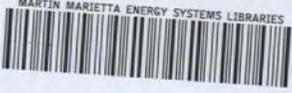


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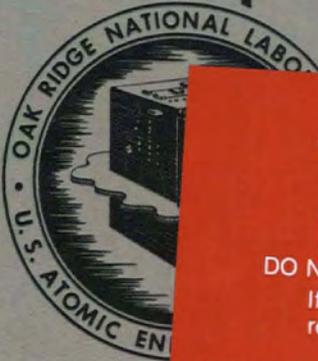
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QUARTERLY PROGRESS REPORT

for Period Ending February 10, 1952

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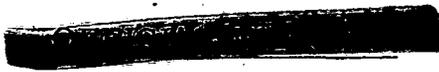


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**BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT
for Period Ending February 10, 1952**

Alexander Hollaender, Director

Edited by E. J. Slaughter

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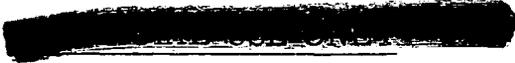
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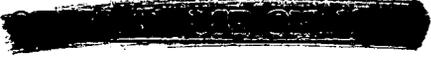




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REVIEW OF RESEARCH PROGRESS

Alexander Hollaender

Cytogenetics. — Several groups in the Division are continuing studies on the mechanism of radiation protection. There is now fairly conclusive evidence that hydrogen peroxide, or some organic peroxides, is a consequential factor in the X-ray sensitivity of Paramecium. Humidity plays an important role in the oxygen sensitivity of isolated pollen grains of Tradescantia, low humidity rendering the pollen grains very much more sensitive to oxygen than high humidity. Studies on Neurospora have shown that oxygen will increase the lethal rate of conidia and it appears, from the data, that the effect is extranuclear. A temperature recovery effect appears after exposure of diploid yeast to X rays, similar to one observed in E. coli. Further studies on the effect of oxygen and X-ray sensitivity of Drosophila have revealed that the temperature effect varies with different gases. The increased solubility of oxygen at lower temperatures cannot account for the entire increase of mutation rate at low temperature in oxygen. Some separation of the indirect from the direct effect of X rays in E. coli has been accomplished by means of the recovery phenomena. Bacteria irradiated in oxygen show a much higher rate of recovery at low temperatures than those irradiated in nitrogen. Observations on the effect of X irradiation on respiration of bacteria show that, in general, the respiration is not affected immediately after irradiation, but decreases below the control an hour or two after exposure. This decrease can be modified by means of the medium in which the organisms are incubated, effects of media differing for different strains of E. coli.

A survey of the effects of different metallic ions on all X-ray sensitivity of E. coli produced no new information. The effects of metal ions on the function of certain enzymes are known; e. g., copper increases the protective activity of BAL. A survey of flavanoids for their protective ability is now in progress.

Mammalian Genetics. — Several investigations are drawing on the excellent material resulting from high mutation rates produced by X rays in mice. Increase of X-ray dosage, up to 1000 r, and also decrease of the energy levels will be made in an effort to determine linearity of mutation rate with dosage. It appears that the genetic constitution of mice has a definite relation to the threshold dose at which embryonic changes are produced by X rays.

Microbiology. — The program of the Fifth Annual Biology Research Conference in Oak Ridge, sponsored by the Biology Division and supported by the Atomic Energy Commission, is given. This Conference deals with problems of special importance to the Microbiology Section. Sessions of this Conference on "Some Aspects of Microbial Metabolism" will again be held in the East Lounge of Ridge Recreation Hall. Dates for the Conference will be April 10 and 11, 1952.

Thursday, April 10 - 9:30 a.m.

C. B. van Niel, Stanford University

"Introductory Remarks on Comparative Biochemistry of Microorganisms"

W. W. Umbreit, Merck Institute

"Terminal Respiration Systems"

Thursday - 1:30 p.m. - ACETYL COENZYMES, TRANSACETYLASES, AND KETO ACID OXIDASES

Chairman: F. Lipmann, Massachusetts General Hospital

G. D. Novelli, Massachusetts General Hospital

"Studies Concerning the Structure of Coenzyme A"

E. R. Stadtman, National Institutes of Health

"The Enzymatic Synthesis of Acyl-CoA Compounds"

I. C. Gunsalus, University of Illinois

"Pyruvate Oxidation Factor: Nature and Mechanism of Action"

Thursday - 7:30 p.m. - METABOLISM AND RADIATION PROTECTION

Round-table discussion, led by Alexander Hollaender

This session will be followed by the Smoker.

Friday - 9:00 a.m. - NEW PATHWAYS IN THE BREAKDOWN OF CARBOHYDRATES

Chairman: W. D. McElroy, McCollum-Pratt Institute, Johns Hopkins University

B. L. Horecker, National Institutes of Health

"The Role of Sedoheptulose Phosphate in Pentose Phosphate Metabolism"

W. A. Wood, University of Illinois

"Alternate Pathways of Hexose Oxidation in Pseudomonas fluorescens"

R. D. DeMoss, Brookhaven National Laboratory

"Routes of Ethanol Formation in Bacteria"

F. Leaver and H. G. Wood, Western Reserve University School of Medicine

"Evidence from Fermentation of Labeled Substrates which is Inconsistent with Present Concepts of the Propionic Acid Fermentation"

Friday - 1:30 p.m. - Continuation of morning session

J. O. Lampen, Western Reserve University School of Medicine

"Pentose and Desoxyntose Metabolism"

PURINE, PYRIMIDINE, AND PTERIDINE METABOLISM

Chairman: C. E. Carter, Western Reserve University School of Medicine

M. Friedkin, Washington University School of Medicine

"Purine and Pyrimidine Metabolism in Microorganisms"

J. R. Totter, University of Arkansas School of Medicine

"The Metabolic Functions of Pteridine Derivatives"

Biochemistry. — Forty components of the ion-exchange separation products of calf-liver and yeast ribonucleic acids are being surveyed for certain determinations in their chemical nature. With the help of ion-exchange resins it has been possible to isolate individual uronic acids in crystalline form. Firefly luminescence is serving as a powerful tool for elucidation of problems under investigation by several groups in the Division.

Plant Physiology. — Urgency of better methods of recognizing different sugars has been brought about by the ability to separate sugars by the ion-exchange method. A program for the development of these methods is now in progress.

Biophysics. — The cobalt source, set up by this section, has proved to be the most reliable radiation source in the Division. As a matter of fact, it is much more suitable than the X-ray machine in microbiological work where large quantities of radiation are essential for continuous exposure. Beta plaques are undergoing further checking for reliability of radiation. The physico-chemical properties of the constituents of the nucleus are the subject of interesting new investigations, the first to be tested being the formation of a nuclear envelope. A detailed study is in progress, designed to compare the efficiency of X, β , and γ rays in affecting the

chromosomes of Tradescantia pollen grains. This section continues to make available not only calibration of instruments for the other members of the Division but also the development of biophysical tools for new approaches to certain biological problems.

PRESENTATION OF RESEARCH RESULTS
TO THE SCIENTIFIC PUBLIC

Publications. Papers published from the Biology Division during this quarter are listed. While actual publication has lagged somewhat during this period, there are forty-four papers now in press, besides a number of abstracts. Much time has been spent in editing and preparing for publication the papers presented at our 1951 Annual Biology Research Conference. This collection of papers is ready to go to press for publication as a supplement to the Journal of Cellular and Comparative Physiology. Publication of the supplement containing the proceedings of the 1949 Conference is expected before the first of April.

<u>AUTHOR (S)</u>	<u>TITLE OF PAPER</u>	<u>PUBLICATION</u>
Carson, S. F.	Lactate and Citrate Biosynthesis	A Symposium on Phosphorus Metabolism, Ed. W. D. McElroy and Bentley Glass, I: 276-282, 1951, Johns Hopkins Press
Furth, J., W. T. Burnett, Jr. (Asst. of W. D. Gude)	Hormone-Secreting Transplantable Neoplasms of the Pituitary Induced by I ¹³¹	Proc. Soc. Exptl. Biol. Med., 78: 222-224, 1951
Hollaender, A. (Director) (E. J. Slaughter, Ed.)	Biology Division Quarterly Progress Report for Period Ending Nov. 10, 1951	ORNL-1167
Hollaender, A.	(Book review) "Progress in Biophysics and Biophysical Chemistry," Vol. I, Ed. J. T. Randall and J. A. V. Butler	Arch. Biochem. Biophys., 34: 229, 1951
Hollaender, A.	Effectiveness of Ultraviolet Radiation in the Control of Air-Borne Infection (report on work previous to ORNL)	Synthèses de Semeiologie et Thérapeutique, (special number Les Journées Internationales de la Lumière) XXI: 127-129, 1951
Mosbach, E. H., E. F. Phares, and S. F. Carson	Conversion of α -Ketoglutaric-1, 2-C ¹⁴ Acid to Malic Acid in Pigeon Breast Muscle	Arch. Biochem. Biophys., 34: 449-452, 1951
Mosbach, E. H., E. F. Phares, and S. F. Carson	The Role of 1-Carbon Compounds in Citric Acid Biosynthesis	Arch. Biochem. Biophys., 35: 435-442, 1952
Noggle, G. R.	Elementary Plant Physiology. Laboratory Manual, Stuart Dunn, 1949 Addison-Wesley Press, Cambridge, Mass. (Book Review)	Quart. Rev. Biol. 25: 332, 1950

<u>AUTHOR (S)</u>	<u>TITLE OF PAPER</u>	<u>PUBLICATION</u>
Storey, R. H., J. Moshman, and J. Furth	A Simple Procedure for the Determination of the Approximate Lymph Volume	Science, 114: 665-667, 1951
Strehler, B. L.	The Luminescence of Isolated Chloro- plasts	Arch. Biochem. Biophys., 34: 239-248, 1951
Conger, A. D. and L. M. Fairchild	The Induction of Chromosomal Aberrations by Oxygen (Abstract)	Genetics, 36: 547-548, 1951
Dent, J. S. and E. L. Hunt	Iodine Distribution in <u>Anura</u> larvae (Abstract)	Anat. Record, 111: 509-510, 1952
Gaulden, M. E.	Prolonged Visible Change Produced by Heat in the Chromatin of Living Grass- hopper Neuroblasts (Abstract)	Genetics, 36: 551-552, 1951
Giles, N. H., Jr., A. V. Beatty, and H. P. Riley	The Relation Between the Effect of Temperature and of Oxygen on the Frequency of X-Ray-Induced Chromo- some Aberrations in <u>Tradescantia</u> Microspores (Abstract)	Genetics, 36: 552-553, 1951
Geckler, R. P. and R. F. Kimball	Effects of Nitrogen Mustard on Cell Division in <u>Paramecium</u> (Abstract)	Anat. Record, 11: 523-524, 1951
Kimball, R. F. and Nenita Gaither	Modification of the Action of X Rays upon <u>Paramecium aurelia</u> (Abstract)	Genetics 36: 558-559, 1951
Kirby-Smith, J. S.	Effects of Infrared Irradiation on the Frequency of X- and Gamma-Ray Induced Chromosomal Aberrations in <u>Tradescantia</u> Pollen Tubes (Abstract)	Genetics, 36: 558-559, 1951
Riley, H. P., N. H. Giles, Jr., and A. V. Beatty	The Oxygen Effect on X-Ray-Induced Chromatid Aberrations in <u>Tradescantia</u> Microspores (Abstract)	Genetics, 36: 572-573, 1951
Russell, L. B., W. L. Russell, and Mary H. Major	The Effect of Hypoxia on the Radiation Induction of Developmental Abnormal- ities in the Mouse (Abstract)	Anat. Record, 111: 445, 1952
Russell, W. L., J. C. Kile, Jr., and L. B. Russell	Failure of Hypoxia to Protect Against the Radiation Induction of Dominant Lethals in Mice (Abstract)	Genetics, 36: 574, 1951
Schwartz, Drew	A Case of Male Sterility in Maize In- volving Gene-Cytoplasm Interaction (Abstract)	Genetics, 36: 575, 1951

Scientific Society Lectures and Traveling Seminars. The Traveling Seminar program has been approaching a seasonal peak during this quarter. Of the thirty-eight lectures given, all but two were presented on this program. All lectures are listed.

Anderson, E. H.	University of Kentucky Lexington	The Recovery of Bacteria after Exposure to Radiation
Arnold, W. A.	Knoxville Science Club Knoxville	Energy Sources for Mankind
Atwood, K. C.	Univ. of Southern Illinois Carbondale	Genetic and Nongenetic Effects of Radiation in <u>Neurospora</u> Heterokaryons
Atwood, K. C.	Washington University St. Louis	The Use of <u>Neurospora</u> Heterokaryons in the Analysis of Radiation Effects
Atwood, K. C.	Univ. of Missouri Columbia	Comparison of the Effects of Ultra-violet and X rays on Heterokaryosis Conidia of <u>Neurospora</u>
Atwood, K. C.	Univ. of Tennessee Knoxville	The Effects of X rays on the Nucleus of <u>Neurospora crassa</u>
Baker, W. K.	Johns Hopkins University Baltimore	Position Effects Causing Variegation in <u>Drosophila virilis</u>
Doherty, D. G.	Washington University St. Louis	Nucleic Acid Chemistry
Doherty, D. G.	Tulane Univ. School of Medicine, New Orleans	Kinetics and Reactions of Proteolytic Enzymes
Doherty, D. G.	Loyola University New Orleans	The Synthesis and Enzymatic Susceptibility of Aldonic Acid-Amino Acid Derivatives (Glyconyl Peptides)
Doherty, D. G.	Southern Regional Research Laboratory Birmingham	As Above
Doherty, D. G.	Louisiana State University Medical School Baton Rouge	Kinetics and Reactions of Proteolytic Enzymes
Doherty, D. G.	Duke University Durham	1. Kinetics of Proteolytic Enzymes 2. Glyconyl Peptides
Furth, J.	Argonne National Laboratory Chicago	The Anemia and Permeability Problems of Acute Radiation Syndrome

Furth, J.	Childrens's Hospital Boston	Capillary Permeability and Lymph Volume
Furth, J.	As Above	Some Fundamental Problems of Leukemia
Furth, J.	As Above	(In charge of open lecture) Conditioned Neoplasms of Endocrine Glands and their Target Organs
Furth, J.	Columbia University New York	Experimental Tumors Related to the Gonads
Furth, J.	National Cancer Institute Bethesda	Conditioned Neoplasms of Endocrine Organs
Furth, J.	S. E. Med. School, Univ. of Texas, Austin	Neoplasia: Induction in Man and Animals by X rays and Radioactive Substances
Furth, J.	Brooke Army Hospital Baylor Univ. Sch. of Medicine, Waco	The Patho-Physiology of Fatal Irradiation, Notably that of the Hemorrhagic Syndrome
Hollaender, A.	Army Med. Res. Group Ft. Knox	Studies on the Protection against Radiation Damage in Biological Materials
Hollaender, A.	Indiana University Bloomington	As Above
Hollaender, A.	Sloan-Kettering Institute New York	Physical and Chemical Factors Modifying the Sensitivity of Cells to Radiation
Jonas, Herbert	Assoc. Sou. Agri. Wkrs. (Am. Soc. Plant Physiol.) Atlanta	Calcium Nutrition and Ascorbic Acid Formation in Peas
Kimball, R. F.	California Inst. of Tech. Pasadena	Modification of the Genetic and Non- genetic Effects of Radiation upon <u>Paramecium aurelia</u>
Kimball, R. F.	Univ. of Texas Austin	Modification of the Effects of X rays upon <u>Paramecium aurelia</u>
Kimball, R. F.	Stanford University	As Above
Noggle, G. R.	UT-AEC Agr. Res. Program Oak Ridge	Ion-Exchange Separation of Sugars in Plant Extracts
Noggle, G. R.	Univ. of Arkansas School of Medicine	Ion Exchange as a Qualitative and Quantitative Method of Investigating the Carbohydrates in Plants

Noggle, G. R.	Assoc. Sou. Agr. Wkrs. (Am. Soc. Plant Physiol.) Atlanta	The Biochemical Effects of Plant Acid Formation in Peas
Sheppard, C. W.	Univ. of North Carolina Chapel Hill	The Role of Potassium in Cell Physiology
Sheppard, C. W.	As Above	Isotopes and Circulatory Mixing
Strehler, B. L.	Vanderbilt Univ. Nashville	Bioluminescence as a Tool for the Study of Energy Metabolism
Upton, A. C.	Univ. of North Carolina Chapel Hill	The Response of Tissue to Ionizing Radiation
Volkin, E.	Univ. of Kentucky Lexington	The Enzymatic Degradation Products of RNA and DNA
Zill, L. P.	Louisiana State University Baton Rouge	The Analysis and Separation of Sugars and Related Compounds by Ion-Exchange Chromatography
Zill, L. P.	Tulane University New Orleans	As Above

Visiting Lecturers. Six visitors have lectured on the Biology seminar program during the quarter. They are as listed:

Dr. C. P. Swanson	Dept. of Biology, Johns Hopkins University, Baltimore, Maryland	The Induction of Activated States in the Chromosomes of <u>Tradescantia</u> by Infrared and X rays, and their Possible Significance in Radio- biological Effects
Dr. Wolf Vishniac	New York Univ. Med. School New York, N. Y.	Problems Connected with the Bio- chemistry of CO ₂ Reduction
Dr. David Shemin	Dept. of Biochemistry, College of Physicians and Surgeons Columbia University, New York	The Mechanism of Porphyrin Formation
Dr. Marcus Rhoades	Department of Botany University of Illinois Urbana, Ill.	Maize Cytogenetics
Dr. N. B. Kurnick	School of Medicine Tulane University New Orleans, La.	A Desoxyribonuclease-Nuclease In- hibitor System in Human Blood and its Patho-Physiological Significance
Dr. W. D. McElroy	Director, McCollum-Pratt Institute, Johns Hopkins University, Baltimore	Bioluminescence

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball (Leader)

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

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

(Kimball, Gaither, King)

In previous reports (ORNL-989, ORNL-1167), it was shown that paramecia irradiated in 0.001 M phosphate buffer to which 3 per cent culture fluid was added (medium D) were considerably more affected by X rays than when irradiated in whole culture fluid (medium C). It has now been shown that this effect is, in all probability, the result of destruction in C by the bacteria of hydrogen peroxide produced in the fluid by X rays.

TABLE 1**

PROTECTION BY BACTERIA AND CATALASE AGAINST THE
EARLY EFFECTS OF X RAYS

Dose (kr)	Medium													
	C		D		Filtered C		Boiled C		D plus bacteria		D plus catalase		D plus boiled catalase	
	d	s	d	s	d	s	d	s	d	s	d	s	d	s
210	47	100	--	0	--	0								
245	40	100	--	0	--	0								
139	85	100	58	26			--	0						
208	70	100	--	0			--	0						
139			71	3					89	100				
134	81	100	23	58							86	96	26	17
138	73	100	50	100							72	100	18	33

** The numbers in the body of the table are percentages. Those under d are the number of divisions in the first day after irradiation expressed as a percentage of the control. Those under s are the percentage surviving for at least 1 day after irradiation. Unirradiated controls were run for each kind of treatment. In no case did less than 90 per cent survive and the divisions expressed as a percentage of the C controls were never more than 110 per cent nor less than 90.

* Research Participant

The bacteria, rather than the other ingredients of C, are implicated, since passage through a sintered glass filter to remove the bacteria and boiling to destroy such enzymes as catalase removed the protective action of C. Bacteria washed off an agar slant into D had a protective effect, showing that ingredients of the lettuce infusion were not needed (Table 1).

More specific evidence that hydrogen peroxide was the active material involved was obtained by adding catalase to D as shown in Table 1. In the concentration used it was as protective as culture medium. The presence of an active material in the irradiated fluid was shown by irradiating boiled D and adding animals, previously washed free of bacteria and concentrated into a small volume (Table 2).

TABLE 2
EFFECT OF IRRADIATED FLUID ON UNIRRADIATED PARAMECIA

Dose to fluid (kr)	Duration of exposure of animals to fluid (minutes)	Per cent survival	Divisions in 1 day as per cent of control
None	20	97	100
154	10	97	73
154	20	13	55
308	10	20	45
308	20	20	8

In order to see whether hydrogen peroxide in the concentrations, which might reasonably be expected to be formed by X rays, could account for these results, paramecia, washed free of bacteria, were exposed for 5 minutes to various concentrations of the compound (Table 3). At the end of the exposure period, culture fluid with living bacteria was added in excess to destroy the peroxide, and the animals were transferred with as little fluid as possible to fresh culture fluid. A concentration of 7×10^{-5} M produced a distinct effect upon cell division. According to Bonet-Maury and Frilley (Compt. rend. 218:400, 1944) this concentration would be produced by a dose of about 40 kr. It seems probable that the amount of peroxide produced under our conditions would be sufficient to account for the results.

Table 3 shows that hydrogen peroxide has no effect upon genetic changes as measured by the percentage of normal clones after autogamy. This agrees with the finding that the presence of bacteria during irradiation has no influence upon genetic changes as shown in Table 4. Paramecium contains catalase, and so it seems unlikely that peroxide can diffuse very

far into the cell. It may be suggested that peroxide does not reach the micronuclei, and so has no mutagenic effect. If this is so, it follows that death before the first division and division delay must be due, at least in part, to alterations in the more superficial parts of the cell.

TABLE 3
EFFECT OF 5-MINUTE EXPOSURES TO VARIOUS CONCENTRATIONS
OF HYDROGEN PEROXIDE

Concentration of H ₂ O ₂ (M)	Per cent survival	Divisions in 1 day as per cent of control	Per cent normal after autogamy
8.8 x 10 ⁻³	0	--	--
4.4	0	--	--
2.2	13	25	98
1.1	13	6	99
5.5 x 10 ⁻⁴	18	10	95
2.8	23	25	96
1.4	57	35	96
6.9 x 10 ⁻⁵	100	61	94
3.4	100	100	97
1.7	100	98	--
8.6 x 10 ⁻⁶	100	100	--
None	100	100	94

TABLE 4*
LACK OF AN EFFECT OF BACTERIA UPON GENETIC CHANGES
PRODUCED BY X RAYS

Dose (kr)	Medium	
	C	D
None	94	--
1.03	87	87
1.93	63	58
3.00	44	44
4.95	32	29

* The numbers in the body of the table are percentage of normal exautogamous clones.

Finally, low oxygen concentration in the medium should decrease the amount of hydrogen peroxide formed by X rays. We have previously reported (ORNL-989, ORNL-1167) that low concentration of oxygen during irradiation, if anything, increased rather than decreased death and division delay although it decreased genetic changes. These experiments were carried out with culture fluid in which there should be little effect of externally produced peroxide. They have now been repeated using medium D; and, in accordance with expectation, low oxygen concentration leads to a distinctly smaller effect (Table 5).

TABLE 5

INFLUENCE OF OXYGEN CONCENTRATION UPON THE EARLY EFFECTS
OF X RAYS WITH AND WITHOUT THE PRESENCE OF BACTERIA

Dose (kr)	Medium C				Medium D			
	Oxygen		Nitrogen		Oxygen		Nitrogen	
	d	s	d	s	d	s	d	s
None	100	100	100	96	100	97	100	100
43	101	99	98	100	49	43	93	98
85	91	100	84	100	--	3	63	94

Breakage of Chromosomes by Oxygen

(Conger, Fairchild)

Continued experiments on the breakage of chromosomes by oxygen in *Tradescantia* pollen grains and flower buds have established several more qualitative and quantitative features of the effect. These are summarized in figures and tables.

Variable, HUMIDITY; constants, 100 per cent oxygen flushed 30 minutes at normal pressure (Fig. 1). The cells very rapidly assume the humidity of the gas. The degree of hydration of the cells as they approach dryness (1-3 hours over Drierite) has a profound influence on the effect. This probably accounts for much of the variability previously found, as was guessed at the time.

Variable, TIME; constant, 100 per cent oxygen flushed at normal pressure (Fig. 2). Aberrations increase with time more rapidly than the first power, probably as the square of time. Normal cells disappear exponentially, but it is believed that they approach an asymptote which is dependent on the oxygen concentration.

Variable, PARTIAL PRESSURE; constants, 50 per cent oxygen at 1 atmosphere for 40 minutes versus 100 per cent oxygen at 1/2 atmosphere (Table 6).

TABLE 6

Treatment	No. of cells	Normal		Aberrations	
		No.	Fraction	No.	Per cell
50% O ₂ , 1 atmos., 40 min.	100	56	0.56	98	0.98
100% O ₂ , 1/2 atmos., 40 min.	100	76	0.76	26	0.26

Apparently effect is not strictly a function of the partial pressure of oxygen.

Variable, TIME (IN OZONE); ozonized oxygen (concentration unknown) at increasing times, and oxygen (Fig. 3). Effect is markedly increased when ozone is used.

Commercial oxygen versus deoxygenated oxygen. Normal pressure, 1-hour flush (Table 7). Cells were exposed to oxygen directly from the cylinder, and after it had run through a deoxygenating column.

TABLE 7

Type of oxygen	No. of cells	Normal		Aberrations	
		No.	Fraction	No.	Per cell
Direct from tank	75	17	0.22	160	2.13
After passage through deoxygenating column	75	9	0.12	166	2.21

This high proportion of aberrations is not caused by the ozone that may be present in commercial oxygen.

Flower buds. Flushed with 100 per cent oxygen for 18 hours at normal pressure over inflorescences. Slides were made 5 days later (Table 8).

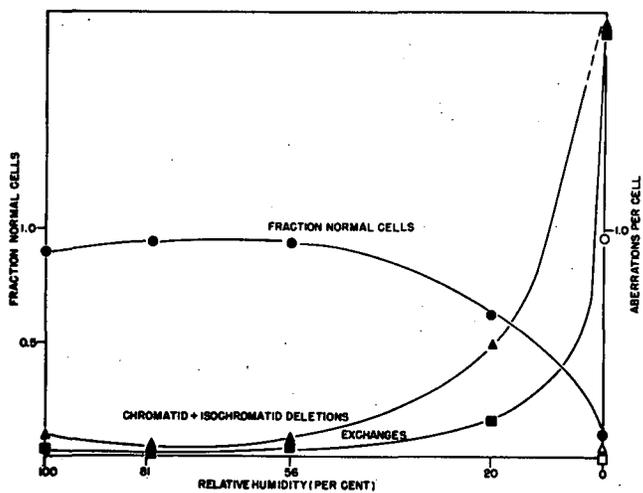


FIG. 1

Influence of moisture content of the cells

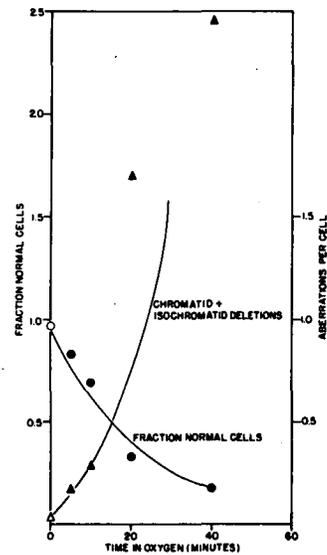


FIG. 2

Time of Exposure

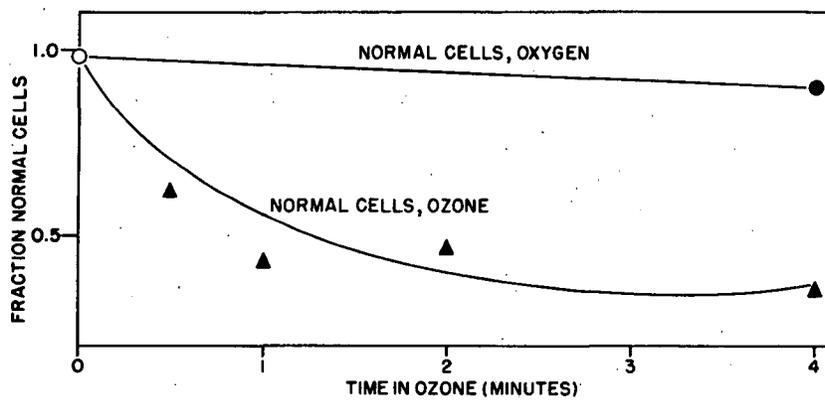
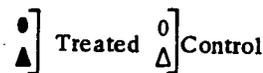


FIG. 3

Time in ozone, and in oxygen



TABLE 8

	No. buds	No. cells	Normal		Chromosome Aberrations	
			No.	Fraction	Dicentrics	Rings
Oxygen	10	715	689	0.96	18	0.025
Control*	10	250	250	1.00	0	--

* Observations of several thousand cells (from other experiments) show the control aberration frequency to be less than one in a thousand.

The cells were in resting stage when exposed. There was a minimum of 3 1/2 days from the end of oxygen exposure to the earliest time at which chromosomes behave as double. Oxygen can therefore break chromosomes in the resting stage when they are still single.

Experiments on the Mutagenic Action of Ionizing Radiation in Maize

(Schwartz, Cheniae)

A study is being conducted to determine whether some of the point mutations produced by ionizing radiations are true gene mutations or if they are all deficiencies. The following setup is being used for these experiments.

The distal half of the most distal chromomere on the short arm of chromosome 9 contains the loci of three chlorophyll genes: w (white), yg (yellow green), and py (pale yellow)(Fig. 4). Any plant

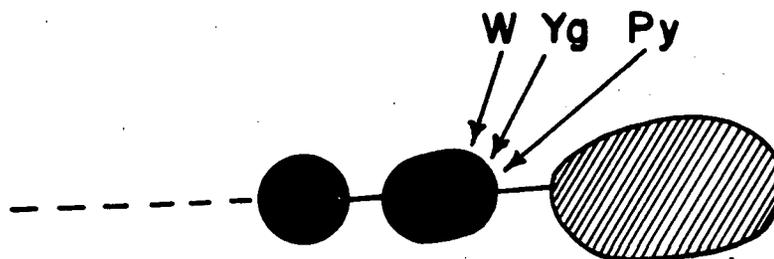


FIG. 4

Chromatin organization of the end of the short arm of chromosome 9

Hatched oval represents terminal knot.
Solid ovals represent two distal chromomeres.

which does not carry the dominant W gene is white. A plant carrying the W gene but not the dominant Py gene is pale yellow. A plant carrying both the W and Py genes but not the dominant Yg gene is yellow green (McClintock, Genetics, 29: 478-502, 1944).

Pollen from plants homozygous dominant for all three genes was irradiated with 1000 r gamma. The treated pollen was then used to fertilize plants heterozygous for a normal chromosome 9 and one carrying a small terminal deficiency which includes all three loci. The resultant seeds were planted in the greenhouse and the seedlings classified according to their chlorophyll phenotype. Seedlings which arise from eggs carrying the normal chromosome 9 will be green, but those seedlings which received a deficient chromosome 9 from the egg parent will show any deficiency or mutation involving the loci under consideration since they will essentially be haploid for those genes. If the radiation caused a break to occur at any point between the centromere and the W locus resulting in a terminal deficiency including all three loci, the seedling will be white. Similarly, if the break occurred distal to the W locus but at any point proximal to the Py locus, the seedling will be pale yellow. Yellow green seedlings must be due either to gene mutation or an internal deficiency where the breaks occurred on both sides of the Yg locus deleting this gene but not W or Py.

From these experiments 70 white, 5 yellow green, 3 green-yellow green mosaic, and no pale yellow seedlings were obtained. The fact that pale yellows were not found suggests that the yellow greens are due to mutations and not deletions. The yellow greens are being tested to substantiate this fact.

The yellow green locus is cytologically very close to Dt (dotted) which is in the terminal knob. However, the frequency of crossing over between these genes is unusually high (8 per cent). Thus, if the yellow greens are due to deficiencies they should result in a substantial decrease in cross-over frequency due to nonhomologous pairing.

The three mosaic plants appear to be due to the formation of unstable or mutable genes at the yg locus. Since W and Py are closely situated on either side of this gene, any plants which are mosaic for yellow green, due to chromosomal aberrations such as rings or dicentrics, should also be mosaic for white and pale yellow tissue. However, the three mosaic plants were sectorial only for green and yellow green. Two of these plants appeared to be $yg^m \rightarrow Yg$ showing green streaks on a yellow green background. The third plant was $Yg^m \rightarrow yg$ with yellow green streaks on a green background.

Oxygen Treatment of Neurospora Conidia

(Atwood, Mukai)

The class of lethal mutations detectable by the heterokaryon method includes chromosomal deficiencies. Therefore, agents which produce unrestituted chromosome breaks should increase the frequency of such mutations. Previous experiments (ORNL-1167:34, 1951) showed that treatment of conidia with oxygen at 100 lb/sq in. for periods up to 100 minutes had no significant effect on survival and mutation. The results of further experiments with the ornithineless + methionineless-amycelial heterokaryon, extending the time to 144 hours, are given in Table 9.

TABLE 9

EFFECT OF 100 PER-CENT OXYGEN AT 100 LB/SQ IN. ON SURVIVAL AND
MUTATION OF CONIDIA ATTACHED TO AERIAL HYPHAE

	Duration (hours)					
	Control	24	48	72	120	144
Survival	1.0	0.52	0.022	0.0015	0.0011	0.00012
Total Isolates	979	120	120	118	147	167
Number of isolates carrying lethals	20	1	3	5	6	5
Per cent lethals	2.0	0.8	2.5	4.2	4.1	3.0

Treatment for 24 hours or more produced marked effects on survival, but the increases in mutation frequency are of doubtful significance. A chi-square test (6 rows, 2 columns) indicates $0.2 > P > 0.1$.

The fraction of homokaryons has been computed, in another experiment, from the difference in colony count in minimal medium and in medium supplemented with arginine and methionine. These results are shown in Table 10. The absence of a marked increase in homokaryons at the lowest survival suggests that the lethal effect of oxygen is mostly extranuclear. The wide fluctuations in survival with similar treatments show that the system is sensitive to uncontrolled factors, among which humidity may play an important role.

TABLE 10
EFFECT OF 100 PER CENT OXYGEN AT 100 LB/SQ IN. ON THE
FRACTION OF HOMOKARYONS

	Duration (hours)			
	Control	24	48	72
Survival	1.0	0.80	0.10	0.03
Per cent homokaryons	34	33	37	47

Genetics of Polyploid Yeast

(Pomper, McKee)

In ORNL-1167, we reported the isolation of yeast cultures, tentatively identified as triploids, from crosses of haploids and diploids. The data now on hand substantiate the original conclusion that a triploid was isolated. An especially strong piece of evidence is that, in a single ascus (from the triploid), only two of four ascospores could be induced to sporulate, suggesting very strongly the segregation from the triploid of two diploids and two haploid ascospores. Radiation survival curves support this analysis.

A tetraploid has been isolated by techniques similar to those described in ORNL-1167, from a diploid x diploid cross. We are engaged in a genetic and radiogenetic analysis to substantiate the state of polyploidy of this isolate. When it is complete, we will be in a position to use both triploids and tetraploids in radiation experiments of various sorts where variation in gene number is essential.

Radiation Studies on Yeast

(Pomper, K. Daniels, McKee)

In our ultraviolet experiments on yeast, attempts have been made to compare killing and mutation between haploid and diploid cultures. The experimental setup consisted in using mixed cultures of haploids and diploids in the same flasks to insure equal exposure to the radiation. The cultures are separable by differential plating, since the haploid used requires methionine (as well as adenine and uracil); whereas, the diploid requires tryptophan (in addition to adenine and uracil). Both survival and

mutation can thus be measured separately for both cultures on cells intimately mixed and simultaneously irradiated. Survival curves from such an experiment are shown in Fig. 5. We have only occasionally obtained an exponential survival curve with the haploid; the diploid curve extrapolates to two in most experiments. The mutation data are shown in Table 11. For equal exposure, the haploid shows a consistently higher mutation frequency than the diploid, a result contrary to expectation.

TABLE 11
COMPARISON OF MUTATION RATES FOR HAPLOID AND DIPLOID YEAST

Time (min)	Haploid ^a		Diploid		Haploid/diploid	
	Ad ⁺ /10 ⁶	Ur ⁺ /10 ⁶	Ad ⁺ /10 ⁶	Ur ⁺ /10 ⁶	Ad ⁺	Ur ⁺
Control	0.11	0.02	0.46	0.02	0.24	1
10 ^b	4.3	0.85	3.6	0.65	1.2	1.3
20	13.9	2.8	8.4	1.3	1.7	2.2
30	25.1	5.2	18.4	2.9	1.4	1.8
40	50.6	8.5	29.9	3.3	1.7	2.6
60 ^c	38.4	59	75.6	9.2		
80 ^c		198	111	11.8		
120 ^c		421	109	13		

^a Mutation rates calculated as number of mutants per 10⁶ surviving cells.

^b Minutes of irradiation at room temperature in a rotating quartz flask exposed to a germicidal lamp.

^c The values obtained for these exposures may be too high. There is a strong possibility that irradiation for these extended periods of time causes release of nucleic acid components from the cells, which on plating at low dilutions, would give rise to falsely high counts. This would cause a particularly large error in the haploid, where the total number of surviving cells is decreasing rapidly with exposure.

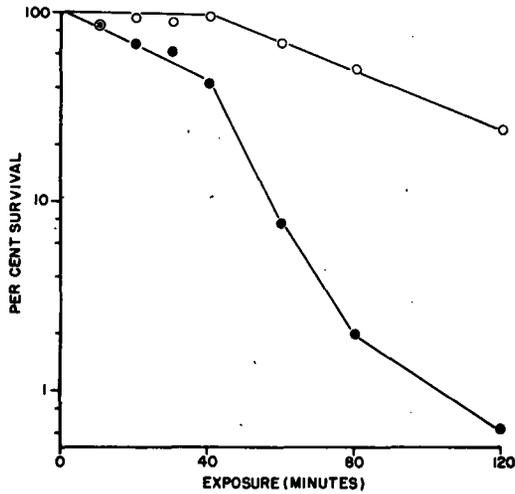


Fig. 5 Survival of Haploid and Diploid Yeast after Ultraviolet Irradiation

● - Haploid
○ - Diploid

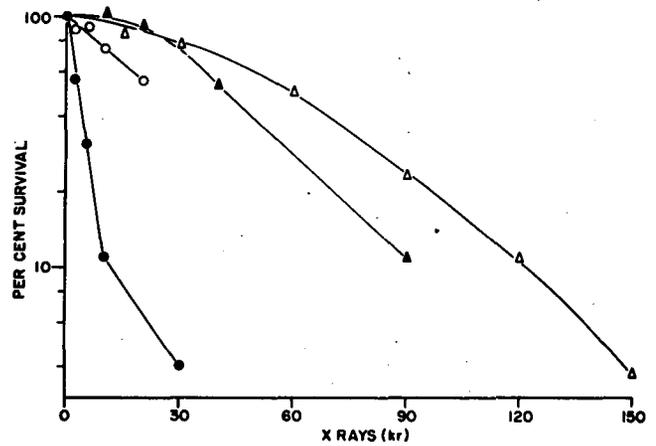


Fig. 6 Survival of Haploid, Diploid, and Triploid Yeast after X Irradiation

● - Haploid (A93.2)
○ - Haploid (A541.2)
▲ - Diploid (# 39)
△ - Triploid P-1 (A176.1 x A555.4)

During the last quarter, we have carried out a number of experiments using X rays. Figure 6 gives survival curves for haploid, diploid, and triploid yeast. The curve labeled "haploid A93.2" has been obtained very reproducibly with this particular culture; however, other haploids may have markedly higher resistance, while still giving an exponential survival curve, as suggested by the curve labeled "haploid A541.2." The possibility of a genetic segregation of radiation resistance is under investigation. No increase in mutation to independence from adenine or uracil requirements has been observed with the haploid A93.2 at exposures up to 30 kr; a slight increase in mutation to adenine independence was noted with a diploid at doses of 40-60 kr. Another very interesting point of difference between haploid and diploid yeast is with regard to recovery from X irradiation. Table 12 gives data from an experiment showing that diploid yeast may recover from radiation damage by incubation at suitable temperatures after irradiation. This table also shows that, at the temperatures tested (12° and 18°C), the haploid shows a decrease in survival. These observations are now being extended to our polyploid yeast clones in an effort to evaluate the role of gene number in these phenomena.

TABLE 12
EFFECT OF LOW-TEMPERATURE INCUBATION ON SURVIVAL TO X
RAYS OF HAPLOID AND DIPLOID YEAST

Temperature (°C)	Haploid (x 10 ⁶)			Diploid (x 10 ⁶)		
	30	18	12	30	18	12
Control	147	126	138	102	101	82
50 kr	2.01	1.84	1.48			
150 kr				7.8	7.6	21.2
Per cent survival (pooled controls)	1.46	1.33	1.07	8.3	8.1	22.6
	F value 9.678 significant at 1 per cent			F value 19.06 significant at 0.1 per cent		

EFFECTS OF RADIATION ON RATE OF MITOSIS

Mary Esther Gaulden (Leader)

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W. K. Baker	Katharine Loemker
Mary L. Alexander†	

A Temperature Effect in Radiation Genetics

(Baker, von Halle)

In some of the early work with Drosophila on the production of mutations and chromosome aberrations by ionizing radiations, it was reported that these genetic effects were induced with a higher frequency when the flies were maintained near 0°C during treatment as compared to those kept at room temperature. Other investigators, however, using more extreme high temperatures, reported no difference. In the work previously reported none of the investigators had made studies at more than two different temperatures, so that the effect over most of the viable range of Drosophila remained unknown. It thus seemed likely that studies at the intermediate temperatures might resolve these conflicting results.

Three groups of wild type D. melanogaster males were exposed simultaneously to 3000 r of 250 kvp X rays while being maintained at a given temperature. In one group, pure oxygen was passed through the irradiation chamber containing the flies for 10 minutes prior to and also during irradiation. In the other two groups air and nitrogen were flushed through their respective irradiation chambers during the same period. The temperature of the gas was controlled by passing it through copper coils immersed in appropriate water baths. The temperature of the gas in one of the irradiated chambers was measured directly by use of a series of small copper-constantan thermocouples. Immediately after treatment the males were removed from the chamber and mated to Muller-5 females. The number of sex-linked recessive lethals induced in the sperm of the treated males was determined by appropriate genetic crosses.

It is obvious from the results presented graphically in Fig. 7 that the temperature effect is not large. This factor in conjunction with the rather low frequency with which recessive lethals are induced make necessary rather extensive experiments at each of the temperatures. Almost

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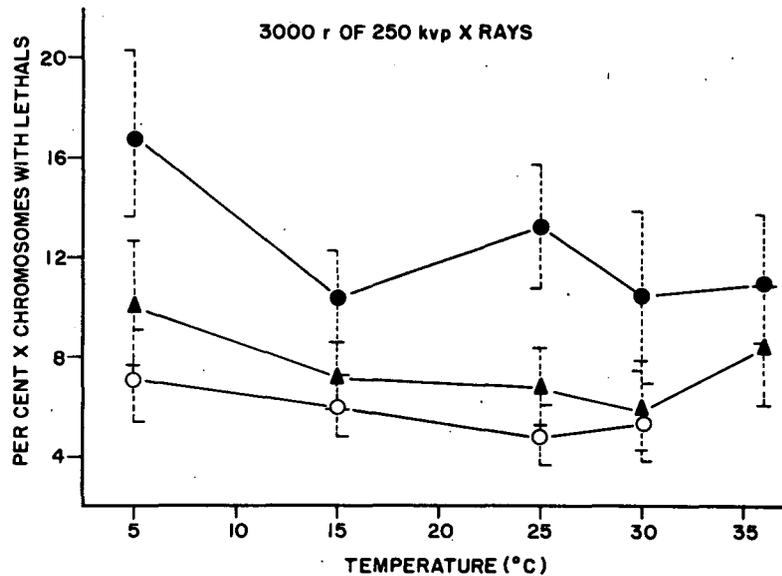


FIG. 7

The relationship between temperature and oxygen concentration (maintained during irradiation) and the induction of sex-linked recessive lethals. The experimental points are bracketed by their 95 per cent confidence intervals.

- - irradiated in oxygen
- ▲ - irradiated in air
- - irradiated in nitrogen

12 thousand X chromosomes were tested for lethals in this study. An analysis of the data show that over 80 per cent of the variation observed within a given temperature and gas can be attributed to binomial variation; the small remainder is caused by extraneous variation. This fact vouches for the accuracy with which given experiments can be repeated.

The fact that temperature is significantly affecting the yield of lethals is indicated by an analysis of variance of the data. From the curves in Fig. 7 it would appear that the flies irradiated in different gases respond differently to temperature. The gas-temperature interaction, although not significant at the 5 per cent level ($0.2 > P > 0.1$), opens the possibility that this might be true. Of particular interest is the apparently U-shaped curve in air since such a relationship would explain the conflicting results of earlier investigators previously mentioned. The experimental points at 5° and at 30°C are very close to being significantly different. More experiments are in progress to reduce further the binomial variation to the point that the experimental points will be more accurately known.

It appears that this temperature effect in Drosophila cannot be wholly explained on the increased solubility of oxygen at low temperatures. If oxygen solubility were the sole factor involved, then a much smaller temperature effect would be observed between 0° and 30°C and, in addition, no increase in yield of lethals with temperatures above 30°C would be anticipated. This substantiates the conclusions which Giles, Beatty, Riley, and Fairchild (ORNL-807) reach after studying the effect of temperature on X-ray-induced aberrations in Tradescantia. They also report that irradiations in an inert gas (helium) produce the opposite effect — an increase in aberration frequency with increasing temperature. Although this latter effect is not observed in the Drosophila irradiated in nitrogen, it should be noted that experiments above 30°C (the region where Giles, et al. find the greatest effect) are not possible in nitrogen, since flies irradiated in this gas and temperature do not survive.

It is interesting to note that not all biological effects of radiation are inversely correlated with temperature, even when the organisms are treated in oxygen. Hollaender, Stapleton, and Martin (ORNL-889) studying the inactivation of E. coli (B/r) report that the cells show increased sensitivity with increasing temperatures both in oxygen- and nitrogen-saturated suspensions.

RADIATION PROTECTION

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Radiation Protection Studies — Metal Ions

(Burnett, Burke)

The effects which certain metallic ions have on the X-ray sensitivity of Escherichia coli, B/r are being examined with particular emphasis on those ions which might increase or decrease the protective action of cysteine and other chemical agents. Added Ca^{++} and Mg^{++} alone, or in combination with BAL, have no effect on cell survival under our test conditions. In addition to its known toxic effect, Cu^{++} increases the X-ray sensitivity; whereas, copper foil alone does not change the survival of either irradiated or nonirradiated cells. On the other hand, copper foil appears to enhance the ability of BAL to protect cell suspensions containing oxygen. This is in agreement with the catalytic effect of copper chloride on the rate of oxidation of dithiols by oxygen (Barron, Advances in Enzymology, XI, ed. F. F. Nord, 1951). Mn^{++} increases the X-ray sensitivity of suspensions containing oxygen. It has been suggested that this effect might be attributed to the radiomimetic properties of Mn^{++} .

In contrast to the benefits which possibly may be derived when certain flavanoids and flavanoid derivatives are given to X-irradiated animals, the survival of X-irradiated E. coli, B/r does not appear to be improved by their presence during the exposure period. Also, cells which are cultured in a broth containing rutin seem to be more radiosensitive. The survival following 60 kr of X radiation in the presence of related compounds, phlobatannin and tannic acid, was of the same order as the survival of irradiated controls, but there were fewer colonies formed on platings of nonirradiated cell suspensions that were exposed to these compounds than on platings given no treatment, indicating that, although toxic, they have some protective action.

Further studies have been made on the protective action of ethanol at 2°C. An examination of the survival of X-irradiated suspensions as a function of ethanol concentration showed that increased concentration of the protective agent up to 7-9 per cent v/v gave markedly increased survival. A concentration of 12 per cent ethanol was not toxic and was as effective as a 9 per cent concentration. The protective effects are not time

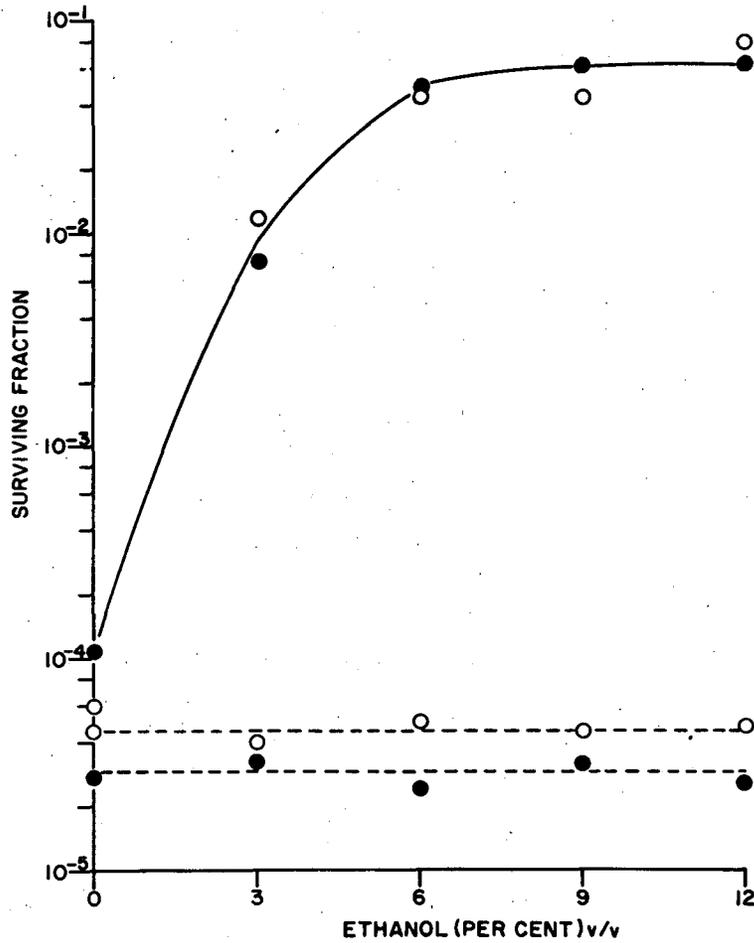


FIG. 8

- - holding period - 5 min.
- - holding period - 2.5 hrs.
- - irradiated - 60 kr
- - - nonirradiated

dependent since the same results were obtained whether the cells were suspended in the alcohol solutions for 5 minutes or for 2.5 hours before exposure to X radiation (Fig. 8).

The Effect of X Radiation on Bacterial Respiration

(Billen, Stapleton, Hollaender)

One phase of our studies on the physiological changes brought about by ionizing radiations in bacterial cells has been concerned with alterations in bacterial respiration following X-ray exposure. In the work reported here, the respiratory characteristics of "resting" Escherichia coli suspensions following exposure have been studied. The data obtained revealed that the respiratory activity of the irradiated cells was inhibited but that the magnitude of the inhibition varied with substrate, temperature, and strain of the organism used.

The cells used in the studies were obtained by growing the organisms in nutrient broth at 37°C with constant aeration. Suspensions, harvested by centrifugation and washed in M/15 phosphate buffer at pH 6.8, were brought to original volume in phosphate buffer. After exposure to 60,000 r at ice bath temperature, the respiratory activity of the suspension was determined by conventional Warburg methods.

It was found that a dose of 60,000 r, while decreasing the number of viable cells of a suspension of E. coli B/r by more than 99.95 per cent, had no apparent effect on the initial respiratory rate of the exposed cells when this rate was compared to the rate obtained with unexposed cells (Fig. 9). However, the initial period of normal activity was followed by a marked decline in oxygen consumption rate as compared to the control cells. It was also observed that the period of normal activity is longer with pyruvate or succinate as substrate than with glucose. With E. coli (Texas) somewhat the opposite was found. With this strain, a period of normal respiratory activity on glucose was found but the activity on pyruvate indicated immediate inhibition. The cause of the increment in pyruvate oxidation rate of the control cells at 75 minutes as noted in Fig. 9 is not known. However, the fact that this does not take place in the irradiated suspension is suggestive of new enzyme formation in the control cells and not in the exposed cells, since it has been shown (ORNL-1167) that an X-ray dose of 60,000 r can completely inhibit the capacity of E. coli B/r to form formic hydrogenlyase.

Both exposed and control cells were found to have a respiratory quotient of 1 on glucose although the former required a longer period of time to complete the oxidation of the substrate. The total oxygen consumed for both the irradiated and nonirradiated cells was 74 per cent of that required for complete oxidation of the glucose to carbon dioxide. On the basis of this admittedly limited data it appears that the X rays affect the rate of oxidation rather than altering the pathways of oxidation.

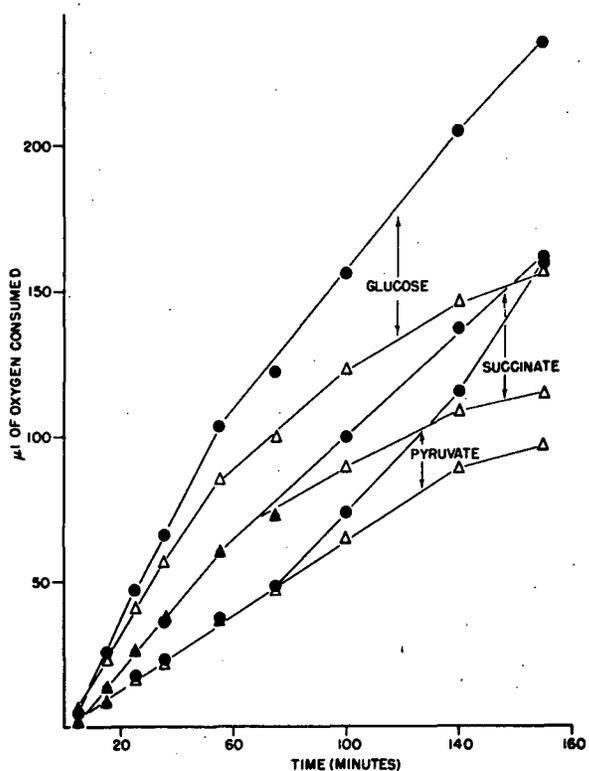


FIG. 9

● = Controls
 Δ = Irradiated cells
 Control contained 20×10^8 viable organisms per cup.
 Experimentals contained 20×10^4 viable organisms per cup.
 $20 \mu\text{M}$ of substrate per cup.

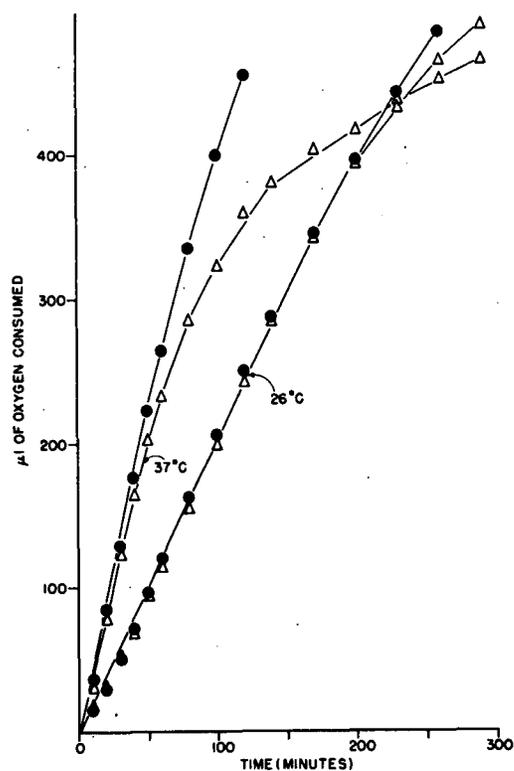


FIG. 10

● = Controls
 Δ = Irradiated cells
 Control contained 54×10^8 viable organisms per cup.
 Experimentals contained 56×10^4 viable organisms per cup.
 $20 \mu\text{M}$ of glucose per cup.

A study of the relationship of X-ray dose to respiratory activity indicated that the duration of normal respiratory activity was relatively unaffected by increasing doses (5000 to 90,000 r), although once the inhibition manifests itself it was most pronounced in those cells exposed to higher doses.

It was also observed that the enzyme activity of the exposed cells was adversely affected by holding at 37°C . Incubating the cells for 60 minutes in the manometer cups before tipping in glucose decreased the duration of normal respiratory activity of the irradiated cells. In relation to this observation it was found that cells kept at ice bath temperature for 3 hours after X-ray treatment possessed the same respiratory activity as those cells studied immediately after exposure.

Since the low temperature appeared to exert a sparing effect on enzyme decay in the absence of a substrate, it was thought feasible to investigate the influence of temperature on the observed accelerated decay of respiratory activity in the exposed cells. A definite retardation of the effects of the X rays was found at 26°C over that observed at 37°C (Fig. 10). Both the length of the normal respiratory period and the oxygen uptake during this period surpassed that of the exposed cells which were held at 37°C. It is also noted that, at the end of 4 hours, the total oxygen uptake is greater with the exposed cells held at 26°C than the total uptake observed during the same period by exposed cells held at 37°C. Apparently, then, the higher temperature accelerates whatever damage the X rays have done to the respiratory system. The relationship of this temperature effect on enzyme activity to the observed beneficial effect of suboptimal growth temperatures on survival of irradiated E. coli (ORNL-1167) is not known.

Bacterial Recovery as Related to the Oxygen Effect

(Stapleton, Grayson)

Results of a preliminary investigation of recovery of bacteria from the lethal effects of X rays were presented in the last quarterly report (ORNL-1167). It was shown that, at reduced incubation temperatures and in the presence of available nutrient, Escherichia coli B/r could partially recover from the damaging effects of X rays.

At about 18°C maximal recovery was obtained relative to that at 37°C. The survival at 18° as well as that at 37°C is an exponential function of X-ray dose, over the dose range used (0 - 60 kr). The ratio of the slopes of the curves at 37° to that at 18°C is about 1.5. This study has now been extended to include an investigation of the conditions which affect the recovery. The effect of the oxygen concentration in irradiated bacterial suspensions has been examined for its possible influence on the recovery phenomenon. After determining that the optimum recovery temperature is the same for suspensions irradiated at high and low oxygen concentrations, survival was studied as a function of X-ray dose, under conditions of high and low oxygen concentration during irradiation, with post irradiation incubation at 18° and 37°C.

Exponential survival curves were obtained for all the following conditions:

1. Oxygen-bubbled suspensions.
2. Nitrogen-bubbled suspensions.

3. Suspensions containing $\text{Na}_2\text{S}_2\text{O}_4$ (0.04 M),
4. Suspensions containing $\text{Na}_2\text{S}_2\text{O}_4$ (0.04 M) plus BAL (0.04 M).

The ratios of the slopes of the survival curves at 18° and 37°C for the conditions mentioned are as follows:

<u>Suspension</u>	<u>Slope at 37°C/ Slope at 18°C</u>
Oxygen bubbled	1.60
Nitrogen bubbled	1.22
Plus $\text{Na}_2\text{S}_2\text{O}_4$ (0.04 M)	1.23
Plus $\text{Na}_2\text{S}_2\text{O}_4$ (0.04 M)+ BAL (0.04 M)	1.25

The reduction in the ratio of the slopes at the two temperatures under conditions of reduced oxygen concentration, indicates that recovery is dependent on the oxygen concentration in the irradiated suspension.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF
RADIATION IN MICE

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Liane B. Russell	Gloria J. Jasny
Josephine S. Gower	Elizabeth M. Kelly
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Radiation-Induced Mutations in the Mouse

(W. L. Russell, Oakberg, Crowell, Cupp, Gower, Jasny, Kelly, Major, Sarvella)

An extensive account of the early results obtained in a large experiment using a 600 r X-ray exposure has been presented (ORNL-989). Data are still being accumulated in this experiment and the mutations obtained are still undergoing tests. The following additional information is now available.

The viability of the homozygotes of twenty-three of the mutations induced at specific loci has now been determined. The results are shown in Table 13. This is not a random sample: the speed with which a test can be completed depends partly on the locus and partly on the nature of the mutation. It is estimated that a final analysis will probably show a somewhat lower proportion of viables than that in the present figures.

TABLE 13

VIABILITY OF HOMOZYGOTES OF MUTATIONS AT SPECIFIC
LOCI IN THE IRRADIATED GROUP

Viability	Locus						Total
	B	C	D	P	S	Se	
Viable	2	2	-	3	-	1	8
Sublethal	-	-	5	2	-	-	7
Lethal	1	2	-	1	2	-	6

In the earlier report it was stated that approximately one-half the original S-locus mutants showed a reduction in body size. With the establishment of descendant generations for each of the mutations at the S

locus, it is now clear that most, or perhaps all, of these mutations will prove to have a detectable effect on size in heterozygous condition. There is evidence that a decrease in viability is associated with the size reduction. It is possible that the S locus is unusual, but the data as they stand, with the S-locus mutations forming a large proportion of the total for the seven loci tested, indicate that induced mutations with marked selective disadvantage in the heterozygous condition may be common and, therefore, that the genetic radiation hazard to immediate descendants in man may not be negligible.

The mean mutation rate for the seven loci being tested is holding at approximately the figure already reported. It has been pointed out that this is considerably higher than the rates obtained by various workers in Drosophila, although exact comparison of results is difficult. As this finding has a significant bearing on the estimation of human hazards, it was felt that more accurate information on the comparison between mouse and Drosophila should be obtained by setting up experiments in Drosophila that would, as closely as possible, duplicate those being made on the mouse. For this purpose, Dr. Alexander of this Division has begun a series of experiments to determine the rates of induced mutation, from X irradiation of spermatogonia, as well as of sperm, at eight autosomal loci in Drosophila.

Since it has been found feasible, with the method used, to obtain a reliable mutation rate for the mouse at the 600 r dose level, it now seems desirable to use the same method in an attempt to test whether or not the relationship between dose and mutation rate is linear for irradiated spermatogonia in the mouse. A new experiment, using a 1000 r exposure, has, therefore, been started.

The Influence of Genetic Constitution on the X-ray Induction of Developmental Changes

(Russell, Russell, Major)

An experiment is under way to determine the effect of genetic constitution on radiosensitivity to the induction of certain developmental abnormalities. Parameters chosen for study are homeotic shifts in the vertebral borders and related changes in the thorax. These characters are known to be affected by environmental factors since they show variation within inbred strains in which variation due to genetic factors is practically zero. Each strain has a different set of characteristic mean values, indicating that genetic constitution determines the location on a scale of developmental potencies, while environmental factors cause individuals

to be continuously distributed about this mean. Because of threshold in expression, however, the final character difference is usually a dichotomy from which the mean can be determined.

Method. - It was determined earlier (see ORNL-807) that the main critical stage for a posterior shift in the thoracolumbar border of the vertebral column and for related changes in the thorax is on day 8 1/2, with day 7 1/2 also highly sensitive; while an anterior shift is achieved by irradiation on day 11 1/2. In order to include all degrees of sensitivity to both the major shifts, radiation was applied to embryos of six different ages, namely, 7 1/2, 8 1/2, 9 1/2, 10 1/2, 11 1/2, and 12 1/2 days post-conception. Three different genetic strains were chosen to give a wide range of mean values for the characters under consideration. There are thus eighteen strain-stage groups. Each group received 100 r. Some of the early irradiations were made with 200 r so that several additional dose points are also available.

All observations are being made in newborns which have been processed for skeletal study by a modification of Dawson's technique. Since large numbers are required for the detection of quantitative shifts, we are observing between 45 - 85 young in each of the groups and have to date obtained a total of 2012 newborns of which 1245 were irradiated as embryos and 767 were controls. Since a few groups are still being filled in and since completion of the skeletal study will require considerably more time, only a sample report can here be given.

Results. - Results for about one-fourth of the animals obtained to date are shown in Fig. 11 for the thoracolumbar shift in the vertebral column only (without correlated thoracic changes) and in Fig. 12 for the lumbosacral border. Although several groups in two of the strains still need to be filled in, the figures already show the following:

1. The direction of the shift for any given stage of irradiation is the same in all three strains. Thus, e. g., all strains shift toward an increased rib number as well as an increased presacral number following irradiation on day 8 1/2; the shift toward a decreased rib number, which begins in the (C57 x NB) F₁ on day 9 1/2 (and comes to a peak on day 11 1/2) is also apparent in strain 129 on day 9 1/2.
2. The apparent radiosensitivity of the three strains is very different. Thus, for example, irradiation on day 8 1/2 post-conception increases the presacral number to 27 in 100 per cent of the B alb C strain animals, in only 3 per cent of the (C57 x NB) F₁'s and in none of the strain 129 mice; the rib number becomes greater than 13 in 100 per cent of the B alb

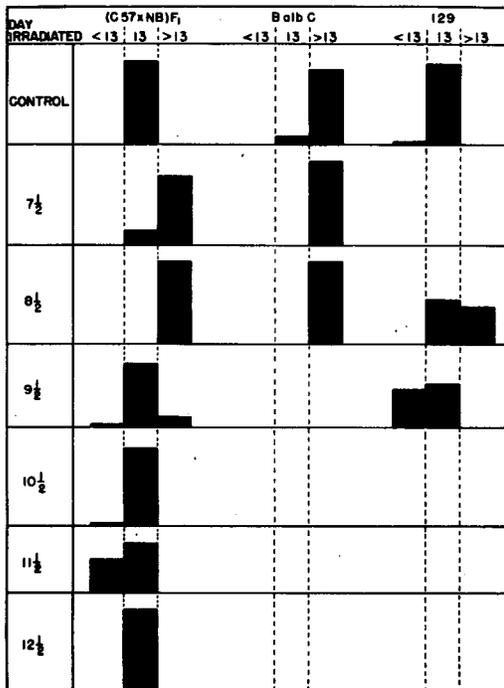


FIG. 11

Incidence of sides of animals having less than 13, 13, and more than 13 ribs in three strains of mice, following irradiation at different stages of embryonic development.

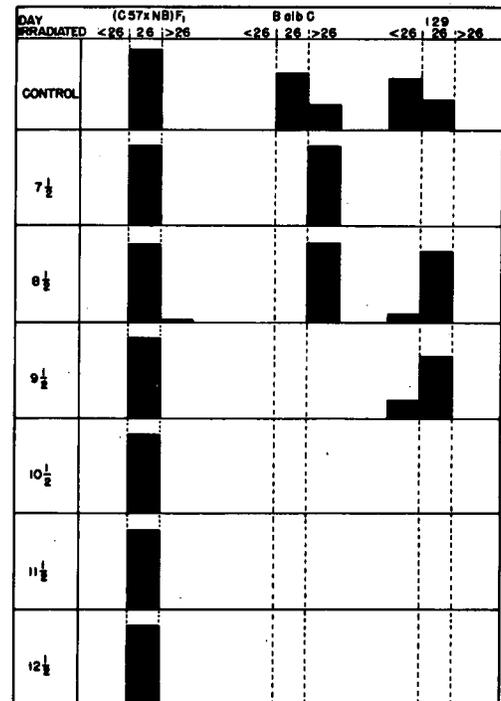


FIG. 12

Incidence of sides of animals having less than 26, 26, and more than 26 presacral vertebrae in three strains of mice, following irradiation at different stages of embryonic development.

C's and (C57 x NB) F₁'s, but in only 46 per cent of strain 129. On the other hand, strain 129 is apparently more sensitive to decrease in rib number than (C57 x NB) F₁ since irradiation on day 9 1/2 gives 46 per cent of the animals with 12 ribs in the former but only 2 per cent in the latter.

3. A consideration of the control distribution of each strain shows that its position with regard to the thresholds is such that an approximately equal radiation-induced shift in the mean of each strain would account for the results as found. Thus, the Balb C and 129 distributions are situated across the 26/27 and 25/26 thresholds respectively for presacral number and the 13/14 and 12/13 thresholds respectively for rib number, while the (C57 x NB) F₁ distribution crosses no threshold for either character.

4. It is therefore provisionally concluded (from as yet incomplete data) that the actual embryological effect of a particular dose of radiation is at least of the same order of magnitude in three different strains but that the position of the strains with regard to the threshold involved is such as to make them differ in the apparent ease of radiation shift.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

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Intermediate Compounds in the Propionic Acid Oxidation System

(Carson, Phares, Long, Gwin)

Previous studies on the "propionic acid fermentation" demonstrated that Propionibacterium pentosaceum contains enzymes which are typical of the tricarboxylic acid cycle (ORNL-989: 79-80). More recent experiments involving C¹⁴-labeled compounds confirmed this view in a reasonably unequivocal manner, and furthermore yielded new information concerning the probable pathway of propionate oxidation (ORNL-1167: 73-75).

Results of present experiments seem to leave little doubt but that the propionate oxidation (aerobic) involves an initial conversion of propionate to succinate (carboxylation in one or more steps?) followed by oxidation of succinate through the C₄ dicarboxylic acid system. A number of experiments have been conducted with propionate-2-C¹⁴ as well as with pyruvate-2-C¹⁴ as tracers, and with propionate as substrate. The bacterial system was allowed to continue the oxidation of propionate until 60 per cent of the theoretical oxygen consumption was observed (manometrically). The following end products and intermediate compounds were isolated, purified and degraded to individual carbon fragments: carbon dioxide, acetate, lactate, pyruvate, malate, succinate, fumarate, and residual propionate.

The results of a variety of such experiments lead us to propose that the oxidation pathway of propionate proceeds to the pyruvate stage via the symmetrical C₄ dicarboxylic acids, rather than through C₃ intermediates only. Experiments with cell-free juices and crude enzyme preparations (isolated systems) are of course necessary, and are in progress.

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BIOCHEMISTRY

STUDIES ON NUCLEIC ACIDS, ENZYMES, AND
ENERGY TRANSFER SYSTEMS

W. E. Cohn (Leader)

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E. Volkin	G. M. Cheniae
Fred Vaslow	Elizabeth H. Brigham

Composition and Structure of Nucleic Acids

(Cohn, Volkin, Jones)

With the exception of the calf-spleen nuclease products, all mononucleotide products of the complete enzymic degradation of ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) seem to have been identified and quantitatively assayed. Attention has therefore been turned again to the problem of identifying the products of partial degradations, such as results from the complete action of ribonuclease or by the partial action of other enzymes.

In the past, ribonuclease digests have defied our ion-exchange techniques (e. g., Carter and Cohn, J. Am. Chem. Soc., 72:2604, 1950). Recently, however, it has been found possible to fractionate such a digest and to recover it completely in well-defined peaks from an ion-exchange column. Half-gram digests of calf-liver RNA and yeast RNA have thus been separated into about forty components, some of which may be multiple in nature.

Detailed analysis of these separated components is in progress and will occupy the bulk of our efforts for several months. Some of them have been shown to be simple dinucleotides (guanylic-cytidylic, adenylic-cytidylic), analogous to these derived from DNA by Sinsheimer and from RNA by Markham and Smith. Others are probably tri- or higher order polynucleotides. With the techniques at hand, we believe we can at last account quantitatively for the material in RNA-RNase digests.

Analysis and Separation of Uronic Acids by Ion Exchange

(Khym, Doherty, Cheniae)

The presence of sugars generally interferes with the methods of analysis for the presence and amount of uronic acids in biological material.

No analytical methods reported so far have effected both a qualitative and quantitative determination of the uronic acids with a complete recovery of the pure individual uronic acids as crystalline material. Such a method will be described here through the use of an anion-exchange resin.

An alkaline solution of glucuronic and galacturonic acids was absorbed quantitatively on the strong base anion-exchange resin Dowex-1 in the acetate form. Under these conditions free sugars were not absorbed. The uronic acids were eluted with 0.15 M acetic acid and the fractions analyzed by a slight modification of the orcinol method described by Brown (Arch. Biochem., 11:269, 1946). The separation of glucuronic acid and galacturonic acid from arabinose and galactose is shown in Fig. 13. Crystalline uronic acids, obtained from the two peaks, were isolated and characterized as the benzimidazole derivative according to the procedure of Lohmar, Dimler, Moore, and Link (J. Biol. Chem., 143:551, 1942).

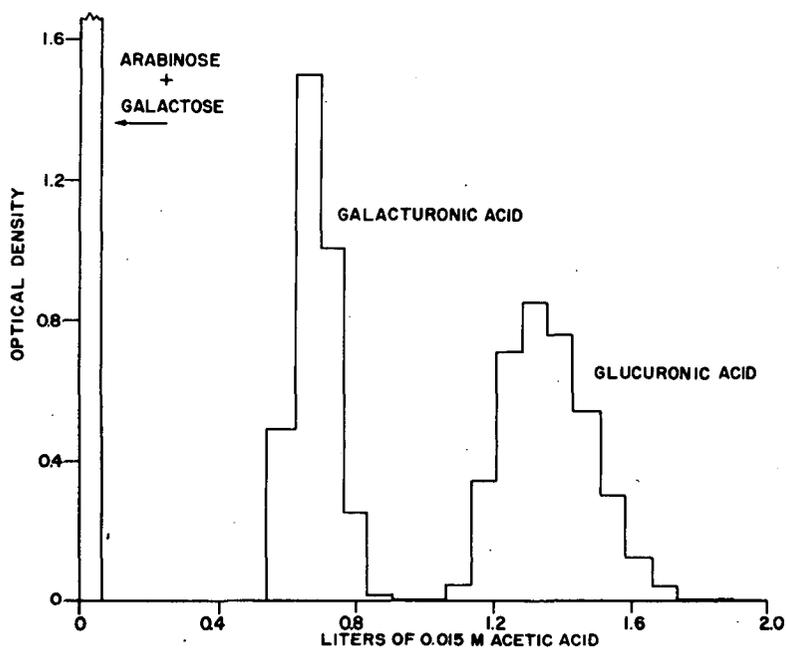


FIG. 13

The Separation of Galacturonic and Glucuronic Acids in the Presence of Sugar

Exchanger: 0.85 sq cm x 12 cm Dowex-1, ca 300 mesh acetate form

Eluting agent: 0.15 M acetic acid at ~2.5 ml/min

Test material: 5.0 mg each of arabinose and galactose, 10 mg each of galacturonic and glucuronic acids in 10 ml of 0.02 M sodium hydroxide (galactose was determined by anthrone method at 620 m μ ; the other materials by orcinol method at 660 m μ .)

Enzyme-Substrate Equilibria

(Vaslow)

The thermodynamic properties of the N-acetyl-3;5-dibromo-D-tyrosine-chymotrypsin complex have been measured. The values of the thermodynamic functions at pH 7.5 for this system, along with those for the L-acid with chymotrypsin and chymotrypsinogen taken from a previous report, are given in Table 14.

TABLE 14

System	F	H	S
Chymotrypsin-D-acid	-1900	-500	+ 5.0
Chymotrypsin-L-acid	-2000	-5600	-11.4
Chymotrypsinogen-L-acid	-2200	0	+ 8.5

Thus the D-acid system behaves similarly to the noncatalytic chymotrypsinogen system rather than the catalytically active system. This behavior is in contradiction to that proposed by Niemann (J. Am. Chem. Soc., 74:101, 1952) for enantiomorphous inhibitors and adds further evidence that the thermodynamic functions depend on the catalytic properties of the enzyme. Some attempt is being made to interpret these results on the basis of the quantum mechanical calculations of Stearn (J. Gen. Physiol., 18:301, 1935).

Biological Energy-Transfer Systems

(Strehler, Brigham)

Green Plant Luminescence. - A phosphoroscope has been built which makes possible the study of the luminescence of green plants very soon (1/100 second) after they have been illuminated. Preliminary studies on both Chlorella pyrenoidosa and Spinacea oleracea indicate that there is a short-lived phosphorescence (1/30-1/100 second) which does not saturate at moderate intensities. The possible relationship of this phenomenon to the Emerson-Arnold flashing-light dark-reaction constant will be investigated.

Firefly Luminescence. - Further studies on some physical properties of luciferin and one of its degradation products have been undertaken. In particular, the pKa has been determined by fluorometric and titration methods as well as the polarographic half waves for the reduction of luciferin (ca + 0.58 v) and one of its degradation products (ca + 0.80 v).

Some Studies on Riboflavin. - The polarographic behavior of riboflavin has been investigated with respect to a half wave which occurs at ca 1.2 volts in the presence but not in the absence of phosphate. The following findings have been made:

1. The height of the half wave is proportional to the logarithm of the phosphate concentration from 10^{-4} to 10^{-1} molar.
2. The height in the presence of phosphate is directly proportional to the H^+ ion concentration.
3. Arsenate is without effect. Whether this phenomenon is related to the oxidation phosphorylation function of riboflavin will be the subject of further investigation.

The chemiluminescent oxidation of riboflavin has been studied with respect to the wave length of the emitted light using a quartz Farrand monochromator and the IP22 Quantum Counter previously described. The color of the light is closely similar to if not identical with the fluorescent emission of this compound, probably indicating a similar electronically excited state for the two processes.

PLANT PHYSIOLOGY

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Studies with the Anthrone Reagent

(Noggle, Schumacher)

The anthrone reagent has been used extensively by this group for following the elution of sugars from the ion-exchange columns. Morris (Science 107:254, 1948) has indicated that the reagent may also be useful for the quantitative analysis of sugars. The reagent is not specific for any particular sugar but if a single sugar is present the reagent can be used to determine the sugar quantitatively. Inasmuch as the ion-exchange method enables one to separate individual sugars from a mixture it would appear that the anthrone method should be applicable to the quantitative analysis of these separated sugars. A rather complete study of the anthrone sugar reaction has been carried out and the important results are here summarized.

Color Development. - The intensity and stability of the colored anthrone-sugar reaction is dependent upon the heat formed during the addition of the anthrone reagent (0.2 per cent anthrone in 95 per cent sulfuric acid) to the sugar solution. This reaction has been stabilized by the following procedure: To 5 ml of the sugar solution in a colorimeter tube (19 x 170 mm) is added 10 ml of the anthrone reagent from an automatic pipette. During the addition of the reagent the tube is immersed in an ice-water bath and vigorously shaken to insure thorough mixing. The tube is then covered with a small glass funnel and placed in a boiling-water bath for 10 minutes. After cooling for 10 minutes in an ice-water bath, the photometric density of the solution is determined in an Evelyn colorimeter with a reagent-water tube as a blank. The 620 filter is used with the colorimeter.

The color so formed is stable for approximately 5 hours, and then gradually fades. The color formed varies with different batches of anthrone reagent so it is necessary to run standards with each batch of reagent.

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Color Formed by Different Sugars. - With any particular bath of reagent, the photometric density of the anthrone-sugar solution is proportional to the concentration of sugar present. The range of concentration of sugar that can be determined is between 1 and 50 μg per ml. The ratio of photometric density-concentration for a number of different sugars was determined and the results are shown in Table 15.

TABLE 15

Sugar	Photometric density	
	Conc. Found	Conc. Calculated
Glucose	0.0274	
Fructose	.0366	
Sucrose	.0336	(.0320)
Galactose	.0195	
Mannose	.0187	
Rhamnose	.0141	
Sorbose	.0235	
Cellobiose (Glucose - Glucose)	.0285	(.0274)
Trehalose (Glucose-Glucose)	.0271	(.0274)
Maltose (Glucose - Glucose)	.0287	(.0274)
Turanose (Glucose - Fructose)	.0319	(.0320)
Lactose (Glucose - Galactose)	.0235	(.0235)
Melibiose (Glucose - Galactose)	.0227	(.0235)
Raffinose (Glucose, Fructose, Galactose)	.0251	(.0245)
Melezitose (Glucose, Glucose, Fructose)	.0315	(.0305)
Stachyose (Glucose, Fructose, Galactose, Galactose)	.0221	(.0208)

The second column of the table gives the ratio of photometric density to concentration of sugar that was actually found. The third column gives

the calculated ratios of the disaccharides, trisaccharides, and tetrasaccharides if one considers that the color given by the individual sugars is additive. There is good agreement between the values actually found and those calculated.

Influence of Borate Ions on Reaction. - Upon analysis of sugar samples from the ion-exchange columns, borate ions are always found. The anthrone-sugar reaction was carried out in the absence and in the presence of borate ions (0.005 M, 0.015 M, 0.030 M, and 0.10 M) and it was found that borate did not interfere with the determination.

Ion-Exchange Chromatography of Sugars

(Zill)

The investigation of the ion-exchange chromatography of sugars and related compounds has been continued both with regard to the behavior of the pure compounds on ion-exchange columns and the application of the method to pertinent problems in the biochemical field.

Since sedoheptulose is not available in the crystalline form, this sugar was studied as an equilibrium mixture of the anhydride and the free sugar. It has been reported that this equilibrium has a composition of 20 parts free sugar to 80 parts of the anhydride form (based on reducing power). Complete separation of the two forms was achieved by chromatography on a column in the borate form, the ratio obtained being identical with the reported value.

Preliminary studies have been made on the effect of temperature on the separation of sugars which are normally eluted quite close together. Using a jacketed column at a temperature of 14°C, no appreciable change was obtained in the separation of sucrose from raffinose. The studies are to be continued using higher instead of lower temperatures.

Application of the method to the analysis of the enzymic hydrolysis of sucrose gave a peculiar ratio of fructose to glucose (after 60 per cent hydrolysis). A ratio of 8 mg of fructose to 12 mg of glucose was obtained instead of the theoretically equivalent amounts. It has been reported several times that a trisaccharide is formed during the action of invertase on sucrose and this might presumably account for the discrepancy. However, in view of a recent paper claiming that no such trisaccharide is formed, the amounts of the sugars formed during the enzymic hydrolysis is being redetermined.

Growth of Callus Culture in Presence of Radioisotopes

(Ball)

Further studies by radioautographs on NTB plates have confirmed the earlier report of accumulation of P^{32} , S^{35} , and C^{14} in meristematic cells of the callus of Sequoia sempervirens when grown on media containing either 1.5 or 1.0 μc per ml of these isotopes. The preparations have also shown something of the relationship between absorption and accumulation. In the tissue of the callus in contact with the agar medium there is a high accumulation of the isotope. Tissues farther away from these absorptive cells show less accumulation. The greatest accumulation of the isotopes occurs in the meristems at the margins of the cells and in the cambia around the tracheid groups. High accumulations were also found in the cells at the top surface of the calli which are the farthest away from the agar medium. There is thus selective accumulation of the isotopes in the tissues of this callus culture. Diffusion of the isotopes from the agar medium cannot be utilized as an explanation of these phenomena.

Growth of the Callus on Various Carbohydrates. - The technique previously described was contained: the callus of Sequoia that had been grown through twenty transfers (forty months) upon autoclaved sucrose medium was transferred to medium containing an equal concentration (w/w) of another sugar that had been sterilized by filtration. In a few cases the sugar was sterilized by autoclaving. Observations were made upon the growth. At the end of two months of culture the callus was removed from the tube and extracted for sugars. These sugars were analyzed and identified by paper chromatography. The culture medium upon which the callus had grown was similarly studied. (Table 16).

TABLE 16

Description of Culture	Sugars Found by Chromatograms
Sequoia shoots from burl germinated 9-1-51	glucose, levulose, sucrose
Sequoia callus 3% sucrose filtered 10-26-51 to 12-26-51	glucose, levulose, sucrose
Knop's medium 3% sucrose filtered on which Sequoia callus had grown 10-26 to 12-26	glucose, levulose, sucrose
Sequoia callus 3% d(-) levulose filtered 2nd tr. 11-7-51 to 1-8-52	glucose, levulose, sucrose

Table 16 cont.

Description of Culture	Sugars Found by Chromatograms
Knop's medium 3% d(-) levulose filtered on which Sequoia callus had grown 11-7-51 to 1-8-52	glucose, levulose
Sequoia callus 3% glucose filtered 10-18-51 to 12-21-51	glucose, levulose, sucrose
Knop's medium 3% glucose filtered on which Sequoia callus had grown 10-18-51 to 12-21-51	glucose, levulose
Sequoia callus 3% d(+) lactose autoclaved 2nd tr. 11-5-51 to 1-9-52	glucose, levulose, sucrose, lactose
Knop's medium 3% d(+) lactose on which Sequoia callus had grown 11-5-51 to 1-9-52	glucose, levulose, lactose
Sequoia callus 3% d(+) raffinose filtered 2nd tr. 11-7-51 to 1-10-52	glucose, levulose, sucrose, raffinose, galactose
Knop's medium 3% d(+) raffinose filtered on which Sequoia callus had grown 11-7-51 to 1-10-52	glucose, levulose, raffinose, galactose
Sequoia callus 3% d(+) galactose filtered 2nd tr. 12-10-51 to 1-16-52	glucose, levulose, sucrose, galactose, raffinose (?)
Knop's medium 3% d(+) galactose filtered on which Sequoia callus had grown 2nd tr. 10-10-51 to 1-16-52	glucose, levulose, galactose, raffinose (?)
Sequoia callus 3% cellobiose autoclaved 2nd tr. 11-30-51 to 1-21-52	glucose, levulose, sucrose, cellobiose
Knop's medium 3% cellobiose autoclaved on which Sequoia callus had grown 11-30-51 to 1-21-52	glucose, cellobiose
Sequoia callus 3% d(-) ribose filtered 10-11-51 to 12-11-51	glucose, levulose, sucrose, ribose
Knop's medium 3% d(-) ribose filtered on which Sequoia callus had grown 10-11-51 to 12-11-51	ribose
Sequoia callus 3% d(+) xylose filtered 10-11-51 to 12-12-51	glucose, levulose, sucrose, xylose

Table 16 cont.

Description of Culture	Sugars Found by Chromatograms
Knop's medium 3% d(+) xylose filtered on which Sequoia callus had grown 10-11-51 to 12-12-51	xylose
Sequoia callus 3% glycerol filtered 10-18-51 to 12-21-51	glucose, levulose, sucrose
Knop's medium 3% glycerol filtered on which Sequoia callus had grown 10-18-51 to 12-21-51	glycerol
Sequoia callus 3% dextrin autoclaved 9-5-51 to 10-29-51	glucose
Knop's medium 3% dextrin autoclaved on which Sequoia callus had grown 9-5-51 to 10-29-51	glucose
Sequoia callus 3% inulin autoclaved 9-5-51 to 10-30-51	glucose, levulose, sucrose
Knop's medium 3% inulin autoclaved on which Sequoia callus had grown 9-5-51 to 10-30-51	no sugars

In a generalization it may be stated that, in addition to growing on any of the three sugars normally found in the shoots, the callus will grow on at least nine other sugars. On these other sugars the callus usually takes up some of the "foreign" sugar from the culture medium and apparently retains it in its living cells. From this "foreign" sugar the callus makes the three normal sugars: glucose, levulose, and sucrose.

BIOPHYSICS

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GENERAL PHYSIOLOGY

Isolated Cell Components

(Anderson)

The permeability and physico-chemical properties of the nuclear envelope are of interest because they are thought to limit or control the exchange of material between the nucleus and the cytoplasm. It has been shown qualitatively that the nuclear envelope of fresh, presumably intact, isolated rat liver nuclei is permeable to certain proteins. However blebs or blisters which arise from the nuclear envelope in response to a variety of conditions do not appear to be permeable to proteins. The implications of this observation are being investigated. Bleb formation has been shown to occur in a wide variety of salt solutions. They are largest and occur most frequently in dilute solutions (0.01-0.001 M) of magnesium chloride and calcium chloride. Sucrose in concentrations as low as 0.05 M has been shown to inhibit effectively bleb formation.

Polyelectrolytes

(Anderson)

A number of attempts have been made to relate the polyelectrolyte heparin to radiation damage. The possibility that heparin release may be a protective mechanism does not appear to have been examined. Twenty-six two-month-old rats were injected with Depo-heparin (2000 T. U.) and then X-rayed (250 r, 2 mm Al, 250 kv, 30 ma). A control group of twenty-six rats was injected with partially degraded gelatin and given a similar dose of X rays. The average survival time for the heparin group was 8.1 days, and for the control group, 9.1 days. Two other experiments in which Depo-heparin was injected at intervals after irradiation and the controls injected with polyelectrolyte heparin show only slight differences in survival time. Heparin does not appear to hasten the appearance of the

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** Research Participant

hemorrhagic syndrome, but rather to cause more rapid death once it becomes apparent. No protection effect was noted.

Comparison of the Effects of X Rays, Gamma Rays, and Beta Rays on Tradescantia Pollen

(Kirby-Smith, D. Daniels)

A number of experiments to determine the relative effects of these radiations on Tradescantia pollen have been completed. In all cases a quantity of pollen sufficient to establish definitely the dose versus aberration rate curves at four or more points has been irradiated and grown. The results for one completely scored experiment show an aberration frequency for X rays approximately twice that observed for γ rays or β rays. A smaller difference has been observed between the aberration rates for γ rays and β rays. All curves have shown a definite nonlinear variation of aberration frequency with dose for isochromatid breaks, contrary to the usual linear curves obtained for isochromatids in Tradescantia microspores. Scoring of slides from other experiments is continuing.

The results being obtained in these studies are based on a large quantity of material and on conditions sufficiently controlled to warrant further analysis. It is apparent that the energy distribution of the secondary electrons in the X-ray and γ -ray experiments, and the energy distribution of the P^{32} β rays used can no longer be ignored. Means for obtaining this information is now being considered, and possibility of obtaining an accurate theoretical energy distribution for the P^{32} β rays has been recently discussed with T. A. Welton of the Physics Division.

Biophysical Instrumentation

(Kirby-Smith, Anderson)

A simple nephelometer operating in the near infrared region has been built for use in blood plasma studies. Scattered radiation is measured by means of a lead sulfide photoconductive detector in a simple bridge circuit or by chopping incident light and reading the detector output on a vacuum tube voltmeter. The instrument appears to have sufficient sensitivity to be useful out to a wave length of 3 microns.



RADIOLOGICAL PHYSICS

(Darden, Kirby-Smith)

Since the transferral to the Operations Division in July 1950 of the surface chamber equipment (ORNL-265) for the routine calibration of P^{32} β -ray plaques an occasional lack of agreement, usually of, 5-10 per cent, has been noted between our calibrations made with the extrapolation chamber and those made by the Operations Division. Since that time several redeterminations of a uranium β -ray rep value have been made here as a check on the operation of our chamber. These recent measurements have agreed consistently within ± 5 per cent of the value obtained when the chamber was first put into operation three years ago. Due to the considerable use and necessary repair undergone by our instrument in the last few months it was deemed advisable to make a more accurate and direct comparison of the two chambers. This was done by carefully calibrating a phosphorus plaque on the extrapolation chamber, after which the plaque was taken to X-10 and measured on the surface chamber. The agreement was within 2 per cent. These results provide satisfactory additional evidence that the quantitative phosphorus β -ray work in this division rests on a firm basis.

Calibration measurements of the large 300-curie distributed type Co^{60} source recently installed here have been largely completed. Along the central part of the axial region of the source exists a field intensity of approximately 1.3×10^5 r/hour. This is in agreement with chemical dosimeter measurements made by K. C. Atwood and bacterial dosimeter determinations by G. E. Stapleton. The field of the intermediate Co^{60} source, originally calibrated in June 1951, has been recalibrated at a number of points and excellent agreement with the original values obtained. It is planned to calibrate, as a secondary standard, a second thimble-type γ -ray dosimeter against our standard thimble chamber and install it permanently in the source for routine use.

Rough initial measurements have been made at the Y-12 cyclotron to ascertain the feasibility of biological experiments with fast neutrons. The preliminary estimates, based on comparison of the readings of shielded and unshielded and condenser-type ion chambers (primarily Victoreen dosimeters) suggest that, under present operating conditions, the maximum fast neutron flux available may be sufficient for experiments involving low-level radiation intensities. In order to refine our estimates a bismuth chamber having a low cross section, similar in design to the beryllium condenser-type chambers used by us to measure γ radiation in the thermal column of the graphite reactor (Darden, Sheppard, Emerson, ORNL-1003) has been designed and is being constructed by the Instrument Department.

