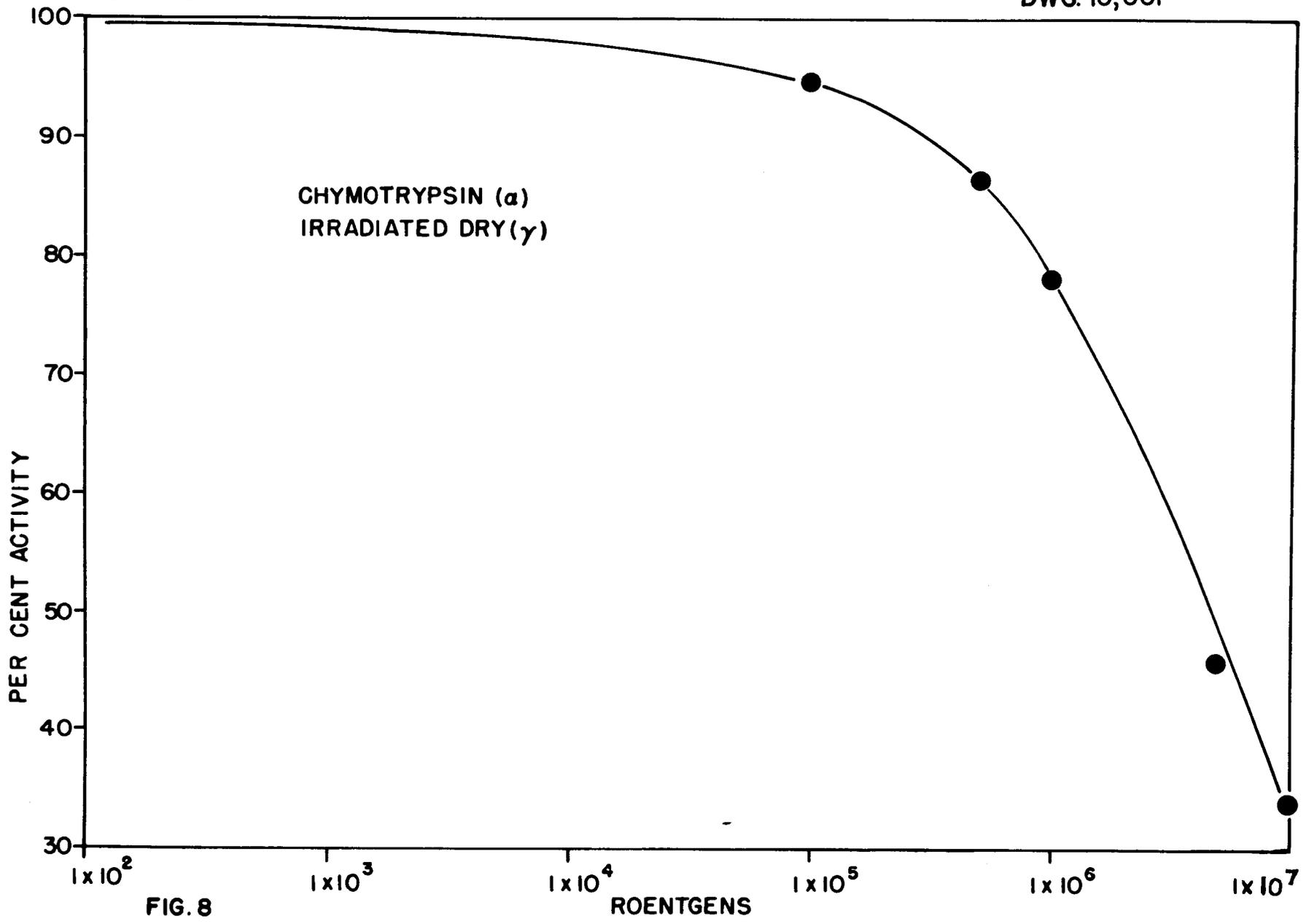
If the carboxyl OH group of acetyl-L-dibromotyrosine is replaced by a methyl group, a ketone is obtained which is an inhibitor for chymotrypsin. This compound presumably has two of the three normal points of attachment to the enzyme and does not have a bond that is split by the enzyme. The thermodynamics of binding of this compound will be studied and compared with the binding of a normal substrate in order to obtain more specific information on the method of action of chymotrypsin. Work has been going on in cooperation with Dr. Doherty on methods for the preparation and purification of radioactive bromine-labeled ketone.

Studies on firefly luminescence. (Strehler). 1. *Attempts at synthesis of luciferesceine.* The work in progress at the time of the last report was continued. A number of other model pterin compounds were synthesized including isoleukopterin derivatives. They include 2-hydroxy, 4-amino-8, 9-dioxypiperidine, 2-hydroxy-4-amino-9-hydroxy propyl carboxylic acid as well as the mesoxalic ester condensate with the corresponding triamino hydroxy pyrimidine.

2. *Application of luminescent system to energy-rich phosphate assay.* The firefly luminescent system was used to assay ATP production by rabbit liver homogenates with various added Krebs cycle intermediates as substrates. Succinic acid and α -ketoglutaric acid were found to be good substrates for ATP production while fumarate, malate and citrate were effective only in the

Unclassified

DWG. 10,001



55

FIG. 8

ROENTGENS

presence of pyruvate. Pyruvate was ineffective in the absence of fumarate or malate. The following table summarizes the results.

SUBSTRATE	ATP PRODUCTION
Succinate	+ + + +
α -Ketoglutarate	+ +
Fumarate, malate, or citrate	0
Fumarate, or malate, or citrate + pyruvate	+ +
Pyruvate	0

RADIOBIOCHEMISTRY

C. W. Sheppard (Leader)	E. B. Darden, Jr.
W. T. Burnett, Jr.	Marian Stewart
J. S. Kirby-Smith	Leon Wish
Arthur Burke	M. L. Morse

Infrared studies. (Kirby-Smith). Investigations on the biological effects of infrared radiation are planned as a continuation of work previously carried out by personnel of this laboratory on the effects of infrared radiation in combination with X rays or ultraviolet. A monochromator for use in the infrared aspects of the problem has been ordered. Long range plans for the application of this instrument to infrared microspectroscopy are being formulated.

Alpha-particle microsources. The development of alpha-particle microsources is in progress. Present indications are that microsources can be developed which will permit the quantitative irradiation of single cells or specific portions of single cells.

Contaminating gamma radiation in the slow-neutron facility. (Darden, Sheppard). The investigation begun by Mr. L. C. Emerson under the Health-Physics Training program has been continued. Following a suggestion by Dr. Snell, an investigation of the ionization effect in the beryllium ion chambers produced by the n-p reaction on the air filling was instituted. The thermal neutron capture cross-section for this reaction is relatively large. Preliminary calculations indicated that the added ionization intensity due to this reaction cannot be neglected. Accordingly, the chamber was modified to permit

it to be filled with various types of gases and the following relative intensities obtained.

Air	100 per cent
O ₂	88 per cent
N ₂	97 per cent
He	84 per cent

With helium, difficulty was experienced in charging the chambers up to about one-third full scale potential (400 volts full scale) with the Victoreen electrometer employing friction charge.

As a means of comparison a similar experiment was carried out with the chamber exposed to the high intensity ⁶⁰Co source with the following results:

Air	100 per cent
O ₂	103 per cent
N ₂	97 per cent
He	84 - 116 per cent

Again difficulty was experienced with He both in charging and in obtaining reproducible intensities as shown. The result clearly shows that the major effect is not due to the n-p reaction but that the latter effect does occur to an extent of about 10-12 per cent of the chamber reading.

The possibility of gamma radiation arising from a contaminant in the bismuth composing the safe surrounding the exposure box was largely discounted when it was observed that a sleeve made of what is believed to be the same lot of bismuth caused a 14 per cent reduction in intensity in the air-filled chamber.

Any effect due to the presence of the graphite exposure box was eliminated by attaching the chamber by means of a teflon jig directly to the slab constituting the lower face of the carrier higher than the corresponding intensity inside the exposure box.

In some early studies, by Stapleton, of the nature of the gamma contamination absorption curves were obtained using a Victoreen chamber surrounded by lead absorbers inclosed in a boron plastic box. However, the Victoreen itself

as well as aluminum attachments on the outside of the box constituted possible sources of unwanted radiation. A larger box of the same material has been constructed with attachments of teflon and bakelite in which it is planned to repeat the experiment using the beryllium chamber and greater thicknesses of absorbers. These absorbers which will have a maximum thickness of almost 4 cm are now in the process of fabrication.

The difficulty of using the chambers when filled with helium led to an investigation of their saturation characteristics. At present a coupling device is being constructed to enable the chambers to be used with our standard gamma thimble chamber extension tube and vibrating reed electrometer assembly so that the variation of ion current with applied chamber voltage can be studied.

Irradiation of peanuts. (Darden, Noggle of Plant Physiology). The work conducted last summer on gamma and X irradiation of peanuts with the Plant Physiology group was completed. Absorption and scattering in thick and thin layers of material were studied in the ^{60}Co source to determine the effect on the intensity of the dose delivered. It was concluded that the biological discrepancies observed between the γ - and X-rayed seeds could not be attributed to differences of more than ± 5 per cent in the total γ - and X-ray doses.

Erythrocyte studies. (Stewart). Methods are being developed for investigating the presence or absence of an oxygen effect in the irradiation of human erythrocytes. It was decided to add to the preliminary work done by Gertrude Beyl a method for the direct determination of the oxygen tension in the cells by the spectrophotometry of the hemoglobin.

A convenient method has been perfected for equilibrating blood in luster-oid tubes whose ends are closed by rubber diaphragm stoppers. Blood is removed from the tubes for spectrophotometry by piercing the rubber with a needle. In this way laboratory air contamination is avoided. Thin lucite absorption cells have been developed for use in a Coleman spectrophotometer and a method has been found for determining the oxygen saturation by measuring the ratio of the absorption at two wave lengths; thus correcting for the variable thickness of absorption of the cell, sedimentation of the blood, and the scattering of light by the erythrocytes. In order to demonstrate the absence of methemoglobin formation a modification of the method of Chase, Lorenz,

Parpart, and Gregg will be employed. Using the spectrophotometric methods, we have begun a series of determinations of the time necessary to achieve an equilibrium saturation of oxygen in blood. Irradiation experiments will follow in the near future.

Radiomanganese studies. (Burnett; Bigelow, Consultant to Pathology Section). It has been previously shown that labeled manganese appears in the pancreatic juice of dogs within one-half hour after intravenous administration. The 24-hour totals for ^{54}Mn secreted by dog 3 over a period of 12 days, given as per cent of the initial intravenous dose, are given in Table 8, together with the 24-hour totals for the volume of pancreatic juice. A mathematical expression has been developed to describe these data.

TABLE 8

DAYS AFTER INJECTION (t)	TOTAL SECRETION (ml) (x)	^{54}Mn SECRETION (% of I.D. $\times 10^3$) (y)
1	68.5	47.7
2	93.0	41.7
3	69.5	34.7
4	63.2	27.5
5	127.0	45.1
6	99.8	29.8
7	75.8	28.4
8	98.6	31.7
9	101.3	23.5
10	47.0	8.2
11	77.5	13.8
12	100.0	16.3

Correlation coefficients computed by the product-moment method indicate that there is a positive correlation between the ^{54}Mn recovered and both the protein and nonprotein nitrogen fractions of the pancreatic juice, all the observed correlations differing significantly from zero at levels of less than 1 per cent. The computed coefficients are given in Table 9.

Dr. Kimball of the Mathematics Panel has given invaluable assistance with the statistical aspects of this study.

TABLE 9

TIME	CORRELATION COEFFICIENT BETWEEN ^{54}Mn AND	
	PROTEIN N	NPN
12 days	0.553	0.544
1st. 4 days	0.786	0.783
2nd. 4 days	0.850	0.650
3rd. 4 days	0.312	0.377

Colloids and radioactive iron. (Wish, in cooperation with Pathology group). Among the basic changes noted in man and animals that have been exposed to massive irradiation are the exaggerated phagocytic activity and the increased permeability of the capillary wall. Neither of these is well understood at the present time. In the initial experiments previously reported all tests were complicated by what appeared to be an increased capillary permeability. These studies were originally undertaken to determine more precisely the effect of ionizing radiation on the reticulo-endothelial function. It became desirable, therefore, to study quantitatively the rate of disappearance from the blood of various substances labeled with radioactive isotopes and to determine their fate by quantitative assays for their presence in different organs by means of isotopic measurement. Some of these substances are plasma and gelatin labeled with ^{131}I , colloidal ^{198}Au , and erythrocytes labeled with ^{32}P .

PLANT PHYSIOLOGY

G. R. Noggle (Leader)

J. H. Taylor*

G. M. Cheniae

L. P. Zill

M. Eleanor Schumacher

Plant biosynthesis. (Noggle, Schumacher, Cheniae). Work has continued on the production of C¹⁴-labeled compounds by plant biosynthesis. A number of sucrose samples have been isolated varying in activity from 0.064 to 4.05 mc/mg sugar. A total of 11 g of sucrose containing 2.7 mc of C¹⁴ were isolated and crystallized. Several samples of glucose, fructose, and raffinose were also isolated.

A study is being made on the identification and isolation of amino acids from plant materials. Both ion-exchange and chemical methods are being used.

An attempt is being made to work out a unified scheme of extracting and isolating compounds prepared by biosynthesis. This will involve an initial separation of pigments and lipids, followed by organic acids, amino acids, and carbohydrates.

A number of plants are being studied as possible sources of inositol. A yeast-assay method has been set up for determining inositol and the plants will be analyzed at various stages of growth.

Yeast biosynthesis. (Zill). For convenience, this project may be divided into two phases: (1) the incorporation of C¹⁴ in yeast cells by the culture of yeast on C¹⁴-labeled substrates to provide stocks of material for the preparative isolation of desired compounds, and (2) the application and combination of experimental techniques to the isolation of such compounds. Part 2 has been approached from the standpoint of obtaining an efficient combination of techniques so that a maximum recovery of incorporated C¹⁴ will be realized for each yeast sample processed. Up to the present time, effort has been directed toward the ultimate isolation of labeled ribonucleic acid, ribonucleotides, inositol, and trehalose (glucose- α -glucoside with a 1,1 linkage).

1. Culture of yeast on C¹⁴-labeled substrates. The yeast, *Torulopsis utilis* var. major, was chosen for culture since it can readily utilize the labeled substrates which are available. Although this yeast can grow on a simple

* Consultant.

salts and sugar medium (modified Williams media) it was found more satisfactory to use a water-soluble vitamin supplement in order to obtain more rapid growth. To obtain the growth characteristics of the yeast, several cultures were grown on nonlabeled substrates. Yeast obtained from these cultures was utilized for the preliminary development of the second part of this study. Nine-liter cultures of yeast were grown at 27° C for 48 hours with continuous, vigorous aeration (ca 150 cc cotton-filtered air per minute). The yeast was then harvested by centrifugation, washed with distilled water, and dried by lyophilization. The yield of dried yeast was 20-25 g per 9 liters of culture.

Incorporation of C¹⁴ by yeast cells was accomplished in the following manner: yeast cultures were grown by the method described above, harvested and washed with distilled water. The yeast was then resuspended in distilled water (183 g in 1500 cc distilled water) and starved for 16 hours at 27° C. The culture flask was then incorporated into a closed system having six sodium hydroxide traps for the capture of labeled respiratory carbon dioxide. The system was so arranged that the aeration gases were captured in successive traps at half-hour intervals for 3 hours. This system was used to determine the time relationship of labeled C¹⁴O₂ production and to determine the feasibility of using a flow counter in future experiments of this type.

The labeled substrate which was used in this experiment consisted of 2 mc of carboxyl-labeled acetate in the form of the sodium salt. The pH was adjusted to neutrality just before addition to the culture (at zero time). At the end of 3 hours the yeast was harvested and dried. Forty-three grams of labeled yeast were obtained in this particular experiment. Although a rough assay of the sodium hydroxide traps indicated that a significant amount of labeled carbon dioxide has been formed, there was considerable incorporation of C¹⁴ by the yeast cells (also by rough assay). Accurate assays on all samples are in progress.

2. Development and combination of techniques for the isolation of trehalose, inositol, ribonucleotides, and ribonucleic acid. The following procedures have all been carried out on nonlabeled yeast cells. Procedures which have been shown to be applicable in these runs will then be applied to the isolation of the labeled components in "hot" yeast.

a. Trehalose and ribonucleic acid. The presence of trehalose in yeasts affords a ready source of a disaccharide which, by hydrolytic cleavage, will

yield 2 moles of glucose. It is expected that by means of the C¹⁴ incorporation technique described, labeled trehalose (and by hydrolysis, glucose) will be found in the yeast. A preliminary extraction of the trehalose in the yeast, *T. utilis* var. major, indicated a trehalose content of less than 1 per cent (ca 700 mg/100 g dry yeast). This value is being checked by repetition of the isolation. Other yeasts are available having trehalose content as high as 16-18 per cent. As soon as starter cultures of these yeasts are obtained, they will be used as a more suitable source of the sugar. Trehalose is obtained from the yeast by an alcoholic extraction and subsequent purification. The residue from the alcoholic extraction is then used for the isolation of ribonucleic acid. This has been carried out by the method of Chargaff (Chargaff, *et al.*, J. Biol. Chem., 186:51, 1950). The yield by this method is rather low and consequently other methods will be investigated for higher yields.

b. Inositol and ribonucleotides. The free form of inositol is present in yeast to a very limited extent. The largest part of the inositol is found in the phospholipid fraction in the bound form. Several attempts at isolation of inositol from this fraction have demonstrated that the use of yeast as a source of inositol is not feasible.

Since the use of labeled inositol will undoubtedly provide evidence for its metabolic role, the desirability of obtaining a labeled inositol is great. Other sources and methods are to be investigated. The yeast fraction remaining after the extraction of acid-soluble and phospholipid fractions was hydrolyzed and used as a source of ribonucleotides in good yield by means of the ion-exchange separation developed by Cohn (Cohn, W. E., J. Am. Chem. Soc., 72:1471, 1950).

Protein fractions obtained by use of the above procedures will be reserved for possible future isolation of labeled amino acids.

BIOPHYSICS

PHOTOSYNTHESIS

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

Luminescence of green plants. (Strehler, Arnold). In an attempt to assay for adenosinetriphosphate production by poke-weed chloroplasts during illumination, it was found that green plants produce light for some time after they are illuminated. The properties of this light and the conditions of its production have been studied in some detail. Briefly, the following findings have been made.

The production of light by green plants saturates at high light intensities just as does photosynthesis. The action spectrum parallels the photosynthesis action spectrum while the reaction shows a typical enzymatic temperature dependence and is inactivated by treatment at 50° C for a few seconds. Illumination at one temperature and light production measurement at another indicate that light energy may be absorbed and stored even at very low temperatures.

Carbon dioxide depresses the production of light reversibility. The effect of various inhibitors such as dinitrophenol, hydroxylamine, menadiione, potassium cyanide, sodium azide, sodium fluoride, thymol and ethyl alcohol has been investigated. Ultraviolet light also inhibits light production to about the same extent as it inhibits photosynthesis. Attempts to measure the color of the light have been made and preliminary studies indicate that it is about the same color as chlorophyll fluorescence. Its intensity is approximately a thousandth of the fluorescent light or about 1.5×10^{-6} of the incident light below saturation. Finally, the decay curves of the light have been studied at various temperatures.