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BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT

for Period Ending May 10, 1951

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Progress Report

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BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT
for Period Ending May 10, 1951

Alexander Hollaender, Director

Edited by E. J. Slaughter

Date Issued: AUG 22 1951

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36. C. D. Susano
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38. S. I. Carson
39. B. L. Strehler
40. S. C. Lind
41. C. W. Sheppard
42. W. L. Russell
43. F. F. Kimball
44. J. R. Noggle
45. Seymour Pomper
46. M. E. Gaulden
47. E. J. Collins
48. H. H. Anderson
49. A. H. Doermann
50. Jacob Furth
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ORNL-807	Period Ending August 15, 1950
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MICROBIOLOGY 43
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INTRODUCTION

Review of research progress. Continuance of significant findings in the work on protection against radiation damage has stimulated an extension of the investigations to include work with *Paramecium* and bacteriophage. Ethyl alcohol, BAL, sodium hydrosulfite, and sodium formate were tested for protective action against X-ray damage. Results show that sodium formate does not protect paramecia, while sodium hydrosulfite and alcohol do give some protection to this organism. Sodium formate and sodium hydrosulfite do not protect bacteriophage against direct inactivation, but significant protection is afforded by glycol, ethanol, BAL, and cysteine. Bacteria remain in the lead as the material yielding the most outstanding data on radiation protection.

Amino acids which are oxidized by *Escherichia coli* will protect the bacteria against X-ray damage if they are incubated for 30 minutes in the presence of the acid. For example, serine, α-alanine, and pyruvate protect very well, while β-alanine and propionic acid give practically no protection. Compounds which protect against radiation damage only after incubation can be blocked in their protective ability if sodium cyanide is added. Contrary to this, BAL, which is not oxidized by *E. coli* is not inhibited in its protective action by sodium cyanide.

Morphine has been found to be a good protector against X-ray damage in mice.

Interesting results coming out of the study of the effects of radiation on the appearance of blood in lymph spaces are continuing.

Carbon-14-labeled citric acid was biosynthesized from sucrose and acetate-2-C^{14} by the mold *Aspergillus niger;* degradation of the citrate to its six individual carbon atoms by a method worked out in this laboratory led to interesting findings concerning citrate metabolism. "Randomization through the C_4 acid shuttle," a generally believed concept in intermediary biochemistry, has been checked by direct experiments.

It has been found by the Biochemistry and Plant Physiology groups that sugars treated with certain borates can be separated by ion-exchange methods. In view of the difficulties and complications involved in the separation of the different sugars, this opening of a new field in sugar chemistry is an important step. Separation of sugars by this new method can be accomplished with ease.

At the present rate of progress, the new gamma source should be available to the laboratory before the end of the summer.

A number of amino acids have been separated from plants grown in carbon-14.

Investigations in the use of polonium as an α-particle source on chromosome breaks in *Tradescantia* and grasshopper neuroblasts is showing satisfactory development. It has been possible to verify the previous finding that infrared and X rays combined will increase the X-ray damage by treating individual pollen tubes of *Tradescantia*.

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTICIC PUBLIC

Publications. Publication of the following papers has been accomplished during the quarter just ended. The backlog of ORNL reports which have also been published in the open literature has been cleared away. Henceforth, these reports will be issued in the reprint form, which is generally considered to be a great improvement, from the standpoints of form of presentation and economy, over the old procedure.

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION
Anderson, E. H.	The Effect of Oxygen on Mutation Induction by X Rays (Abstract)	Bacteriological Proc., May 1951, p. 64
Arnold, W. A., and W. G. Stone	Stable Isotopes (Abstract)	Proc. Assoc. Sou. Agri. Wkrs., 48th Ann. Conven., p. 134
Bigelow, R. R., J. Furth, M. C. Woods, and R. H. Storey	Endothelial Damage by X Rays Disclosed by Lymph Fistula Studies	Proc. Soc. Exper. Biol. & Med., 76; 734-736, 1951 (April)
Bizzell, O. M. W. T. Burnett, Jr., P. C. Tompkins, and L. Wish	Properties of Phosphorus-Bakelite Beta-Ray Source	Nucleonics, 8: 17-27, 1951 (April)
Burnett, W. T., Jr., G. E. Stapleton, and A. Hollaender	A Study of the Protective Action of Some Sulfur-Containing and Sulfur-Free Compounds Against X-Ray Damage in Bacteria (Abstract)	Fed. Proc., 10: 22, 1951 (March)
Carter, C. E.	Enzymatic Evidence for the Structure of Desoxyribose Nucleotides	J. Am. Chem. Soc., 73: 1537-1538, 1951 (April)
Carter, C. E.	Partial Purification of a Nonphosphorylytic Uridine Nucleosidase from Yeast	J. Am. Chem. Soc., 73: 1508-1510, 1951 (April)
Christenberry, K. W., and J. Furth	Cataract Induction by Slow Neutrons and X Rays (Abstract)	Fed. Proc. 10: 351, 1951 (March)
Cohn, W. E.	The Isolation and Identification of Desoxy-5-Methyl-Cytidylic Acid from Thymus Nucleic Acid	J. Am. Chem. Soc., 73: 1539-1541, 1951 (April)
Cohn, W. E., and E. Volkin	Nucleoside-5′-Phosphates from Ribonucleic Acid	Nature, 167: 483-484, 1951 (March)
Cohn, W. E., and E. Volkin	The Isolation of 5′-Nucleotides from Nucleic Acid Digests (Abstract)	Fed. Proc., 10: 173, 1951 (March)
Furth, J., E. J. Beale, L. Wish, and M. M. Knoohuizen	Organ Blood Volumes of Tumor-Bearing Mice; Oligemia of Neoplasms (Abstract)	Scientific Proc., 2: 249, 1951
Foster, J. W., E. F. Phares, and S. F. Carson	Formate in the Synthesis of Organic Acids by Fungi (Abstract)	Bacteriological Proc., May 1951, p. 133
Gottschalk, R. S., and J. Furth	Polycythemia with Features of Cushing's Syndrome Produced by Luteomas.	Acta Haematologica, 5: 100-122, 1951

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION
Holt, A. S., I. A. Brooks, and W. A. Arnold	Some Effects of 2537 A on Green Algae and Chloroplast Preparations	J. Gen. Physiol., 34: 627-645, 1951 (May)
Holt, A. S., R. F. Smith, and C. S. French	Dye Reduction by Illuminated Chloroplast Fragments	Plant Physiol., 26: 164-173, 1951 (Jan.)
Khym, J. X., and L. P. Zill	The Separation of Monosaccharides by Ion Exchange (Communication to the Editor)	J. Am. Chem. Soc., 73: 2399-2400, 1951 (May)
Kohn, H. I.	Changes in Composition of Blood Plasma in the Rat During Acute Radiation Syndrome and their Partial Mitigation by Dibenamine and Cortin	Am. J. Physiol., 165: 27-42, 1951 (April)
Kohn, H. I., Nancy Swingley, W. J. Robertson, and Marian Kirslis	The Effect of Immaturity, Hypophysectomy and Adrenalectomy upon the Changes in the Blood Plasma During the Acute Radiation Syndrome in the Rat	Am. J. Physiol., 165: 43-56, 1951 (April)
Kohn, H. I.	The Effect of X Rays upon Hemolysin Production in the Rat	J. Immunol., 66: 525-533, 1951 (May)
Mosbach, E. H., E. F. Phares, and M. V. Long	Degradation of Alpha-Ketoglutaric, Glutamic, and Citric Acids (Abstract)	Fed. Proc., 10: 226, 1951 (March)
Noggle, G. R.	Radioisotopes in Plant Physiology Studies (Abstract)	Proc. Assoc. Sou. Agri. Wkrs., 48th Ann Conven., p. 134
Noggle, G. R., and R. A. Bolomey	The Biosynthesis of Carbon-14 Labeled Compounds. I. The Chromatographic Separation of Glucose and Fructose	Plant Physiology, 26: 174-181, 1951 (Jan.)
Oginsky, E. L., P. H. Smith, N. E. Tonhazy, W. W. Umbreit, H. C. Lichstein, and S. F. Carson	The Influence of Vitamin B 12 on Oxidations by a Mutant Strain of *E. coli*	J. Bact., 61: 581-590, 1951 (May)
Pomper, Seymour	A Complex, pH-Sensitive Yeast Mutant (Abstract)	Bacteriological Proc., May 1951, pp. 51-52
Pomper, Seymour	Recent Developments in Yeast Genetics	Wallerstein Lab. Commun., 14: 31-42, 1951
Sheppard, C. W., and Gertrude Beyl	Cation Exchange in Mammalian Erythrocytes. III. The Prolytic Effect of X Rays on Human Cells	J. Gen. Physiol., 34: 691-704, 1951 (May)
Sheppard, C. W., P. F. Hahn, and Gilbert Jordan	The Disappearance of Isotopically Labeled Gold Colloids from the Circulation of the Dog	Am. J. Physiol., 164: 345-350, 1951 (Feb.)
Sheppard, C. W., and A. S. Householder	The Mathematical Basis of the Interpretation of Tracer Experiments in Closed Steady State Systems	J. Applied Physics, 22: 510-520, 1951 (April)
Sheppard, C. W., 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., 34: 411-429, 1951 (March)
Sheppard, C. W., and Marian Stewart	The Lack of an Oxygen Effect in the Disturbance by X Rays of Selective Potassium Accumulation in Human Erythrocytes *in vitro* (Abstract)	Fed. Proc., 10: 125, 1951 (March)
Stapleton, G. E., and A. Hollaender	Differential X-Ray Sensitivity of Bacterial Cells During the Growth Cycle (Abstract)	Bacteriological Proc., May 1951, p. 62

AUTHOR(S)	TITLE OF PAPER	OPEN PUBLICATION
Strehler, B. L., and W. Arnold	Light Production in Green Plants (Abstract)	Fed. Proc., 10: 225, 1951 (March)
Teas, H. J.	Effect of Canavanine on Mutants of *Neurospora* and *Bacillus subtilis*	J. Biol. Chem., 190: 369-375, 1951 (May)
Totter, J. R., E. Volkin, and C. E. Carter	Incorporation of Isotopic Formate into the Nucleotides of Ribo- and Desoxyribonucleic Acids	J. Am. Chem. Soc., 73: 1521-1522, 1951 (April)
Volkin, E., and C. E. Carter	The Incorporation of Isotopic Phosphate in the Mononucleotides of Liver Nucleic Acids	J. Am. Chem. Soc., 73: 1519-1520, 1951 (April)
Volkin, E., and C. E. Carter	The Preparation and Properties of Mammalian Ribonucleic Acids	J. Am. Chem. Soc., 73: 1516-1519, 1951 (April)
Volkin, E., J. X. Khym, and W. E. Cohn	The Preparation of Desoxynucleotides	J. Am. Chem. Soc., 73: 1533-1536 (April)
Volkin, E., and H. I. Kohn	A Factor in the Plasma of the Irradiated Rat Which Changes the A/G Ratio	Arch. Biochem., 30: 326-332, 1951 (Feb.)

Editing and preparation for publication of the collection of papers and open discussions presented at the 1949 Biology Conference on "Radiation Microbiology and Biochemistry" is nearing completion. Following the same plan of publication of the proceedings of the 1948 ("Radiation Genetics") and 1950 ("Biochemistry of Nucleic Acids") conferences, these papers will be published as a supplement to the Journal of Cellular and Comparative Physiology. The table of contents follows.

Introduction - Alexander Hollaender

Howard B. Newcombe. A comparison of spontaneous and induced mutations of *Escherichia coli* to streptomycin resistance and dependence.

C. P. Swanson. The effect of supplementary factors on the radiation-induced frequency of mutations in *Aspergillus terreus*.

Walter M. Dale. The indirect action of ionizing radiations on aqueous solutions and its dependence on the chemical structure of the substrate.

L. H. Gray. The energy transfer from ionizing particles to an aqueous medium and its bearing on the interpretation of radiochemical and radiobiological change.

R. E. Zirkle. Exponential and sigmoid survival curves resulting from alpha and X irradiation of *Aspergillus* spores.

G. E. Stapleton, Alexander Hollaender, and F. L. Martin. Mechanism of lethal and mutagenic action of ionizing radiation on *Aspergillus terreus*. I. Relationship of relative biological efficiency to ion density.

G. E. Stapleton and Alexander Hollaender. II. Use of modifying agents and conditions.

ABSTRACTS OF PAPERS

Albert Kelner. Experiments on photoreactivation with bacteria and other microorganisms.

S. E. Luria. Reactivation of ultraviolet-irradiated bacteriophage by multiple infection.

Renato Dulbecco. Experiments on photoreactivation of inactive bacteriophage.

Scientific society lectures and traveling seminars. A total of seventy-three lectures have been given by Division members during this quarter. Sixty of these presentations have been given on the Traveling Seminar program co-sponsored by the Oak Ridge Institute of Nuclear Studies and the Oak Ridge National Laboratory; thirteen oral reports have been delivered before scientific society meetings.

Anderson, E. H.	Virginia Poly. Inst.	Factors modifying the X-ray survival and mutation induction in bacteria
Arnold, W. A.	N. E. Tenn. Sec. ACS, Johnson City	Some problems in photosynthesis
Arnold, W. A.	Vanderbilt University	Problems connected with photosynthesis
Arnold, W. A.	University of Georgia	Same subject
Arnold, W. A.	University of Tenn., Memphis	Same subject
Arnold, W. A.	University of Maryland	Light production in plants
Burnett, W. T., Jr. (G. E. Stapleton, A. Hollaender)	Am. Physiol. Soc. (Fed. Am. Soc. Exper. Biol.), Cleveland	A study of the protective action of some sulfur-containing and sulfur-free compounds against X-ray damage in bacteria
Carson, S. F.	Winthrop College	1. Organisms: their transformation of raw foodstuffs into cell material and energy 2. Isotope experiments concerned with problems in intermediary metabolism
Christenberry, K. W. (J. Furth)	Am. Soc. for Exper. Pathol. (Fed. Am. Soc. Exper. Biol.), Cleveland	Induction of cataracts in mice by slow neutrons and X rays
Cohn, W. E.	Atlanta University	The application of ion exchange to nucleic acid chemistry

Cohn, W. E.	Emory University	Recent developments in the chemistry of nucleic acids
Cohn, W. E.	Alabama Poly. Inst.	The application of ion exchange to nucleic acid chemistry
Cohn, W. E.	University of N. C.	Some recent developments in the chemistry of nucleic acids
Cohn, W. E.	Duke University	1. Application of ion exchange to nucleic acid chemistry
		2. Some recent developments in the chemistry of nucleic acids
Cohn, W. E. (E. Volkin)	Am. Soc. Biol. Chem. (Fed. Am. Soc. Exper. Biol.), Cleveland	The isolation of 5′ nucleotides from nucleic acid digests
Darden, E. B., Jr. (C. W. Sheppard)	S. E. Sec. Am. Phys. Soc., Chattanooga	Verification of the value of the standard γ-ray r unit with a thimble type ion chamber
Doermann, A. H.	La. State University	1. The genetics of bacteriophage
		2. Same as above continuation
Doermann, A. H.	University of Texas	Genetics as a tool in the study of bacteriophage reproduction
Doermann, A. H.	Tulane University Medical School	Same subject
Doherty, D. G.	University of Tenn., Memphis	Proteolytic enzyme studies
Doherty, D. G.	University of Oklahoma	Same subject
Doherty, D. G.	Oklahoma A. & M.	Same subject
Doherty, D. G.	Alabama Poly. Inst.	Kinetics and reactions of proteolytic enzymes
Furth, J.	La. State University	Radiation sickness; physiological and pathological effects of radiation
Furth, J.	Tulane Univ. Med. School	Human leukemias in the light of experimental studies of leukemias in animals
Furth, J.	University of Florida	Neoplasia by irradiation
Furth, J.	Meharry Med. College	The etiology and nature of the leukemias
Furth, J. (R. R. Bigelow, J. B. Kahn, M. M. Knoohuizen, M. H. Ross)	Am. Assoc. of Pathol. & Bacteriol., Cleveland	Morphological changes in the lymph: estimation of permeability and lymphopoiesis
Furth, J. (E. J. Beale, L. Wish, M. Knoohuizen)	Am. Assoc. for Cancer Research, Cleveland	Organ blood volumes of tumor-bearing mice: oligemia of neoplasms
Gaulden, M. E.	Duke University	The effects of radiation and chemicals on cell structure and mitosis as observed in living cells
Gaulden, M. E.	Vanderbilt University	Same subject
Harrington, N. H.	University of Tennessee	Quantitative cytochemical determinations by the photometric method
Harrington, N. H. (R. W. Koza)	Assoc. of S. E. Biol., Tuscaloosa	Effect of X radiation on the desoxyribonucleic acid and on the size of grasshopper embryonic nuclei
Hollaender, A.	Ciba Foundation Conf., London	Modification of X ray damage by chemical compounds and oxygen

Hollaender, A.	USDA, Beltsville	1. Biological applications of high-energy radiation
		2. Factors modifying sensitivity of living cells to radiation
Hollaender, A.	Howard University (Sigma Xi)	Studies on the biological effects of radiation
Kimball, R. F.	Virginia Poly. Inst.	A comparison of genetic and nongenetic effects of radiation on *Paramecium aurelia*
Kimball, R. F.	Howard University	Same subject
Mosbach, E. H. (S. F. Carson, E. F. Phares *et al.*)	Am. Soc. Biol. Chem. (Fed. Am. Soc. Exp. Biol.), Cleveland	Degradation of alpha-ketoglutaric, glutamic, and citric acids
Noggle, G. R.	Duke University	1. Chromatographic methods as applied to problems in plant physiology
		2. The transformation of sugars in plants
Noggle, G. R.	University of N. C.	The transformation of sugars in plants
Noggle, G. R.	N. C. State College	Same subject
Noggle, G. R.	University of Texas	Same subject
Noggle, G. R.	University of Oklahoma	Same subject
Noggle, G. R.	University of Georgia	Same Subject
Noggle, G. R.	Emory University	Chromatographic methods as applied to problems in plant physiology
Noggle, G. R.	Alabama Poly. Inst.	The transformation of sugars in plants
Noggle, G. R.	University of Tennessee	Chromatographic methods as applied to problems in plant physiology
Pomper, S.	Emory University	Biochemical genetics of yeast
Russell, L. B.	Texas A. & M.	Radiation as a tool in mammalian embryology
Russell, L. B.	Tulane Univ. Med. Sch.	Effects of radiation on prenatal development of mammals
Russell, L. B.	La. State Univ. Med. Sch.	Same subject
Russell, L. B.	Emory University	Radiation as a tool in mammalian embryology
Russell, L. B.	Atlanta University	Effects of radiation on prenatal development of mammals
Russell, L. B.	University of Georgia	Same subject
Russell, W. L.	University of Texas	Mammalian radiation genetics
Russell, W. L.	Rice Institute	Same subject
Russell, W. L.	Tulane Univ. Med. Sch.	Same subject
Russell, W. L.	La. State Univ. Med. Sch.	Same subject
Russell, W. L.	Emory University	Same subject
Sheppard, C. W.	Alabama Poly. Inst.	The interpretation of isotope experiments
Sheppard, C. W.	Emory University	Radiobiology of the erythrocyte
Sheppard, C. W.	La. State University	Tracer uses in medicine
Sheppard, C. W.	Tulane Univ. Med. Sch.	The interpretation of isotope experiments

Sheppard, C. W.	Rice Institute	Same subject
Sheppard, C. W.	Prairie View A. & M.	Same subject
Sheppard, C. W.	Univ. of Ark. Sch. of Med.	The radiobiology of the erythrocyte
Sheppard, C. W. (L. J. Savage)	S. E. Sec. Am. Phys. Soc., Chattanooga	The random walk in relation to the physiology of circulatory mixing
Sheppard, C. W. (Marian Stewart)	Am. Physiol. Soc. (Fed. Am. Soc. Exp. Biol.), Cleveland	The lack of an oxygen effect in the disturbance by X rays of selective potassium accumulation in human erythrocytes *in vitro*
Strehler, B. L. (W. A. Arnold)	Am. Soc. Biol. Chem., (Fed. Am. Soc. Exp. Biol.), Cleveland	Light production in green plants

Visiting lecturers. The Biology Division has been fortunate in securing the services of the following leaders in the field of biology and medicine for weekly Biology seminars.

Dr. Luther Smith - State College of Washington - "Environmental Factors Influencing X-ray Sensitivity"

Dr. George Wald - Harvard Biological Laboratory - "The Chemistry of Rod Vision"

Dr. J. L. Bollman - Mayo Foundation, University of Minnesota Graduate School - "The Physiologic Factors Affecting Lymph Formation"

Dr. Jonas S. Friedenwald - Johns Hopkins University - "Problems in Enzymatic Histochemistry"

Dr. Eugene F. Oakberg - Iowa State College - "Genetic Differences in Growth of Organs and Tissues of Chickens, Particularly in Reference to Leukosis Mortality"

Dr. L. J. Stadler - University of Missouri - "Gene Mutation in Maize"

Dr. David Merrell - University of Minnesota - "Selection in Populations of *Drosophila*"

Dr. Warren Spencer - Wooster College - "Genetic Variability in *Drosophila* Populations"

Dr. Bernard Strauss - University of Texas - "Acetate Formation by an Acetate-requiring mutant of *Neurospora*"

Dr. James A. Miller - Emory University - "Effects of Temperature Variations Upon Asphyxial Resistance in Guinea Pigs"

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

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

Protective action of various agents upon radiation damage in Paramecium. (Kimball, Gaither, King) Hollaender, Burnett, Stapleton, *et al.* (ORNL-969: 23-32, 1951) have found a variety of substances which protect *Escherichia coli* against inactivation by X rays when they are present in the medium during irradiation. Four of these substances are being tested for their protective action against various types of radiation damage in *Paramecium aurelia.* The tests are not complete but several preliminary findings are of interest.

The four substances tested were ethyl alcohol, British anti-lewisite (BAL), sodium hydrosulfite, and sodium formate. The first three of these substances proved to be quite toxic to paramecia, and in consequence it was not possible to use concentrations as high as those used by Hollaender *et al.* This fact makes it difficult to evaluate negative results with these compounds.

A series of tests were carried out using the Maximar 250 machine operated at 250 kvp and 30 ma with a filter of 3 mm of aluminum. The doses of X rays ranged from 2500 to 5000 r at a rate of approximately 400 r per minute. At these low doses, the only effect which can be measured is the per cent of reduced vigor after autogamy which is a measure of the amount of mutation in the micronucleus.

Other tests were carried out for the effect of the X rays upon survival through the first day, and upon division rate. The doses used were estimated to be of the order of 10^4 r. The unfiltered beam of the Maximar 250 machine operated at 250 kvp and 30 ma was employed, and directed upward through the thin mica bottom of a specially designed dish. The animals were placed in a small drop of water in the center of the dish under a 2-cm-deep layer of a light grade mineral oil. The intensity in air at the same position as the drop of water was estimated to be approximately 60,000 r per minute using a nylon Victoreen chamber.

The measures used with the high intensity radiation were per cent survival through the first 24 hours after irradiation, average number of divisions in the first 24 hours among the survivors, and, in some cases, the per cent of exautogamous descendants of the treated animals which failed to survive 4 days following isolation. As yet, the last measure has not been investigated very thoroughly to ascertain the nature of the effect which it measures. The survivors among animals exposed to doses around 10^4 r regained the normal rate of cell division within a few days, after which they were sent through autogamy and 25 autogamous animals isolated from each. Almost all these autogamous animals were of reduced vigor by the 4-day total-growth test mentioned in previous quarterly reports. However, only a portion of them failed to survive for the 4 days. The per cent of those which die in the 4-day period presumably reflects some sort of inherited change, possibly in the micronucleus. There is some reason to suspect that the nature of the effect is different from that causing the reduced vigor at lower doses.

Solutions of the desired concentrations of the substances were made up and the animals were added to them by means of a capillary pipette with as little fluid as possible. All solutions except formate were made up as near as possible to using time, and the animals added 5 to 15 minutes before irradiation. Animals were allowed to remain in the formate for a half hour before irradiation. As soon as possible after irradiation, animals were removed from the solutions, washed, and put individually into containers.

TABLE 1

The effect of chemical substances upon the mutagenic action of low doses of X rays as measured by the per cent of reduced vigor after autogamy

COMPOUND	CONCENTRATION	NUMBER OF EXPERIMENTS	NUMBER OF ANIMALS*	AVERAGE PER CENT OF REDUCED VIGOR
Ethyl alcohol	0.7 *M*	5	100	41.6
	none	5	100	61.6
BAL	0.001 *M*	3	100	58.4
	none	3	100	66.3
Sodium hydrosulfite	0.001 *M*	4	80	70.6
	none	4	80	71.1
Sodium formate	0.01 *M*	3	60	55.1
	none	3	60	59.6

*The number of animals isolated immediately after irradiation. From each of these animals 25 autogamous animals were obtained so that the percentages are based upon twenty-five times the number in this column.

Table 1 shows the results for mutational changes after low doses of X rays. Tests of significance were carried out using the arc sine transformation and analysis of variance. Only the results with alcohol show a significant decrease (protective action) of reduced vigor. However, the results with BAL and hydrosulfite were quite variable from one experiment to the next. In each case, there is one experiment which suggests a protective action. It is possible that both substances have a small protective action at the very low concentrations which had to be used and that the amount of protection was subject to uncontrolled variation. This would agree reasonably well with the finding of Hollaender *et al.* that the maximum effect with both substances was produced at considerably higher concentrations than those used here. There has been no evidence of a protective action of formate at the relatively high concentration used. This result would appear significant since the concentration used was quite comparable to those used for bacteria. In summary, there is good evidence for *Paramecium* that ethyl alcohol exerts a protective action against the mutagenic action of X rays. It is possible that BAL and sodium hydrosulfite also afford such protection but this has not been clearly demonstrated. There is considerable reason to believe that formate lacks this protective ability, at least under the conditions of our experiments.

Table 2 summarizes the results with high doses of X rays. Both ethyl alcohol and sodium hydrosulfite appear, rather clearly, to exert a protective action against the immediate lethal effects of X rays. The apparent increase in protection with decrease in concentration of the hydrosulfite may be spurious. Doses used were not the same in the various experiments. Lower concentrations involved experiments in which there was relatively more death in the irradiated controls than in the experiments with 0.001 *M* hydrosulfite. Moreover, experiments with this concentration include the only one in which the combination of hydrosulfite and X rays resulted in more death than with X rays alone.

This latter phenomenon was of regular occurrence for both BAL and formate. In only one case, the experiment at 0.0001 *M* concentration, were there more survivors in the group exposed to BAL and X rays than in that exposed to X rays alone; and, even in this case, the difference was small. It is, of course, impossible to decide from this kind of evidence whether the BAL and formate sensitize to the X rays or whether the X-ray damage makes the cells more susceptible to these compounds. The great toxicity of BAL makes the latter hypothesis attractive for this compound.

TABLE 2

The effect of chemical substances upon the action of high doses of X rays on Paramecium

COMPOUND	CONCENTRATION	IRRADIATION	NO. OF EXPERIMENTS*	NO. OF ANIMALS	PER CENT SURVIVAL FOR 1 DAY	AVERAGE NO. OF DIVISIONS IN FIRST DAY	NO. OF EXPERIMENTS*	PER CENT DEAD IN 4 DAYS**
Ethyl Alcohol	0.7 *M*	yes	4	120	80.8	2.04	2	71.0
	none	yes	4	112	63.4	1.69	2	80.2
	0.7 *M*	no	4	120	100.0	3.93	-	--
BAL	0.001 *M*	yes	2	53	0.0	--	-	--
	none	yes	2	56	39.3	--	-	--
	0.001 *M*	no	2	60	48.0	--	-	--
BAL	0.0005 *M*	yes	2	60	33.3	0.65	1	82.0
	none	yes	2	52	80.8	1.30	1	82.4
	0.0005 *M*	no	2	60	98.3	4.46	-	--
BAL	0.0002 *M*	yes	2	60	40.0	1.21	-	--
	none	yes	2	60	48.3	3.14	-	--
	0.0002 *M*	no	2	60	100.0	4.97	-	--
BAL	0.0001 *M*	yes	1	30	20.0	--	-	--
	none	yes	1	30	0.0	--	-	--
	0.0001 *M*	no	1	30	100.0	--	-	--
Sodium hydrosulfite	0.001 *M*	yes	4	118	43.2	1.66	1	79.6
	none	yes	4	112	37.5	1.29	1	82.4
	0.001 *M*	no	4	120	95.8	4.31	-	--
Sodium hydrosulfite	0.0005 *M*	yes	2	56	62.5	2.92	1	83.4
	none	yes	2	56	39.3	1.32	1	82.4
	0.0005 *M*	no	2	60	100.0	4.97	-	--
Sodium hydrosulfite	0.00025 *M*	yes	1	30	90.0	--	-	--
	none	yes	1	30	0.0	--	-	--
	0.00025 *M*	no	1	30	100.0	--	-	--
Sodium formate	0.01 *M*	yes	2	60	5.0	--	-	--
	none	yes	2	56	35.7	--	-	--
	0.01 *M*	no	2	53	84.9	--	-	--

*The number of experiments was different for the immediate effects and the postautogamous effect, partially because of the death of animals, and partially because not all material was carried through to autogamy.

**The percentages in this column are based upon groups of 500 autogamous animals derived from 20 original treated animals.

The effect upon division rate parallels closely the effect upon death. Survivors from the combination of alcohol or hydrosulfite and X rays have, on the average, higher division rates than the corresponding irradiated controls, while the survivors of the combination of BAL and X rays have a lower rate. No meaningful division rate data are available for the formate group because of the small number of survivors.

Only a few tests have been made for effect of the substances upon the per cent of death in 4 days among the exautogamous descendants of the irradiated animals. No clear effect was revealed although this measure is known to increase as a function of the dose of X rays. However, further tests will be needed before this negative conclusion can be taken too seriously.

In summary, the protective action of ethyl alcohol against the mutagenic action of relatively low doses of X rays and the protective action of both alcohol and sodium hydrosulfite against the immediate lethal and division-retarding action of high doses of X rays appears to be established. The combination of BAL and X rays appears to have a greater immediate lethal and division-retarding action than either alone, possibly because the X rays increase the sensitivity to BAL, which is a toxic agent. An effect of BAL and of hydrosulfite upon the mutagenic action of X rays is possible but has not been established. Work with formate has not shown a protective effect upon the mutagenic action of X rays even though the formate was added in quite high concentrations. Data suggest the possibility that these different agents might protect more effectively against certain effects of X rays than others.

Yeast genetics. (Pomper, K. Daniels) An investigation of the genetic status of ultraviolet-induced "reversions" has been started. A haploid clone which required adenine and uracil was irradiated, and plated on agars lacking (respectively) each factor. Due to the radiation, an increased number of colonies appears on such minimal agar plates, as compared to the spontaneously appearing independent cultures (see ORNL-807 for some representative curves). The identification of the genetic status of such revertant colonies has been carried out as follows:

> Ten colonies (varying in size) were isolated from each type of minimal agar, and were crossed to a culture of opposite mating type that was independent for both adenine and uracil. Prototrophic diploid recovery was possible by use of methionine and tryptophan deficiencies (one in each stock). The diploids were then induced to sporulate and dissections carried out.

The study is not yet complete, since it is a time-consuming undertaking and germination of ascospores has not been very good. We have data on four adenine-independent isolates, which indicate that they are all true back mutations at the same locus, since nothing but adenine-independent ascospores were obtained. Our data on three uracil-independent isolates indicate that two are back mutations, while the third is either a case of a suppressor mutation or a mutation at the same locus which has not returned to wild-type efficiency. Preliminary information on two more revertants suggests that they are also back mutants, but more data are needed.

The *p*H-sensitive complex mutant that has been reported in previous reports continues under study. We have found that tyrosine may replace the PAB requirement of this mutant, and that phenylalanine is inhibitory. Phenylalanine has no effect on the mutant grown on PAB. The tyrosine replacement effect has been found with two commercial samples of tyrosine, and with tyrosine prepared from tyrosine ethyl ester (through the courtesy of Dr. D. G. Doherty). An apparent stimulation has been noted with lysine with one of the commercial samples, but the evidence suggests that this is an artifact perhaps brought about by an inhibitory impurity in the tyrosine preparation which is antagonized by lysine.

Studies on a multiple unit nephelometer. (Doermann, with the cooperation of J. R. Davidson, ORNL Instrument Department) The nephelometer designed by Underwood (see ORNL-807:36) was connected to a Brown recorder and its characteristics studied. As light scatter specimens, chemoplastic standards of the sort described by Underwood were used. Under these conditions two difficulties with the instrument became apparent. First, random fluctuations amounting to approximately 10 per cent of the scale in the readings were observed, particularly in the high sensitivity range. Such fluctuations become magnified by factors of 5 to 10 under conditions where background light scatter cannot be eliminated, but must be bucked out by an opposing signal. This difficulty is not, as suspected earlier, due to electronic disturbances in the photomultiplier tubes, but to fluctuations in the incident light intensity of the lamp, operated on alternating current regulated by a Sola constant voltage transformer. By operating both the light and the bucking current from storage batteries, this difficulty was completely eliminated, the fluctuations being reduced by at least fiftyfold. The second difficulty, which has not yet been resolved, concerns itself with the fact that the signal obtained from a steady

amount of scattered light drops continuously over a period of at least 24 hours. It is suspected that the source of the difficulty lies in fatigue of the photocathode and dynode surfaces of the photomultiplier tube which results in lowered sensitivity. Experiments are being continued in an effort to trace down and eliminate the difficulty.

X-ray sensitivity of bacteriophage T2H in the presence of various selected chemical compounds. (Doermann, Hill) Using bacteria as a test organism, the Radiation Protection Group of the Biology Division has screened numerous chemical compounds and discovered among them certain groups which exhibit the ability to decrease X-ray sensitivity of *Escherichia coli*, B/r. In the hope that bacteriophage might be helpful in elucidating the mechanisms by which these substances exert their protective effect, we have begun some experiments testing their ability to protect bacterial viruses. Watson (Dissertation, Indiana University, 1950) has previously shown that X rays inactivated phage by several mechanisms. We have started by testing the ability of the protective compounds to protect phage from "direct inactivation." With bacteriophage, one can eliminate the indirect effects from experimental results, and thus study exclusively the direct effects by suspending the virus in nutrient broth media. Therefore, our standard control experiments consist of measuring survival of phage T2H after different X-ray exposures in nutrient broth (0.8 per cent Difco N. B. and 0.5 per cent sodium chloride). The substances in question were dissolved in nutrient broth with careful control of *p*H, and these solutions added to broth suspensions of bacteriophage for the experimental tests. Survival of the virus in the various solutions was compared to survival in broth. Results of our preliminary experiments are shown in Table 3, where the protective effectiveness of a compound is given as the dose-reduction factor (see Kelner, J. of Bact. 58:511-522, 1949). Dose-reduction factors differing from unity by 0.2 or more are considered significant. Significance of smaller differences depends on the number of points on which comparisons are based. It is seen that sodium formate and sodium hydrosulfite, which are clearly protective to bacteria, do not protect phage against direct inactivation. However, ethanol, glycerol, BAL, and cysteine all give significant protection. No discussion of the mechanism is warranted before more experiments are done, and particularly before the protective effect of these compounds against the indirect effects is studied.

TABLE 3

The ability of selected chemical compounds to protect bacteriophage T2H from direct inactivation by X rays

EXPERIMENT	SUBSTANCE	CONCENTRATION*	DOSE REDUCTION	NO. OF POINTS ON CURVES**
5-2	Nutrient broth	0.8% (standard control)	1.00 (by definition)	-
5-2	Nutrient broth	3.2%	1.10	5
5-2	Nutrient broth	0.1%	0.90	5
4-24	Sodium formate	0.01 *M*	1.00	4
4-26	Sodium formate	0.1 *M*	1.12	3
4-24	BAL	0.04 *M*	1.59	4
4-26	Sodium hydrosulfite	0.04 *M*	0.95	3
4-26	Glycerol	1 *M*	1.32	3
4-26	Ethanol	1 *M*	1.26	3
5-7	Ethanol	1 *M*	1.23	6
4-24	Ethanol	3 *M*	1.45	4
4-26	Ethanol	3 *M*	1.50	3
5-7	Ethanol	3 *M*	1.54	6
5-3	L-Cysteine	0.01 *M*	1.41	6
4-26	L-Cysteine	0.04 *M*	2.22	3
5-3	L-Cysteine	0.04 *M*	1.67	6
5-8	L-Cysteine	0.04 *M*	1.60	7

*All concentrations used were tested and found to be nonlethal to T2H. These concentrations are protective to bacteria (information from Radiation Protection Group).

**Each point consists of a survival determination from a single aliquot, irradiated with a given dose. The survival determination consists of one to three separate assays of the aliquot. The number of points given was determined for both control curves and for the experimental curves. The point of origin is included as one point.

EFFECTS OF RADIATION ON RATE OF MITOSIS

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Chromosome breakage. (Gaulden) Experiments have been completed to determine the frequency of X-ray-induced chromosome breaks at various times after irradiation with a view toward learning the stage or stages of mitosis at which chromosomes are most susceptible to fragmentation. Grasshopper embryos were treated with 64 r of X rays (32 r/minute) and then made into culture preparations immediately and placed at 38° C. They were fixed and stained with acetocarmine at 22, 44, 154, 198, 270, and 374 minutes after irradiation. Only those cells in late anaphase were studied for fragments, because at this stage acentric fragments can be clearly distinguished—they do not go to the poles of the spindle. A dose of 64 r of X rays produces only simple or one-hit breaks, therefore the fragments can be recorded as single fragments or as double fragments (two fragments obviously produced by the same ionization).

The data are summarized in Table 4. They were analyzed statistically by Moshman of the ORNL Mathematics Panel, the statistic used being the χ-square test of homogeneity of k samples (Kendall, Advanced Theory of Statistics, Vol. II, page 119). The frequency of fragments at 22 and 44 minutes after irradiation is quite low. These intervals fall in the period prior to complete mitotic inhibition and cells which reach anaphase during this time were probably irradiated in late or very late prophase. On the other hand, the incidence of fragments at 374 minutes after radiation is significantly higher than at all other times. There are no statistically significant differences in the number of single fragments manifest at 198, 270, and 374 minutes after treatment, but the frequency of double fragments at 374 minutes is quite different from the frequency at 198 and 270 minutes. The ratio of single to double fragments at 198 minutes after treatment is significantly greater than at other intervals. Before these data can be interpreted, a determination must be made of the stage of mitosis at which cells are irradiated which reach anaphase at these intervals after irradiation. This study is now in progress.

Cytological effects of methylene blue. (Gaulden, Nix) A study, begun last quarter, to determine the cytological effects of substances affecting the oxidation-reduction potential of the cell has been extended to include methylene

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TABLE 4

Fragmentation of chromosomes at various times after irradiation with 64 r of X rays

TIME AFTER IRRADIATION (minutes)	NO. OF EMBRYOS	TOTAL CELLS	TOTAL CHROMOSOMES	CELLS WITH FRAGMENTS						
				NO. OF CELLS	TOTAL CHROMOSOMES	SINGLE BREAKS	DOUBLE BREAKS	RATIO S/D	TOTAL BREAKS	PER CENT BREAKS
22	14	52	1208	1	24	0	1	-	1	0.08
44	18	72	1703	3	72	3	0	-	3	0.1
154	2*	6	139	2	46	1	1	1	2	1.4
198	11	64	1483	19	449	26	4	6.5	30	2.0
270	18	103	2402	44	1027	41	19	2.2	60	2.4
374	14	131	3042	70	1645	64	50	1.1	114	3.7

*Thirty-one embryos were fixed but only two had cells in late anaphase.

blue. Grasshopper embryos were made into culture preparations containing the desired concentration of the dye. The results can be classified as follows:

5-0.1 × 10^{-3} *M*. All activities of the cells are irreversibly stopped. The cells are presumably dead.

0.01-0.005 × 10^{-3} *M*. Progress of all stages of mitosis is slowed. Spindle structure is sometimes altered. Chromatin is rendered refractive. Complete separation of chromatids at anaphase is inhibited.

0.001 × 10^{-3} *M*. Mitotic rate is only slightly retarded. Cell structures show no abnormalities.

EFFECTS OF RADIATION ON MICROORGANISMS

A. Hollaender (Leader)

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E. H. Anderson	A. W. Burke**
Daniel Billen	Ruth Whittle
W. T. Burnett, Jr.*	R. B. Grayson

Mechanism of protective action of some chemical substances. (Hollaender, Stapleton, Billen, Grayson) The protective action of a wide variety of chemical substances in *Escherichia coli* B/r has been reported (ORNL-969). Some of these compounds, chiefly those falling in the general category of "metabolic compounds," have been further investigated from the point of view of elucidating the mechanism by which they protect bacterial cells.

In previously reported experiments, several amino acids were tested for protective ability at low temperature (ice-bath temperature), results indicating that only cysteine afforded significant protection under those conditions. Recently we were able to show that the carboxylic acids, formate, succinate, fumarate, malate, lactate, and pyruvate, afford excellent protection if washed-cell suspensions containing these compounds are incubated for a short time before irradiation at room temperature or at 37° C. Moreover, low concentrations (10^{-3} *M*) of carboxylic acids are required to give maximum protection under these conditions. The amino acids, glycine, α-alanine, serine, threonine, and glutamic acid, have been investigated for protective capacity with incubation, and have been found to give equally as good protection as the carboxylic acids used.

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**Member of Radiation Protection Group.

It is of interest that parallel studies on respiration with some of the compounds used indicate that protective capacity is dependent to some extent on the rate at which these compounds are oxidized by the bacterial cells. Two typical examples of this can be shown at present. Comparing respiration and protective capacity of β- and α-alanine, it was found that β-alanine, which was oxidized at an insignificant rate as compared with α-alanine, afforded little or no protection, while α-alanine gave excellent protection if cells were incubated with it prior to irradiation. Similar but not so striking results were obtained by comparing propionic and pyruvic acids. Propionic acid being respired slowly, gives little protection, while pyruvate being rapidly oxidized, gives good protection. Our bacterial cells show no significant respiration with cysteine or BAL as substrates, nevertheless these sulfhydryl compounds afford maximum protection to bacteria, interestingly enough, at low or high temperatures (2° C or 37° C).

These preliminary findings suggest that incubation of washed cells in the presence of an oxidizable substrate may produce protection in part, by as simple mechanism as depletion of oxygen from the bacterial cell, thereby preventing the formation of some of the decomposition products of water at critical locations within the cell. However, cysteine and BAL protection must be through a different mechanism, perhaps involving their sulfhydryl groups.

Further confirmation of the proposed mechanism has recently been obtained by use of a respiratory inhibitor, sodium cyanide, which should prevent cellular oxygen uptake through the iron-containing enzyme carrier systems. The technique consisted essentially of incubating washed-cell suspensions in the following ways for 30 minutes at 37° C, and then irradiating with X rays at several doses:

1. Incubated with buffer
2. Incubated with buffer + 0.002 *M* cyanide
3. Incubated with compound in buffer
4. Incubated with compound in buffer + 0.002 *M* cyanide

The results of four compounds tested are given in Table 5.

These results appear to bear out the hypothesis presented earlier that incubation of cells in the presence of substrate depletes partially the oxygen from the cell, and also that the sulfhydryl compounds probably exert their protection through a different mechanism.

Other chemical substances which have been shown to protect will be investigated from this point of view, especially the glycols and alcohols.

TABLE 5

SUBSTRATE CONC. = 10^{-2} *M*	Dcpd/D BUFFER	INHIBITION OF O_2 UPTAKE BY ADDED CN (per cent)	Dcpd/D BUFFER WITH CYANIDE
Formate	2.5	~90	1.0
Serine	2.5	~50	1.2
Cysteine	2.5	-	2.0
BAL	3.5	-	3.5

Dcpd/D buffer = ratio of mean lethal dose (MLD) in presence and absence of compound during incubation.

Comparative sensitivity of various strains of E. coli. To investigate the generality of the oxygen-effect, that is the relative sensitivity of bacteria to X rays in suspensions of high and low oxygen concentrations, ten available strains of *E. coli* have been studied. A somewhat surprising variation in sensitivity between strains was found. Although half of the strains tested show a similar sensitivity, some extreme differences were found. Table 6 shows the MLD's for the various strains tested in suspensions of high and low oxygen tension.

TABLE 6

STRAIN	OXYGEN MLD IN (r)	NITROGEN MLD (r)	RATIO MLD's N_2/O_2
B	3.5×10^3	1×10^4	2.8
Tennessee, B/r, Gratia, 86-G	$6.0 \rightarrow 6.5 \times 10^3$	$1.8 \rightarrow 2.0 \times 10^4$	3.0
H-52, Texas, 10-B-3, β-alanineless	$8.0 \rightarrow 9.0 \times 10^3$	$2.4 \rightarrow 2.6 \times 10^4$	3.0
Crook	1.75×10^4	5.0×10^4	2.9

A difference of fivefold in relative X-ray sensitivity was found for these strains, however, the ratio of sensitivities of suspensions of high and low oxygen concentrations is the same, about three times the dose required in nitrogen-saturated suspensions as in oxygen-saturated suspensions. The innate variation in X-ray sensitivity between strains is unexplainable at present, but should make interesting investigation from the point of view of metabolism.

Postirradiation effects on respiration during growth. (Billen) A study of the respiration of resting *E. coli* B/r cells (185×10^7/ml), grown under aeration, that have received an X-ray dose sufficient to cause killing greater than 99.9 per cent (60,000 r) reveals an initial respiration on glucose equal to that of nonirradiated cells for the first 30 minutes, followed by a more rapid decrease in rate of oxygen uptake by the irradiated cells. Information concerned with whether the drop in respiration of the exposed cells is due to the dying off of the individual cells or the dying is due to a drop in respiratory ability of the cells has remained inconclusive.

Irradiated and nonirradiated cells were grown in a peptone-glucose broth in colorimeter tubes and the growth recorded by observing increase in turbidity readings on a photoelectric colorimeter (3.5 ml of medium + 0.5 ml of cell suspension). It was found that the irradiated cells were capable of growth equal to that of nonirradiated cells for a short period of time (30 minutes). This was followed by a decreasing rate of growth which ends 60 minutes after inoculation (Fig. 1). Respiration studies made simultaneously with the growth studies under exactly the same conditions, with the exception that Warburg vessels were used, showed that although there was an increase in growth of irradiated cells there was no related increase in respiration as found in the control cells (Fig. 1A). It may be noted from the figure that, although there was a 60 per cent increase in turbidity in the culture of irradiated cells during the interval between 30 - 60 minutes incubation, there was no increase in oxygen utilization, whereas the control cells showed a 115 per cent increase in turbidity during the same time interval coincidental with a 65 per cent increase in oxygen uptake. Since less than 0.01 per cent of the irradiated cells produce observable colonies on plating, the growth must be due initially to all the cells actively partaking in cell synthesis (using an inoculum containing viable cells, equal in number to those which show up as survivors, did not give any turbidity increase in 90 minutes). This is followed by a rapid decrease in rate of turbidity increase until it finally ceases, in contrast to the nonirradiated cells.

RELATIONSHIP BETWEEN RESPIRATION AND GROWTH IN E. COLI B/r EXPOSED TO X RAYS

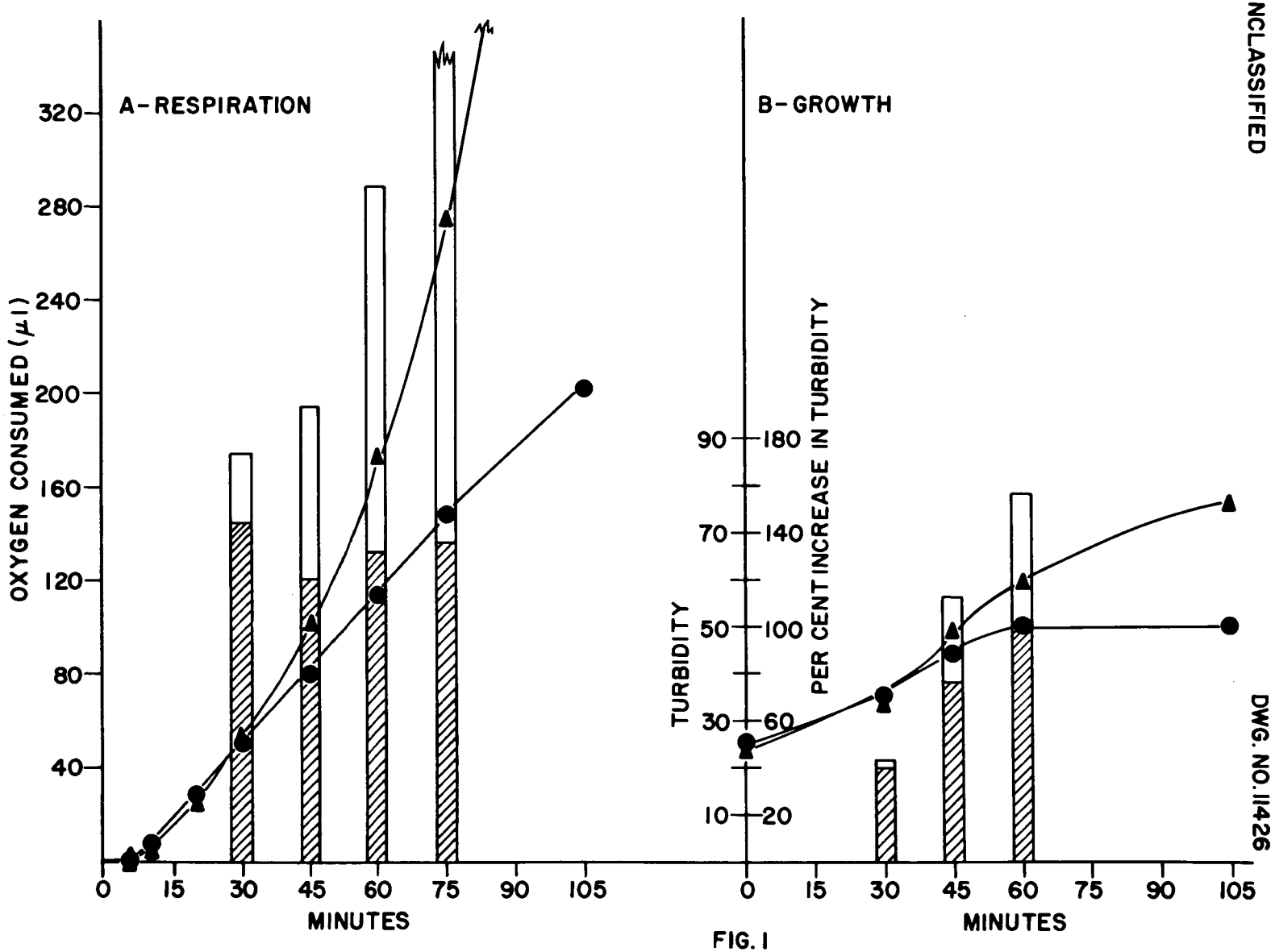

FIG. 1

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As there is no increase in the respiration rate of these exposed cells, it must follow that on a cellular basis (no increase in cell size was noted at 75 minutes) there is a decrease in a respiratory enzyme system or systems so that, although the cells increase in number, the over-all respiration does not. This suggests the possibility that either a part or all of the respiratory system is incapable of being replaced, upon deterioration, by new synthesis in the exposed cells so that, following several divisions, the respiratory rate per cell is insufficient to support active cell synthesis.

Relationship between viability and respiration in nonproliferating cells following X-ray exposure. During an investigation concerned with the fall in respiration of irradiated resting cells incubated at 37° C over that of nonirradiated cells, a study was made of the viable cells, based on colony counts, during the period of experimentation.

It was found that there was a rapid decrease in viable cells in the irradiated suspension (60,000 r) in comparison to the decrease in the nonirradiated cells (Table 7). Moreover, the drop in the irradiated cells was accelerated when the cells were allowed to respire on a glucose substrate. This increased rate of death, of the actively metabolizing cells, was most pronounced during the early period of respiration. It may be observed from the table that at the end of 3 hours of respiration a drop of 97 per cent from the initial viable count at the time of glucose addition had occurred. The drop in nonirradiated metabolizing cells was only 14 per cent during the same period and that of the nonmetabolizing exposed cells 82.

The drop in respiration exhibited by irradiated cells cannot be accounted for by the decrease in viable cells, since oxygen uptake by a cell concentration less than 10^7 cells/ml would not be detected under the conditions used here. The possibility exists that glucose itself may be responsible for the increased death of the exposed cells. This will be checked in future studies by using other substrates for respiration studies.

Modification of the X-ray sensitivity of bacteria by chemical agents. (Burnett, Morse, Burke) At the 0.02 *M* concentration level, cysteine, BAL, and sodium hydrosulfite increase the survival of *Escherichia coli* B/r exposed to 60,000 r of X rays (250 kvp, 30 ma, 3 mm Al added filtration) by a factor of 50 or more (Burnett, Stapleton, and Hollaender, Fed. Proc. 10: 22, 1951) The modification of the X-ray sensitivity of *E. coli* B/r by a variety of other chemical agents is shown in Table 8. Under the conditions studied, most of

TABLE 7

Postirradiation decrease in viable cells of E. coli B/r

TIME* (minutes)	METABOLIZING CELLS				NONMETABOLIZING CELLS			
	IRRADIATED		NONIRRADIATED		IRRADIATED		NONIRRADIATED	
	VIABLE CELLS ($\times 10^3$/ml)	PER CENT DECREASE**	VIABLE CELLS ($\times 10^7$/ml)	PER CENT DECREASE	VIABLE CELLS ($\times 10^3$/ml)	PER CENT DECREASE	VIABLE CELLS ($\times 10^7$/ml)	PER CENT DECREASE
0	510	--	109	--	545	--	135	--
30	185	64	--	--	421	22	--	--
60	94	82	--	--	368	33	--	--
120	41	92	--	--	190	65	--	--
180	16	97	92	14	100	82	92	32

*Addition of glucose from side arm taken as zero time.

**Per cent based on zero time or time of addition of glucose. This was 50 minutes after cells were removed from X-ray machine.

TABLE 8

Modification of X-ray sensitivity of E. coli B/r by various chemical agents at 2° C

COMPOUND	CONCENTRATION MOLAR	RELATIVE SURVIVAL * AFTER 60,000 r OF X RADIATION
Tetrathiodiacetic acid	0.02	0.06
2(2-Mercaptoethoxy) ethanol	0.02	5
Sodium formaldehyde sulfoxylate	0.02	1.2
Mercaptopyruvic acid, ammonium salt	0.02 0.005	53 9
Uracil	0.02 0.005	5 3
Diethylmercaptal of glucuronic acid, sodium salt	0.02 0.005	3 3
Glucuronic lactone	0.02 0.005	1.6 1.4
Pyruvic acid	0.02 0.004	3 2
Mercaptopyruvic acid	0.02 0.004	4 3
Sodium hypophosphite	0.04 0.02 0.01 0.004 0.0004	8 1.4 0.7 0.5 0.8
Thiourea	0.04 0.02 0.01 0.004 0.0004	12 4 2 1.6 1.6
Sodium dihydrogen versenate	0.02 0.005	1.2 1.2
N-(2-Nitroisobutyl)L-cysteine	0.02 0.005	0.8 1.2
Acrylonitrile	0.02 0.005	0.6 1.2
Acetonitrile	0.02 0.005	1.6 3.7
Methyl formate	0.02 0.005	4 5
Cyclohexanol	0.02 1%	1.7 2
Propylene oxide	0.02 1%	0.9 0.9
Resorcinol	0.02 0.005	1 1.7
Methylal	0.02 0.005	0.5 1
Hydroquinone	0.02 0.005	Toxic Toxic
Formaldehyde	0.02 0.005	Toxic Toxic

$$* \frac{\text{Number of survivors in suspension containing chemical agent}}{\text{Number of survivors in control suspension}} = \text{relative survival.}$$

these compounds were not very effective in increasing the survival. The experimental procedures have been described in previous reports.

The effect of the low molecular weight alcohols on the survival of X-irradiated *E. coli* B/r suspensions is shown in Table 9. High alcohol concentrations were not toxic to the cells at 2° C. There are no marked differences in the protective abilities of methanol, ethanol, propanol, and butanols. The effect of incubation with ethanol on the survival of X-irradiated cells is discussed elsewhere.

Factors to be considered in examining the protective action of the chemical agents listed in previous reports and in Tables 8 and 9 are:

(1) The ability of the compound to combine with dissolved oxygen in the suspension

(2) The reducing capacity

(3) The functional groups, e.g., hydroxyl, cyano, sulfhydryl, etc.

(4) The penetration into the cells.

Since we now have available a variety of chemical agents which will increase the survival of *E. coli* B/r, experiments are being designed to elucidate their mode of action. One question which should be answered is whether the protective agents produce changes in the cells or simply interfere with the production of radicals in the medium. For example, it is believed that the benefits attained by the presence of sodium hydrosulfite may simply be due to the removal of oxygen. Data supporting this view have been obtained by resuspending hydrosulfite-exposed cells in phosphate buffer and then exposing to X radiation. Such irradiated resuspensions exhibit the same survival as suspensions not exposed to hydrosulfite.

An examination of the protective action of various combinations of chemical agents is being continued. The results with BAL, sodium hydrosulfite, and ethanol at a constant dose (60,000 r) are summarized in Fig. 2. The numbers at the apices of the triangle represent the per cent survival of *E. coli* B/r in the presence of the indicated compound. The numbers in the middle of each leg represent the per cent survival using the compounds indicated at both ends of the leg. The center number represents the survival, using all the compounds, the benefits in this case, however, being no greater than with any two of the chemical agents.

TABLE 9

Modification of X-ray sensitivity of E. coli B/r by alcohols at 2° C

COMPOUND	CONCENTRATION PER CENT (V/V)	CONCENTRATION MOLAR	RELATIVE SURVIVAL* AFTER 60,000 r OF X RADIATION
Methanol	0	0	1
	0.05	0.013	0.77
	0.1	0.026	1.2
	0.5	0.13	1.8
	1.0	0.25	8.6
	5.0	1.25	56
	10.0	2.5	202
Ethanol	0	0	1
	0.05	0.009	2.7
	0.5	0.09	2.1
	1.0	0.18	6.4
	5.0	0.88	45
	10.0	1.76	94
	20.0	3.52	170
iso-Propanol	0	0	1
	0.1	0.01	2.2
	1.0	0.13	4.2
	5.0	0.66	47
	10.0	1.31	133
n-Butanol	0	0	1
	0.05	0.005	2.5
	0.5	0.05	4.9
sec-Butanol	0	0	1
	0.05	0.005	1.3
	0.5	0.05	5.5
iso-Butanol	0	0	1
	0.05	0.005	1.3
	0.5	0.05	4.5
tert-Butanol	0	0	1
	0.05	0.005	1.6
	0.5	0.05	5.3

* $\frac{\text{Number of survivors in suspension containing chemical agent}}{\text{Number of survivors in control suspension}}$ = relative survival.

Figure 2

The concentrations used are as follows: ethanol-20%, sodium hydrosulfite 0.04 *M*, and BAL, 0.04 *M*.

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PER CENT SURVIVAL–60 KILOROENTGENS

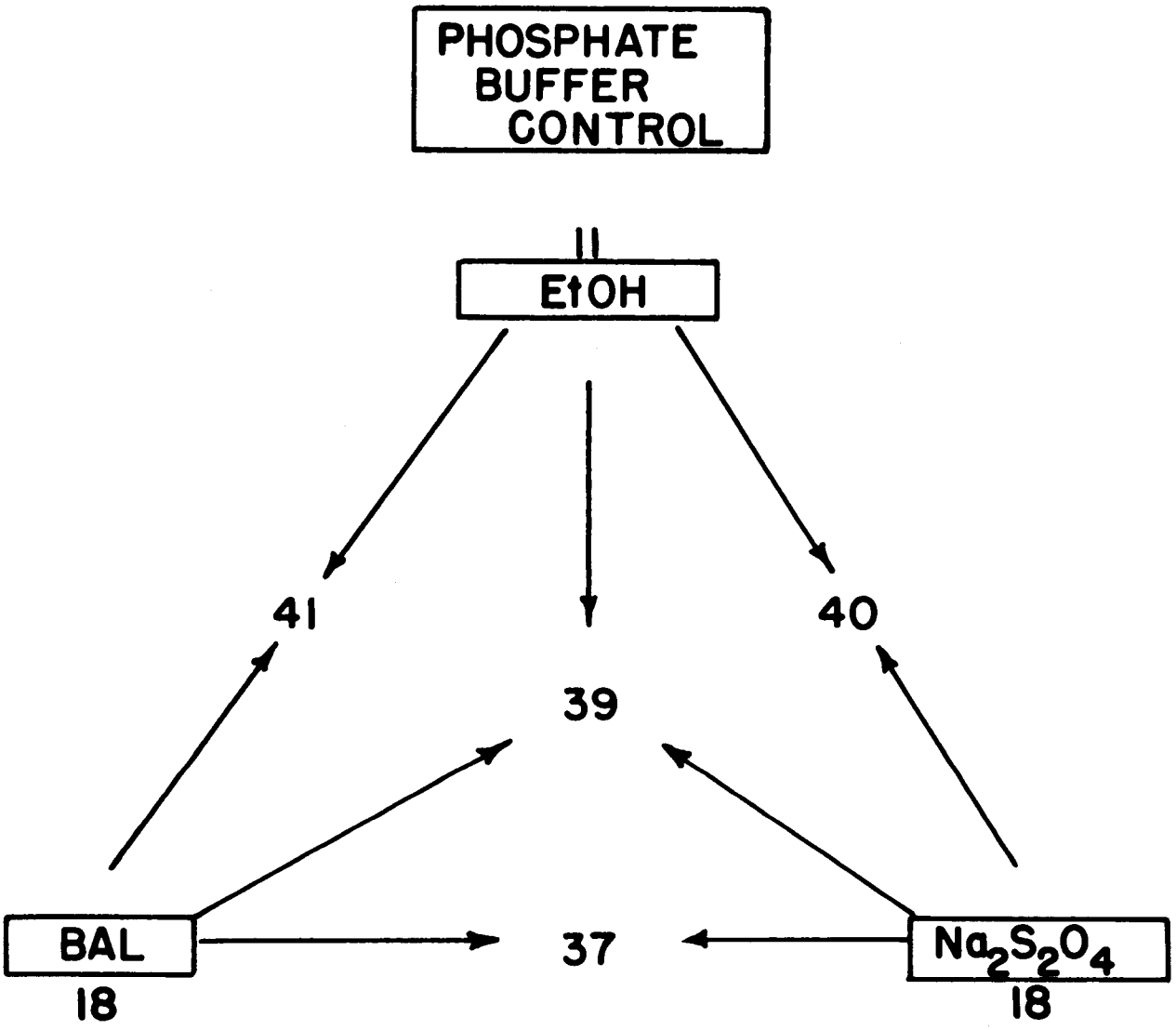

FIG. 2

Several compounds, including ethanol, sodium hydrosulfite, triethylene glycol, and mercaptosuccinic acid have been turned over to the Argonne National Laboratory (A. M. Brues and H. M. Patt) for further testing, using mice. The preliminary results of this cooperative project, stated elsewhere, are encouraging. The results of a small-scale screening project in this laboratory indicate that mecholyl bromide at therapeutic levels will increase the survival of X-irradiated mice. This, however, is to be confirmed by more extensive testing.

Effect of secondary factors modifying the X-ray induction of bacterial mutations. (Anderson, Whittle) The study of secondary factors modifying the X-ray induction of mutations in bacteria, using biochemical mutants of *Escherichia coli,* has been continued. Preliminary investigations with BAL, sodium dithionite, and sodium formate, which have been shown by Hollaender, Stapleton and Burnett to protect the B/r strain of coli against the lethal action of X rays, have been carried out to determine the ability of these compounds to modify the mutagenic action of X rays on two radiation-resistant biochemical mutants of strain B coli. These two mutants have been used previously in extensive studies on the effect of oxygen on the X-ray induction of bacterial mutations (ORNL-807). Although the sensitivity of both mutants to the lethal effects of X rays was similarly decreased by a low oxygen tension during irradiation, one of them, a streptomycin-dependent mutant, was found to have its induced mutation rate essentially unchanged by the presence or absence of oxygen during irradiation. The second, a purineless mutant, exhibited the same degree of decrease in induced mutation rates as for lethal effects when the suspension was irradiated under a low oxygen tension. These results are interpreted as indicating that, although the lethal effect of X rays is the result of the same process in the two strains of coli studied, the genetic effect examined for each strain is the result of different reactions.

In studies on the ability of the three protective compounds to modify the mutagenic effect of X rays on the streptomycin-dependent and purineless mutants, concentrations of the compounds were used at their plateau levels for protection against the lethal effects of X rays on cells exposed in air. Preliminary results indicate that the induced mutation rates of each mutant are reduced by each compound to a degree closely approximating that obtained by exposure under reduced oxygen tension.

Seventeen additional biochemical mutants of another strain of *E. coli* have been screened for their sensitivity to the lethal effect and response to the mutagenic action of X rays under high oxygen tension. More extended studies on one of them, an isoleucine + valine-dependent mutant, indicate that, although both the lethal and mutagenic efficiencies of X rays are increased for this strain by a high oxygen tension during irradiation, the dose-reduction ratio of oxygen for the mutagenic effect is about two-thirds that for the lethal effect. This is a further indication that oxygen may show a spectrum of magnitude in increasing the efficiency of X rays in producing different genetic effects in *E. coli*.

PATHOLOGY AND PHYSIOLOGY

Jacob Furth (Leader)
M. C. Woods
J. B. Kahn
R. H. Storey
Mary H. Ross
J. J. Lane
R. R. Bigelow*
K. W. Christenberry**
W. D. Gude
Peggy Ledford
Germaine Click
Mary M. Knoohuizen

Chronic changes by slow neutrons and equivalent doses of X rays. (Ophthalmology, Christenberry; assistance, Lane, Gude, Ledford) The experimental setup has been outlined in earlier reports. Leukemia now appears in large numbers also in the control animals. The type of leukemia and the incidence rates are now being studied to determine if irradiation has merely hastened the induction of leukemia or whether it causes the appearance of a type of leukemia absent in the controls.

The possibility that a stress factor (adrenal-pituitary mechanism) plays a role in modifying the leukemia induction rate is being tested experimentally in specially designed experiments.

The examination of mice for cataract incidence and time of induction have been continued by Christenberry with the slit-lamp microscope. All recent examinations have been made by him as unknowns. Therefore, the summary of his recent examinations, shown in Table 10, leaves no doubt that the

TABLE 10

Eyes examined as "unknowns"

INTENSITY OF CATARACT	14-15 MONTHS*						16-19 MONTHS	
	O	N 5**	X 5***	N 10	N 20	X 20	O	N 80 & X 80
			(32 r)			(128 r)		
O	10	1	3	-	-	-	1	-
±	3	9	1	2	-	-	3	-
+	7	8	7	2	1	2	1	-
++	-	4	-	6	6	3	-	-
+++	-	-	-	-	-	-	-	4
Total No.	20	21	11	10	7	5	5	4

*After exposure
**5 minutes exposure in the thermal column
***5 minutes exposure to 250 kv X rays at a rate of 6.4 r/min.

* Consultant, Surgery.
** Consultant, Ophthalmology.

threshold single dose of X rays producing opacities of the lens within 14 to 15 months is within the neighborhood of 32 r and that the equivalent doses of slow neutrons (equivalent on the basis of LD-50) is definitely below that dose. In evaluating these data the report of Sheppard and associates has to be considered, indicating that the neutron flux in the biological tunnel of the Oak Ridge reactor is heavily contaminated by γ rays, accounting for about one-half the biological effects in this flux.

Shifts in fluid and cell compartments after massive irradiation. (Surgery, Bigelow; chemistry, Woods, Storey; assistance, Knoohuizen, Ross) The essential results of the experiments are being described in a preliminary report which will appear in the Proceedings of the Society for Experimental Biology and Medicine.

Earlier studies of the rate of disappearance of various substances from the blood of rabbits and mice led to the assumption that capillary permeability is greatly increased in these species by exposure to large (LD-50) doses of X rays. Tagged plasma, homologous and heterologous erythrocytes, and other tagged large-molecular substances when injected into the blood disappeared from the blood stream of X-rayed rabbits and mice faster (2 to 14 days after irradiation) than from that of normal control animals. In order to bring direct evidence for heightened capillary permeability and to ascertain its magnitude, lymph fistulas were prepared in dogs and rats and the cellular and chemical composition of the lymph was studied. In addition, the rate of appearance in the lymph of intravenously injected tagged homologous plasma was assayed in both X-rayed and normal animals.

Lymph fistulas were prepared in dogs by a technique similar to that described by Brown and Hardenbergh. Polyethylene and polyvinyl tubing was inserted into the thoracic duct at the left supraclavicular region and another into a large tributary of the external jugular vein. The connecting loop was exteriorized. In rats the procedure was that used by Bollman and associates. The dogs used were mongrels of both sexes. The rats were Wistar rats weighing approximately 240 to 370 g. By disconnecting the loops all injections and samplings could be done without further surgical intervention. Intravenous injections were made through the venous cannula. Aureomycin was given routinely on 3 consecutive days after operation. In the final phase of the work the cannulas were flushed with heparin but, since this is inadequate to prevent clotting in normal dogs and exaggerates the bleeding tendency in irradiated dogs, it was abandoned.

In normal dogs the lymph-venous anastomosis remained serviceable for about 2 to 3 days only, when clotting caused closure of the fistulas. In X-rayed animals the fistulas remained patent for as long as 16 days after operation. Closure of the lymph cannula was the usual cause of the cessation of flow in the anastomosis. The venous fistulas remained open or could be readily reopened. The maintenance of flow through the fistulous anastomosis in X-rayed dogs was due in part to the clotting disturbance caused by irradiation and in part to the increased lymph flow in X-rayed animals.

In normal dogs the rate of flow through the fistula was less than 1 cc per minute, while in several X-rayed dogs it exceeded 1 cc and in one dog reached 5 cc per minute. That increased flow, itself, is evidence of heightened permeability was at first not appreciated by us and, therefore, exact flow rate determinations were not made except in the clearance studies.

The most striking finding was the presence of blood in the lymph of X-rayed rats and dogs. As an illustration the data on Dog Scot are presented in Fig. 3. In the lymph of normal rats the number of erythrocytes is a small fraction of that of lymphocytes. In X-rayed rats the lymph was grossly bloody, and this was noted before insertion of the cannula. In the normal dogs studied, the number of erythrocytes in the lymph was also much below that of lymphocytes. The slightly excessive number of erythrocytes, occasionally caused by operative trauma, dropped within a day. In the lymph of six irradiated dogs studied 3 to 17 days after exposure the erythrocyte count was ten- to a hundredfold that of the lymphocyte count. At 2 days after irradiation there was little evidence of passage of erythrocytes into the lymph. Permeability as evidenced by the very high erythrocyte counts seemed to increase with time, reaching a maximum at about 7 to 14 days.

There is evidence that X rays within the dose tolerated by a mammal do not injure erythrocytes. Yet, the anemia after massive irradiation is in excess of that caused by death of aging cells in absence of erythrogenesis. The findings here reported indicate widespread extravasation with flooding of the lymph compartment by erythrocytes. The well-known finding of erythrophagocytosis and later massive hemosiderosis in lymph sinuses indicates that many of the extravasated erythrocytes are destroyed. Postmortem examinations confirmed these long-known characteristic radiation changes. Every dog autopsied exhibited a hemorrhagic tendency. Red-colored lymph nodes were a common finding; the thoracic duct of several dogs dissected was red like a vein as were numerous lymphatics leading to lymph nodes.

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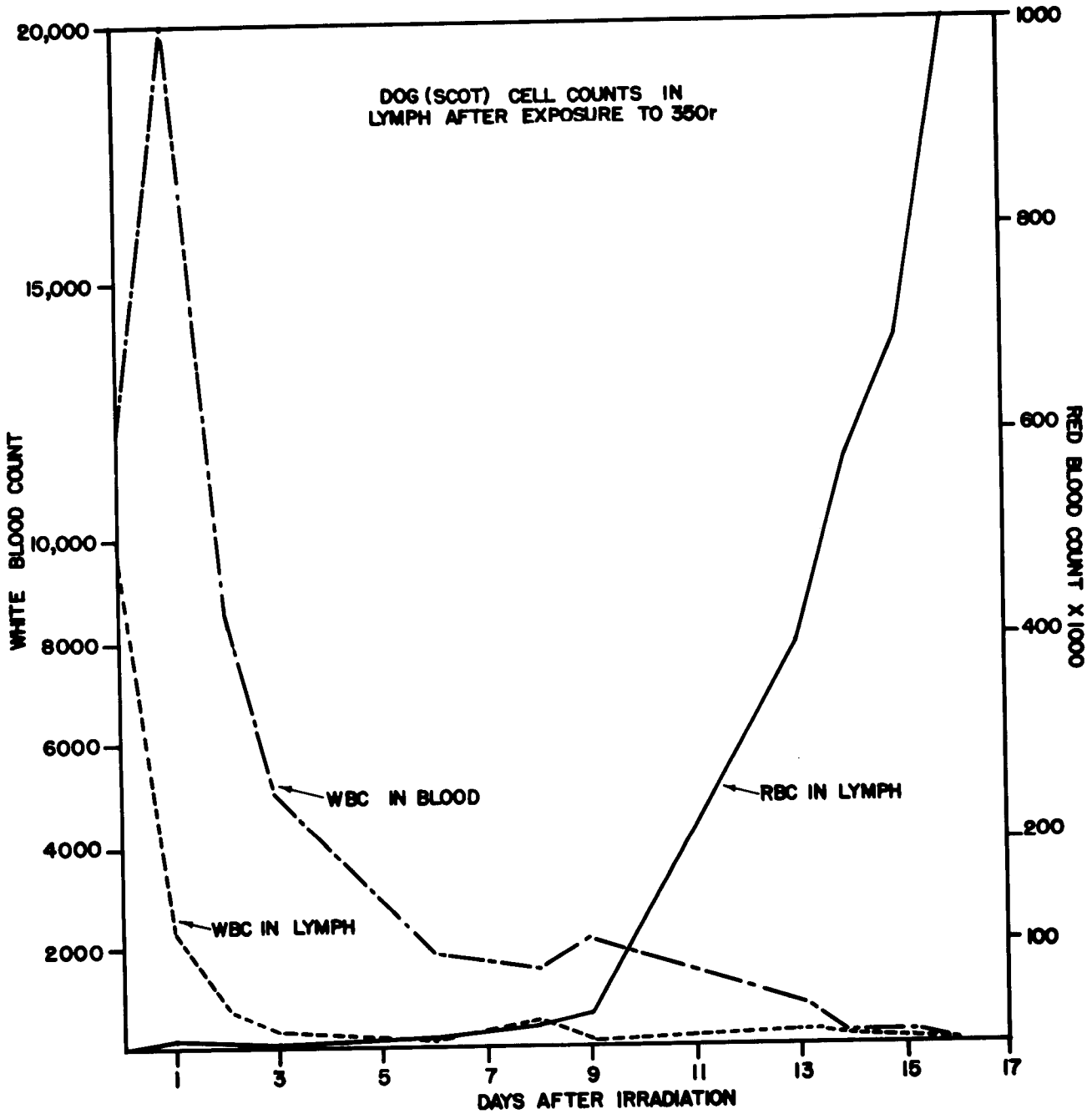


FIG. 3

Pathogenesis of radiation anemia. (Kahn, Storey; assistance, Click) The basic design of this experiment and the salient findings have been outlined in our earlier reports which have since been confirmed by further experiments. These studies are now being drafted for publication.

Radiation protection. (Kahn; assistance, Lane, Click) In previous reports it was pointed out that morphine sulfate affords protection against X radiation in ZBC male mice. Analysis of the data by the probit method (by Kimball, ORNL Mathematics Panel) indicates that 50 mg/kg of morphine sulfate injected intraperitoneally into ZBC male mice 30 minutes before exposure to radiation increases the LD-50 of the radiation from 600 r to 800 r (33 per cent). The probability that this increase is due to chance factors alone is vanishingly small ($p \ll 0.001$). This work is being prepared for publication.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson (Leader)
E. H. Mosbach — Betty Ann Gwin
E. F. Phares — F. W. Densison, Jr.*
Mary V. Long — J. M. Adams*

Wolff-Kishner reduction of pyruvic and 3-formylpropionic acids. (Mosbach, Phares, Long) The Wolff-Kishner reduction has been applied to a large number of organic compounds (Adams, "Organic Reactions," Vol. III, Ch. 8, John Wiley and Sons, Inc., 1946), but very few investigations have been reported concerning the reduction of low molecular weight aldehydo- and keto-acids. We have found that pyruvic-2-C^{14} acid and 3-(formyl-C^{14})-propionic acid could be reduced to propionic-2-C^{14} acid and butyric-4-C^{14} acid, respectively, in good yield. These and similar transformations have been used in this laboratory for the synthesis and degradation of biochemically important compounds.

Table 11 summarizes specific radioactivity data obtained from a synthesis

TABLE 11

Synthesis and degradation of propionic-2-C^{14} acid

COMPOUND OR CARBON	SPECIFIC RADIOACTIVITY* cts./min./mg $BaCO_3$	
	CALC.	FOUND
Pyruvic-2-C^{14} acid	-	126
Propionic-2-C^{14} acid	15.8**	16.2
Carbon 1	0.0	0.1
Carbon 2	47.4	46.1
Carbon 3	0.0	0.1

*$BaCO_3$ from wet combustion, G-M counting at infinite thickness, estimated over-all precision of radio-assay ± 5 per cent.

**Eightfold dilution with nonradioactive carrier.

*ORINS fellow.

of propionic-2-C^{14} acid. The compound was degraded stepwise (Phares, Abstracts of Papers, 117th Meeting of Am. Chem. Soc., 1950, p. 4C; completed manuscript submitted to journal), in order to determine whether any rearrangement of the carbon skeleton had occurred during the reduction.

Table 12 shows similar data for the preparation of butyric-4-C^{14} acid

TABLE 12

Synthesis and degradation of butyric-4-C^{14} acid

COMPOUND OR CARBONS	SPECIFIC RADIOACTIVITY * cts./min./mg $BaCO_3$	
	CALC.	FOUND
Glutamic-1,2-C_2^{14} acid	-	7.50
3-(formyl-C^{14})-propionic acid	4.69**	-
Butyric-4-C^{14} acid	4.69	4.72
Carbon 1	0.0	0.05
Carbon 2	0.0	0.04
Carbon 3	0.0	0.24
Carbon 4	18.8	18.1

*See footnote for Table 11

**Calculated from specific activity of glutamic-1,2-C_2^{14} acid

from glutamic-1,2-C_2^{14} acid. This reaction was carried out by converting glutamic acid quantitatively to 3-formylpropionic acid with chloramine T, followed by a Wolff-Kishner reduction of the aldehydo-acid. As before, a stepwise degradation (Mosbach, Phares, and Long, Federation Proc., 10:226, 1951; completed manuscript submitted to journal) of the fatty acid was carried out to determine the position of the radioactive label.

The data of Tables 11 and 12 indicate that under the conditions of the Huang-Minlon modification of the Wolff-Kishner reduction there occurred no

detectable rearrangement of the carbon skeleton of pyruvic and 3-formylpropionic acids. It is believed that the appreciable radioactivity found for carbon 3 of butyric-4-C^{14} acid was probably not caused by the reduction or the degradation procedure, but may have been introduced during the synthesis of the labeled starting material (Kögl, Halberstadt, and Barendregt, Rec. trav. chim., 68:387, 1949).

Biosynthesis of citric acid. (Carson, Mosbach, Phares, Gwin, Long; J. Bact., in press) In connection with carbon isotope experiments on the mechanism of biosynthesis of citric acid by microorganisms (Foster and Carson, J. Am. Chem. Soc., 72:1865, 1950; Weinhouse and Millington, ibid, 69:3089, 1947) and animal tissues, one prime difficulty has always been the inability to distinguish by direct determination between isotopic carbon concentrations within two pairs of carbon atoms, namely, 1 and 5, and 2 and 4 (I. U. C. numbering system for glutaric acid and derivatives). Experimental proof (Potter and Heidelberger, Nature, 164:180, 1949; Wilcox, Heidelberger and Potter, J. Am. Chem. Soc., 62:5019, 1950) of the Ogston concept (Ogston, Nature, 162:963, 1948), relating to the asymmetry of the citric acid-enzyme complex, together with availability of a new stepwise degradation method worked out in this laboratory (Mosbach, Phares, and Long, Fed. Proc., 10:226, 1951), now made it feasible to determine individually the isotopic content of each of the six carbon atoms of citric acid.

Labeled citric acid was produced from sucrose by washed resting mycelia of *Aspergillus niger* in the presence of acetic acid-2-C^{14} and $C^{13}O_2$; "submerged-aerobic" conditions were maintained by placing the Erlenmeyer flask containing the mycelial suspension in a 10-liter desiccator, which was continuously shaken for 40 hours. The citric acid was isolated by continuous ether extraction and separated from other fermentation acids by reisolation through a chloroform-butanol partition chromatogram (Peterson and Johnson, J. Biol. Chem., 174:775, 1948; Marvel and Rands, J. Am. Chem. Soc., 72:2643, 1950). The labeled citric acid was shown to be isotopically pure by the finding that several widely separated fractions from the citric acid band had identical specific activities. The labeled citric acid was then degraded stepwise (Mosbach, Phares, and Long, Fed. Proc., 10:226, 1951), and the carboxyl carbons were also determined on a separate aliquot by the sulfuric acid method (Weinhouse, Medes, and Floyd, J. Biol. Chem., 166:691, 1946), which yielded the 6-carboxyl as carbon monoxide, and the 1- and 5-carboxyls as carbon dioxide, and hence furnished an independent check on the degradation. The results of the degradations are presented in Table 13.

TABLE 13

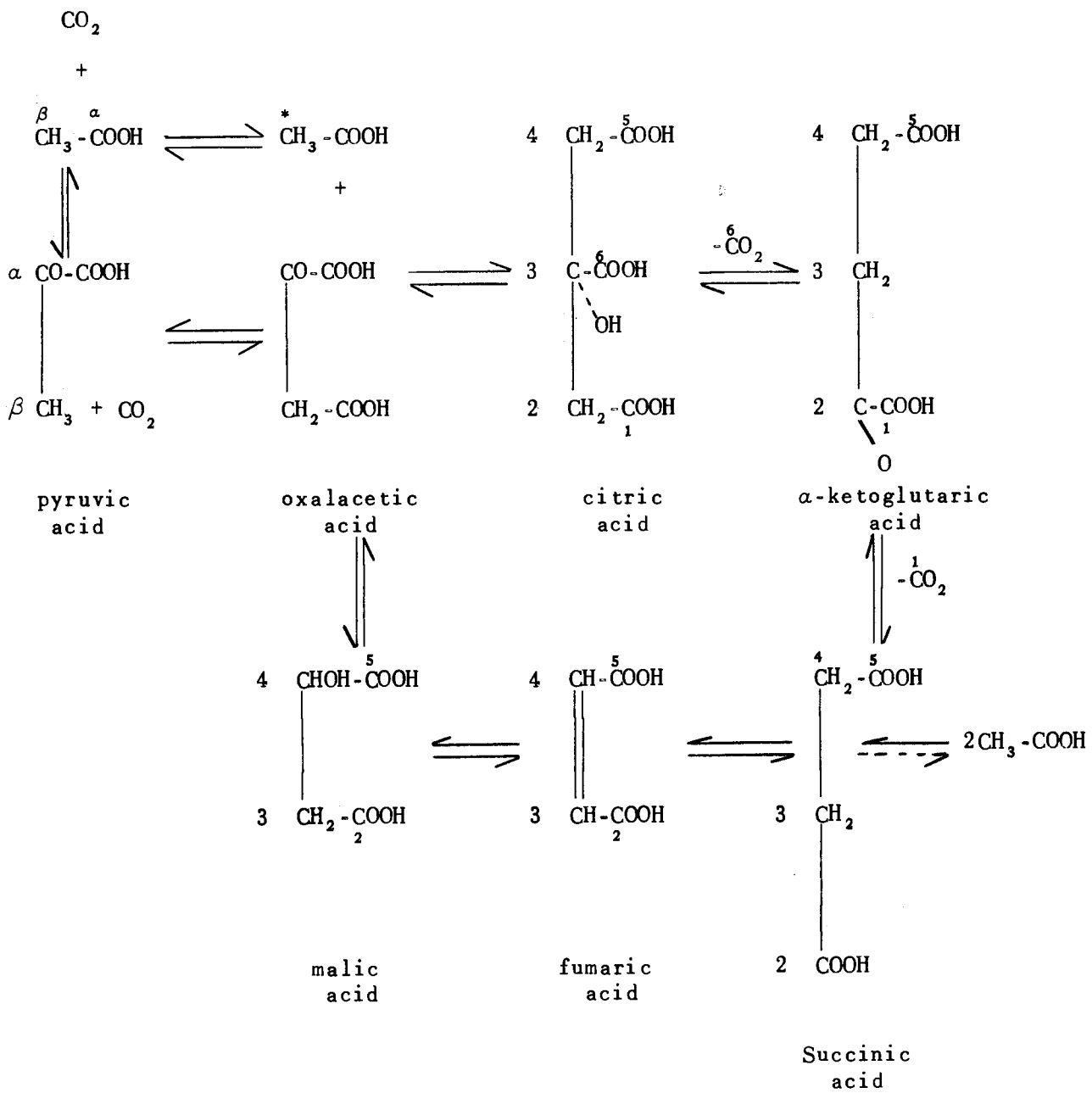
Isotopic composition of labeled citric acid

	C^{14} cts./min./mM C X 10^{-2} (CARRIER CITRATE ADDED)	C^{13} ATOM PER CENT EXCESS (NO CARRIER CITRATE ADDED)
5 COOH	132	
4 CH_2	560	
6 COOH	476	2.345 ± 0.003
3 C–OH	598	
2 CH_2	592	
1 COOH	463	
Total citric acid		
found	463	1.035 ± 0.005
calculated	470*	1.067
1 + 5 carboxyls		
found	283	2.030 ± 0.006
calculated	279**	

*Average of the six independently found values
**Average of the two independently found values

The finding of appreciable C^{14} activity in the 5-carboxyl indicated existence of reactions leading to formation of doubly-labeled acetate (acetate-1,2-C^{14}). For example, condensation of acetate and oxalacetate (Stern, Shapiro, and Ochoa, Nature, 166:403, 1950), can give rise to citrate (labeled in the 4-position by using acetate-2-C^{14}); continual C_6 cycling of such citrate eventually yields oxalacetate labeled in the 2 central carbon atoms (see diagram). Oxalacetate of such labeling can yield acetate-1,2-$C_2{}^{14}$ by the following series of reactions:

$$\text{oxalacetate} \longrightarrow \text{pyruvate} + CO_2 \longrightarrow \text{acetate} + CO_2.$$


CO2
+
β α
CH3-COOH
*
CH3-COOH
+
α CO-COOH
CO-COOH
β CH3 + CO2
CH2-COOH
4 CH2-COOH
5
3 C-COOH
6
OH
2 CH2-COOH
1
-CO2
6
4 CH2-COOH
5
3 CH2
2 C-COOH
1
O
pyruvic acid
oxalacetic acid
citric acid
α-ketoglutaric acid
-CO2
1
4 CHOH-COOH
5
3 CH2-COOH
2
4 CH-COOH
5
3 CH-COOH
2
4 CH2-COOH
5
3 CH2
2 COOH
2CH3-COOH
malic acid
fumaric acid
Succinic acid

Eq. 1

The doubly-labeled acetate would then give rise by means of the condensation reaction to citrate labeled in the 4 and 5 positions.

The gas phase was found to contain $C^{14}O_2$, therefore, one would expect to find labeling in the 1- and 6-carboxyls of citrate (but not in the 5-carboxyl) from carbon dioxide fixation and/or exchange reactions. The labeled carbon dioxide (from C_6 cycling) can enter into carbon dioxide fixation or exchange reactions at the β-carboxyl of oxalacetate; subsequent C_4-acid shuttling will tend to randomize the labeling with the oxalacetate α-carboxyl (Martin, Wilson, and Burris, Arch. Biochem., 26:103, 1950). The oxalacetate carboxyls can then give rise to the 1- and 6-carboxyls of citrate by the condensation reactions. Carbon dioxide fixation or exchange reactions were confirmed by the finding of excess C^{13} (from $C^{13}O_2$) in the citrate carboxyls. The C^{13} excess was not high enough to permit separate determination of the C^{13} content of carboxyls 1 and 5, whereas this was accomplished in the case of the C^{14} labeling with addition of carrier citrate.

Consequently, the isotopic composition of the labeled citric acid indicates the existence of extensive C_4- or C_6-acid cycling during biosynthesis. The data make it appear possible that citric acid itself is involved in one or more cyclic processes, although it is the end product of carbon metabolism in this organism. The widely different specific radioactivities of the primary carboxyl groups apparently confirm the Ogston concept for the case of a microbial system.

Conversion of α-ketoglutaric-1,2-$C_2{}^{14}$ acid to malic acid in pigeon breast muscle. (Carson, Phares, Mosbach, Long, Gwin) In studies with carbon isotopes on intermediary metabolism, it has frequently been suggested that the resultant isotope distribution found in the end products is at least in part due to recycling and "randomization" through the C_4-acid shuttle (Wood, Cold Spring Harbor Symposia Quant. Biol., 13:201, 1948). As far as we are aware, no definitive experiments have been undertaken to establish this contention.

The present experiments were conducted with pigeon breast muscle (Krebs and Johnson, Enzymol., 4:148, 1937) which does not exhibit any detectable carbon dioxide fixation or exchange reactions. This preparation is, therefore, eminently suitable to test for "randomization" during the conversion of α-ketoglutarate to malate through succinate and fumarate.

Fifteen grams of pigeon breast muscle were minced for 7 seconds in a Waring blendor in 40 ml of 0.02 *M* phosphate buffer (*p*H 7.4). Sodium α-ketoglutarate-1,2-C_2^{14} (0.34 m*M*; 64000 c/m/m*M*) and 1.0 m*M* unlabeled sodium malate, both adjusted to *p*H 7.4, were added. The gas atmosphere was composed of 5 per cent carbon dioxide (containing 40 atom per cent C^{13}) in oxygen. The muscle preparation was shaken in two 160-ml Warburg vessels at 37° C for 35 minutes.

At the end of the incubation period the reaction mixture was treated with 1.0 ml concentrated sulfuric acid; the proteins were centrifuged off and the supernatant concentrated *in vacuo* to 25 ml. Deproteinization was completed by addition of 25 g of Celite 535, followed by extraction of the organic acids with 45 per cent butanol in chloroform (Isherwood, Biochem. J., 40:688, 1946). The acids were extracted from the organic phase by titration with 1 *N* sodium hydroxide and the aqueous phase concentrated for partition chromatography. Malic acid, 0.51 m*M*, obtained from the partition column (Phares *et al.*, this report, Section 4) was shown to be isotopically pure by the finding that widely separated column fractions had identical specific radioactivities within the precision of measurement.

The pure malic acid was degraded by the sulfuric acid method (Utter, J. Biol. Chem., 188:847, 1951) which yields quantitatively the carboxyl α to the carbinol group as carbon monoxide. The carboxyl β to the carbinol group was obtained as carbon dioxide in 25 per cent yield. As a check, another aliquot of the malic acid was degraded to carbon dioxide (β-carboxyl) and lactic acid with *Lactobacillus casei*; the lactate was then degraded to acetate and carbon dioxide in order to obtain the α-carboxyl (Carson *et al.*, Arch. Biochem., in press). The results are presented in Table 14.

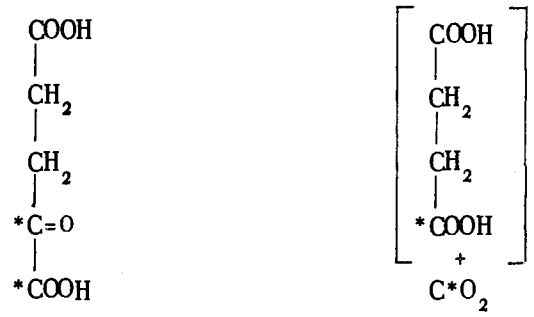
TABLE 14

Specific activities of malate and degradation products

	cts./min./m*M* C × 10^{-1}
Total malate	118
H_2SO_4 degradation	
α-COOH	246
β-COOH	214
L. casei degradation	
α-COOH	221
β-COOH	254
acetate *	7
Average values from the two degradations	
α-COOH	234
β-COOH	239
Calculated average carboxyl specific activity (from value for total malate)	236

*Central carbon atoms of malate; lactate from *L. casei* fermentation oxidized with $KMnO_4$ to acetate + CO_2.

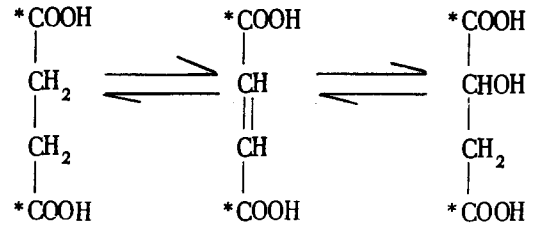
The results clearly indicate that within the limits of experimental error (± 5 per cent) complete randomization occurred between labeled carbon in the carboxyl groups of fumarate and succinate, as indicated in the following equations.



α-ketoglutaric acid

succinic acid

Eq. 2



succinic acid

fumaric acid

malic acid

Eq. 3

The data do not exclude the possibility that an unsymmetrical derivative of succinate, e.g. succinylmonophosphate or succinic acid semialdehyde, is formed during the oxidative decarboxylation of α-ketoglutarate (Ochoa, Physiol. Rev., 31:56, 1951). However, such an intermediate must have been converted to a symmetrical C_4 compound at some stage during its conversion to malate via fumarate.

Partition chromatographic separation of biosynthetic organic acids. (Phares, Mosbach, Carson, Denison, Gwin, Long) Radioactive tracer studies of

the role of organic acids in various biological systems require clean-cut separations of isotopically pure compounds. Although satisfactory methods have been described for the isolation of some of the intermediates of the tricarboxylic acid cycle (Isherwood, Biochem. J., 40:688, 1946) and a number of other acids (Marvel and Rands, J. Am. Chem. Soc., 72:2642, 1950) by partition chromatography, we have found that several pairs of acids were obtained which could not be separated on the chloroform-butanol chromatograms reported in the literature. This difficulty was encountered particularly in the cases of succinic and lactic acids, malic and oxalic acids, and glycolic and oxalic acids. Preliminary studies of ion-exchange methods (Anthony, *et al.*, unpublished results) indicated that such procedures were not entirely satisfactory for the isolation of labeled compounds on a 0.1 to 1.0 m*M* scale, mainly because of two considerations: (1) the introduction of organic matter into column eluates by partial decomposition of the resin employed, resulting in lowered specific radioactivity values upon wet combustion, and (2) the difficulty of devising accurate analytical methods for the determination of organic acids in acidic or buffered column effluents.

Consequently a method was developed, using partition chromatography exclusively, which allowed the separation from biological materials of a number of organic acids. The following acids were examined: acetic, α-ketoglutaric, citric, formic, fumaric, glucolic, iodoacetic, kojic, lactic, malic, malonic, oxalic, perchloric, pyruvic, phosphoric, succinic, sulfuric, and tartaric. Satisfactory separations and isolations were obtained by the use of two solvent systems, butanol in chloroform - 0.5 *N* sulfuric acid and ethyl ether - 0.5 *N* sulfuric acid. Celite 545 was used as support for the immobile phase. In general, it was found that of the acids studied those not resolved by chloroform-butanol could be separated by rechromatographing on an ether column. Recovery data obtained indicated that the method also afforded a fair quantitative estimation of these acids in animal tissues and fermentation liquids.

EXPERIMENTAL

The standard columns employed were made of pyrex glass, 45 × 1.0 cm. For preliminary studies and small amounts of material (less than 0.1 m*M*) 10 × 1.0 cm columns were often found satisfactory. For separating succinic, α-ketoglutaric and malonic acids slightly larger columns, 65 × 1.5 cm, were found to give more satisfactory isolations.

Both chloroform butanol (CB) columns and ether columns were prepared in an identical manner. The stationary phase, 0.5 *N* sulfuric acid, was mixed very thoroughly with the inert support, Celite 545 (100 to 200 mesh). For most applications a ratio of 8 ml of 0.5 *N* sulfuric acid to 10 g of Celite was found to be satisfactory. The moist Celite was then slurried with the organic phase and packed into the column by tamping (Peterson and Johnson, J. Biol. Chem., 174:775, 1948). The samples to be analyzed were placed on the column as follows: the aqueous solution of the sodium salts of the organic acids was evaporated *in vacuo* to a volume of about 0.25 ml in a small, widemouthed conical flask. For some of the less stable acids, e.g., α-ketoglutaric acid, the evaporation was carried out at neutral *p*H and room temperature. The concentrated aqueous phase (in the conical flask) was mixed with enough Celite (0.5 g or less) to make a fairly dry slurry. This mixture was then acidified with 4 to 8 drops (0.1 to 0.2 ml) of 50 per cent sulfuric acid, transferred quantitatively to about 2 ml of solvent on top of the Celite and tamped down. The appropriate organic phase was added from a reservior attached to the top of the column. Care was taken not to let the column go dry at any time after packing. Flow rates of approximately 0.5 to 1 ml per minute were found to be satisfactory for all types of columns. The effluent was collected with an automatic fraction collector, and the organic acids were determined by titration with standard (0.100 *N*) alkali. Individual acids were identified by comparison with known compounds run on standard columns, and also by the application of specific reactions (Umbreit *et al.*, Manometric Techniques, 1946), colorimetric procedures (Long, Biochem. J., 36:807, 1942; Dische, Biochem. Z., 189:77, 1927), and paper chromatography (Lugg and Overell, Austr. J. Sci. Res., 1:98, 1948).

RESULTS AND DISCUSSION

Fig. 4A shows a composite diagram of the position of the various acids on a standard CB column, 45 × 1.0 cm. Elution was started with 10 per cent butanol in chloroform (CB-10) and continued with CB-35, as indicated. The inorganic acids, perchloric, phosphoric, and sulfuric are included because they are often encountered through deproteinization procedures. The figure shows that a number of acids are resolved either partially or not at all by this method.

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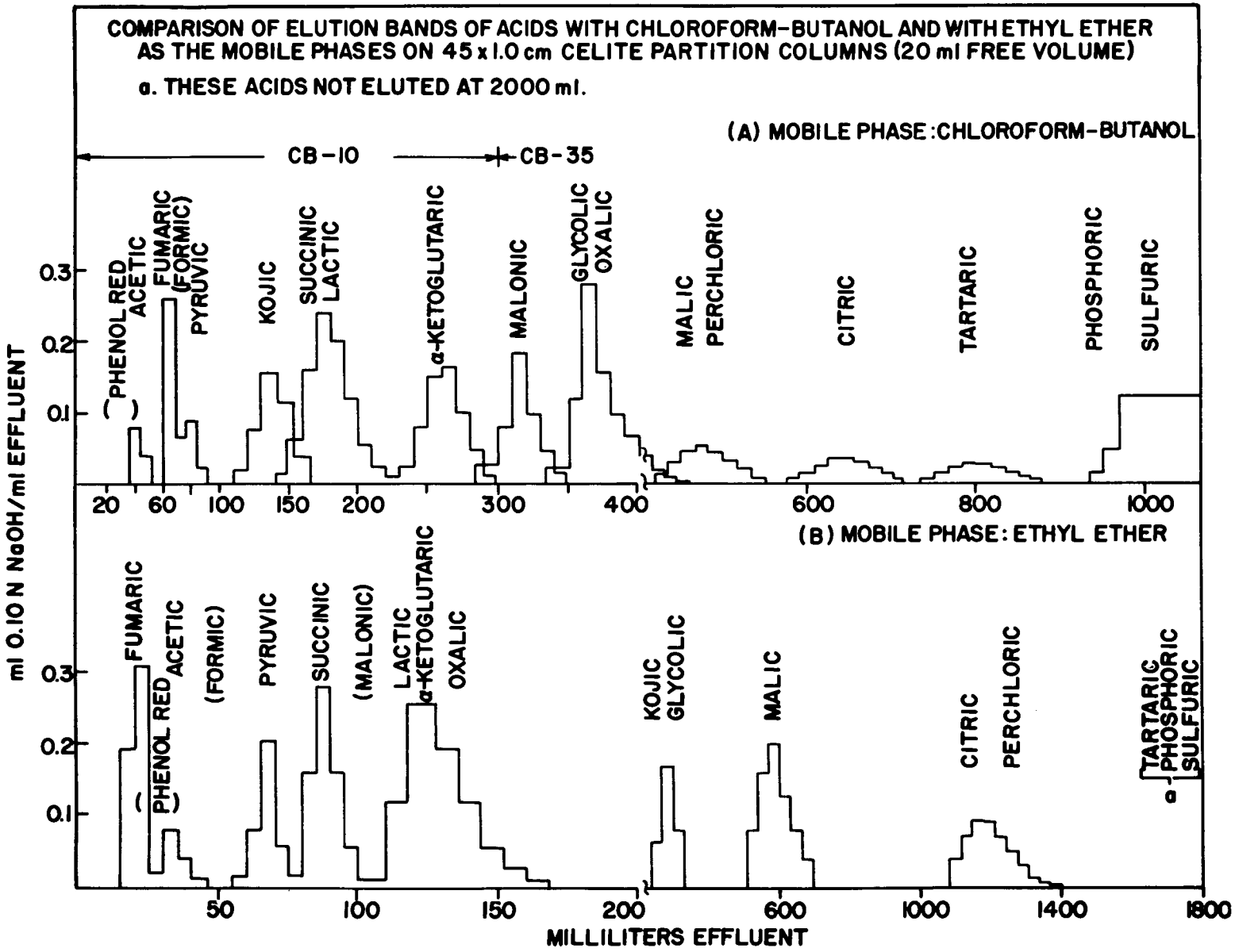

FIG. 4

Fig. 4B shows a composite diagram for the standard ether column, 45 × 1.0 cm. It is evident that most of the pairs not resolved on CB separated satisfactorily on ether columns. The differences in partition coefficients from CB to ether are particularly striking for the following pairs of acids: succinic and lactic, fumaric and pyruvic, glycolic and oxalic, and malic and oxalic. For the separation of the two latter pairs, short ether columns, 10 × 1.0 cm were particularly convenient.

Fig. 5 shows the separation of α-ketoglutaric, succinic, and malonic acids on a large CB column. These acids frequently must be separated in connection with inhibition studies on animal tissues. Radioactive tracer studies (Mosbach *et al.*, Federation Proc., 10:226, 1951) indicated that by the use of large columns, α-ketoglutaric acid could be obtained with a very high degree of purity.

Table 15 summarizes recoveries obtained for various acids on both ether

TABLE 15

Recoveries of organic acids from known mixtures

ACID	CB COLUMNS*	ETHER COLUMNS
α-Ketoglutaric	77.2*	93.9
Acetic	95.0	-
Citric	90.8	-
Formic	95.0	92.5
Fumaric	81.0	92.6
Glycolic	90.0	94.0
Iodoacetic	87.2	-
Kojic	87.5	-
Lactic	-	88.0
Malic	84.3	-
Malonic	89.9*	-
Oxalic	92.0	84.0
Pyruvic	85.7	90.0
Succinic	96.0	97.5
Tartaric	95.0	-

45 X 1.0 cm columns, except those marked with an asterisk which were 1.5 X 65 cm.

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SEPARATION OF SUCCINIC, α-KETOGLUTARIC, AND MALONIC ACIDS ON LARGE CELITE COLUMN (40 ml FREE VOLUME)

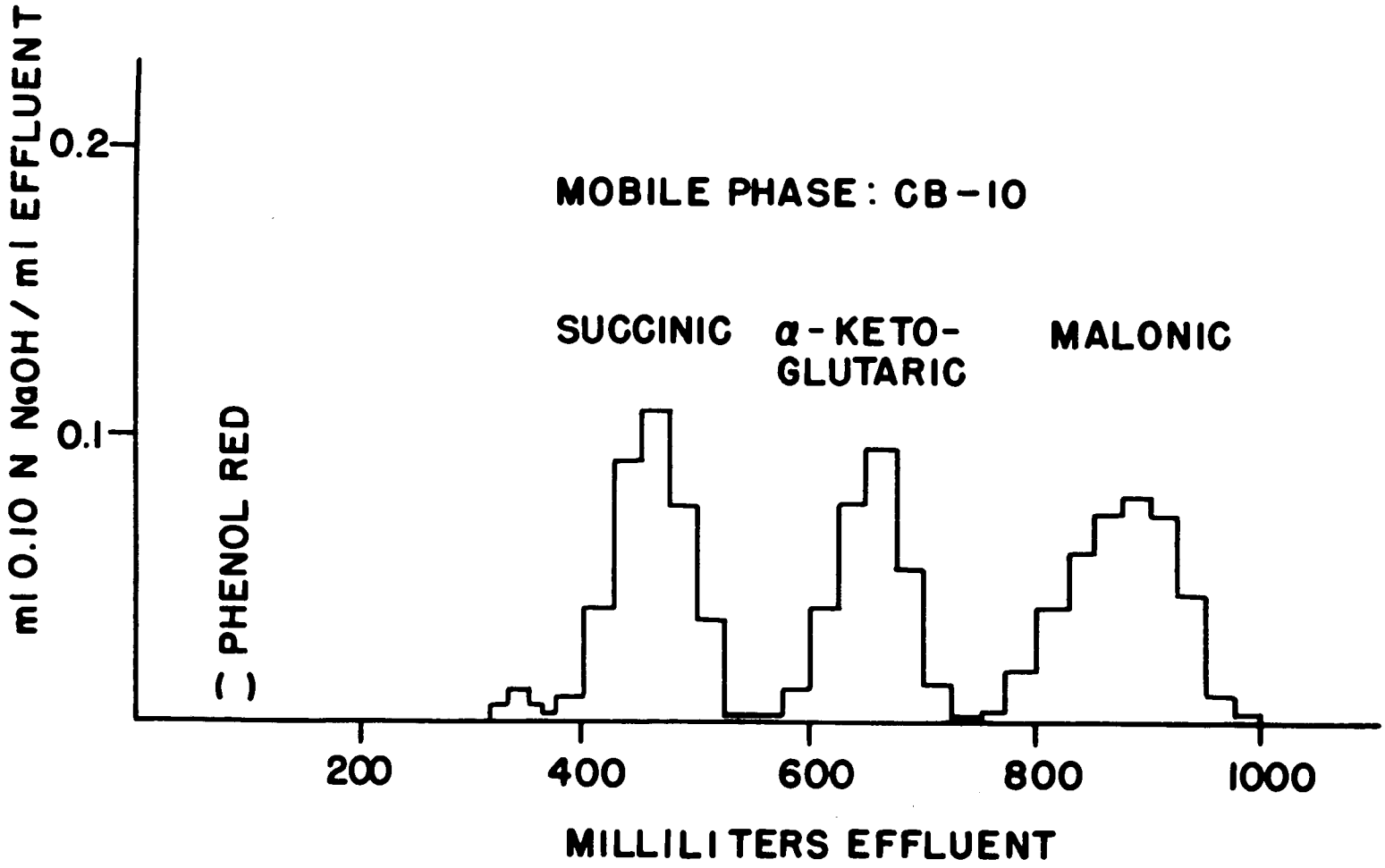

FIG. 5

and CB columns. The data indicate that the recoveries from Celite columns may be slightly less than those obtained with silica gel (Isherwood, Biochem. J., 40:688, 1946). Nevertheless, the recoveries obtained in this study allow a fair quantitative estimation of the organic acids present in the biological systems studied.

These methods have been applied in this laboratory in numerous carbon isotope studies of intermediary metabolism (Mosbach *et al.*, Fed. Proc., 10: 226, 1951; Carson, *et al.*, J. Bact., in press, July, 1951; Mosbach, *et al.*, J. Am. Chem. Soc., in preparation; see also Section 3 of this report). It was found that under the experimental conditions employed, chemically and isotopically pure acids were isolated from a number of biological systems.

BIOCHEMISTRY

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

W. E. Cohn (Leader)

D. G. Doherty
E. Volkin
B. L. Strehler
F. Vaslow
M. Helen Jones
J. X. Khym
J. A. Lloyd
E. Jane Beale

Composition and structure of nucleic acids. (Cohn, Volkin, Khym, Jones) The discovery of 5′-nucleotides in enzymatic digests of calf-liver ribonucleic acid (RNA) was reported last quarter. We have now extended this observation to yeast ribonucleic acid which makes it appear that the 5′ linkage is a more general phenomenon than the previous observations alone might indicate.

The two pyrimidine diphosphates, reported in ORNL-969, are still incompletely characterized, due to insufficient material. Resistance to acid hydrolysis indicates the absence of a simple pyrophosphate linkage; titration data, indicating two groups at about *p*K 6 per mole of pyrimidine, are also in harmony with this concept. (The titration curves show a hysteresis for which we have as yet no explanation.) Neither compound loses phosphate with any 5′-nucleotidase, including that of Kornberg which removes the 5′-phosphate from his diphosphoadenosine, while other phosphatases hydrolyze one or both phosphates yielding mixtures of *a* and *b* mononucleotides, as judged by ion-exchange behavior. These facts incline us to the belief that these compounds are 2′,3′-diphosphocytidine and 2′,3′-diphosphouridine.

Utilizing slow flow rates, a successful ion-exchange separation of several polynucleotide components from a ribonuclease digest of RNA has been achieved on a large scale. Chemical and enzymatic degradation studies of these fragments are in progress.

Ion-exchange separation of carbohydrates. (Khym, Zill of Plant Physiology) The biosynthetic preparation of C^{14}-labeled sugars requires a method for the preparative isolation of individual sugars from their mixtures. A method having decided advantages over existing methods has been developed through the joint efforts of the Plant Physiology and Biochemistry groups. A preliminary report concerning this work appeared recently (J. Am. Chem. Soc., 73: 2399-2400, 1951) and two other papers are in preparation.

Since borate ion reacts with sugars to produce negatively charged sugar-borate complexes, it occurred to us that such complexes might be separable by the techniques of ion-exchange in a fashion similar to that used in separating nucleotides and related compounds.

It was found that fructose, glucose, mannose, and galactose dissolved in dilute sodium borate solutions, are quantitatively adsorbed on strong base anion exchangers. Elution was carried out with dilute sodium borate solutions and the effluent fractions were analyzed for sugar by the quantitative anthrone method. Identification of the sugars was accomplished by paper chromatography.

A separation of glucose, galactose, and fructose on a strong base anion exchanger is shown in Fig. 6. Recoveries of the individual sugars were essentially quantitative.

The method has been further extended to include the separation of other sugars. A summary of the sugars studied appears in Table 16.

TABLE 16

Volume to peak position of various sugars; exchanger-0.85 sq cm × 12 cm. Dowex-1

SUGAR	ELUTING AGENT	ml TO PEAK
Xylose	0.015 *M* $K_2B_4O_7$	2400
Ribose	" "	250
Arabinose	" "	1400
Mannose	0.006 *M* $K_2B_4O_7$ in 0.05 *M* H_3BO_3	600
Fructose	" " " " "	1150
Sucrose	0.005 *M* $K_2B_4O_7$	175
Trehalose	" "	370
Cellobiose	" "	660
Maltose	" "	750
Lactose	" "	810

Figure 6

Exchanger: 0.85 sq cm × 11 cm Dowex-1, ca.300 mesh, borate form.

Eluting agent: sodium borate as shown at 1 ml/minute

Test material: 10 mg fructose, 12.5 mg galactose, 12.5 mg glucose in 10 ml of 0.01 *M* sodium borate.

Recovery: ~99 per cent based on optical density at 620 mμ.

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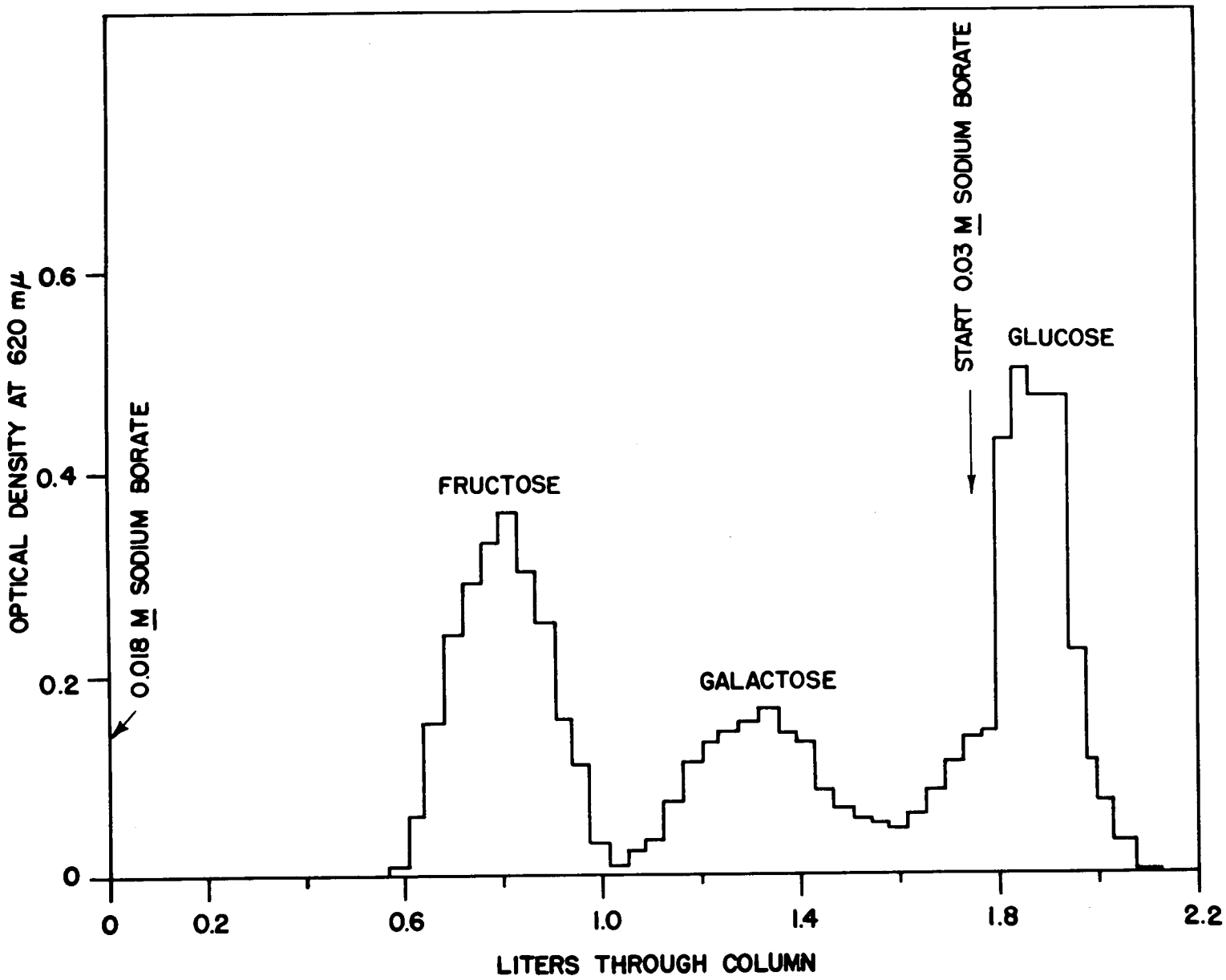

FIG. 6

Melibiose (not listed in table) was found to be more firmly attached to the exchanger than any other sugar thus far investigated. This sugar required 4050 ml of 0.015 *M* $K_2B_4O_7$ followed by 850 ml of 0.025 *M* $K_2B_4O_7$ to reach the peak position. No previously reported borate complex of a disaccharide has been shown to have properties which would account for this binding capacity. Consequently, melibiose complexes with borate will be further studied by means of polarimetric and conductimetric methods.

Several applications of the method have been carried out. The simplest of these was the complete preparative separation of glucose and fructose from a sucrose hydrolysate. Separation of the sucrose from the glucose and fructose in an incomplete hydrolysate was also achieved. The separation of radioglucose and radiofructose from a labeled plant extract (prepared by G. R. Noggle) was carried out quantitatively. Four other peaks which appeared in this run are in the process of being identified. The method has been further applied to studies on nucleic acids. The separation of the ribonucleosides can be accomplished by adsorbing them on an anion exchanger in the borate form and eluting with a mixture of 0.03 *M* KCl and 0.02 *M* $K_2B_4O_7$. Thymidine and uridine can be separated in a similar fashion.

Further studies to be carried out include the following: (a) concentration of the effluent fractions and removal of the borate, (b) extension of the method to all sugars which form a borate complex, (c) correlation of ion-exchange patterns with previously reported structural configuration studies by means of borate complexes, (d) application of the method to the preparative isolation of labeled sugars from plants, and (e) application of the method to the preparative isolation of labeled glucose and mannose from the yeast, *Torulopsis utilis* var. major.

Enzyme-substrate binding studies. (Doherty, Vaslow) A new class of carbohydrate-amino acids of biochemical interest has been synthesized. It is an aldonic acid coupled through the carboxyl group to the amino group of an amino acid in a stable $CO\text{-}NH_2$ linkage. The generic name, *glyconyl peptide,* is proposed for this type of compound. These glyconyl peptides will be of value in studies of (a) their possible role in the synthesis of nucleic acids and the origin of ribose, (b) as a means of obtaining soluble Br^{82}-labeled substrates for enzyme-binding studies, (c) as a source of soluble peptide derivatives for kinetic studies in proteolytic enzyme systems, (d) as a means of

solubilizing and possibly detoxifying compounds used in protection studies, and (e) as an excellent means for investigation of the stereochemical specificity of enzyme reactions, since aldonic acids of widely varying configuration are available.

The glyconyl peptides are prepared by coupling the acetylated aldonyl chloride with a free amino acid ester to give an excellent yield of acetylated aldonyl amino acid ester. This compound can be catalytically deacetylated to yield the aldonyl amino acid ester, and either de-esterified to the free aldonyl amino acid or converted to the amide with methanolic ammonia. This series of reactions has been carried out with the following aldonyl chlorides and amino acids and esters: penta-acetyl-D-gluconyl chloride, tetra-acetyl-D-arabonyl chloride, tetra-acetyl-D-ribonyl chloride; glycine, L-tyrosine, L-glutamic acid, and glutamic acid, and glycylglycine. The glyconyl peptide esters and amides crystallized readily and were appreciably more water soluble than the corresponding acetyl and benzoyl derivatives, providing soluble substrates for the investigation of enzymatic reactions.

Studies on the biosynthesis and bioluminescent assay of adenosinetriphosphate. (Strehler) Work was continued on the projects summarized in the last report. In particular, the limits of sensitivity of the firefly enzyme - quantum counter combination to added adenosinetriphosphate (ATP) have been determined. Under proper conditions it is possible to detect ATP in concentrations of 1/10 - 1/200 μg/7 ml total solution. This method has been used to measure ATP in illuminated and nonilluminated *Chlorella pyrenoidosa*. Preliminary results indicate large increases (up to 1000 per cent) in ATP content after illumination for 15 seconds if the cells had previously been allowed to become anaerobic. An examination of the effect of other possible variables is now being pursued. It has been found that a preparation of nucleic acid (isolated by Volkin from a growing culture of *Torulopsis utilis*) exhibits ATP activity toward the firefly enzyme. The significance of this finding is being investigated.

Studies on the luminescence of green plants. Work has been completed for the present on the luminescence of chloroplasts. In addition to the results summarized in the last quarterly report, it has been found that there is no induction period for chloroplast luminescence and no effect of carbon dioxide on the luminescence as contrasted to results with intact *Chlorella*. These

findings strongly suggest that the photosynthesis-fluorescence-luminescence induction phenomena are intimately related to the fixation of carbon dioxide and reflect an accumulation of early intermediates in photosynthesis which are then displaced to steady state values when carbon dioxide fixation can proceed. A comparison of the effect of inhibitors on *Chlorella* and chloroplast luminescence has also been completed.

Studies on the luminescence of bacterial extracts and of riboflavin. (with C. S. Shoup, AEC) An attempt has been made to obtain luminous extracts from the luminous bacterium, *Achromobacter fischeri*. It has been found possible to obtain light detectable with the quantum counter from acetone dried bacteria upon wetting them. It was also found that boiled bacterial extracts emitted light when hydrogen peroxide was added to them. Of the known constituents of these organisms, riboflavin was found to emit light upon the addition of peroxide. The effect of various environmental factors on this chemiluminescence has been studied.

RADIOBIOCHEMISTRY

C. W. Sheppard (Leader)

W. T. Burnett, Jr. E. B. Darden, Jr.
J. S. Kirby-Smith Marian Stewart
Arthur Burke M. L. Morse
(See also Cytogentics - Hollaender)

Erythrocytes. (Sheppard, Stewart) Studies of changes in sugar, lactic acid, and adenosinetriphosphate (ATP) content have been made, in conjunction with Strehler (of Biochemistry), in irradiated and control blood. This work was planned in order to determine whether a relationship exists between the glycolytic cycle and potassium accumulation by the red cell.

The possibility that acetylcholine metabolism is vitally concerned in this potassium accumulation is being investigated. Studies of choline esterase activity have been made on cells suspended in sodium bicarbonate buffer. The observations of Greig (Vanderbilt University)that potassium leakage from cells suspended in sodium bicarbonate buffer can be delayed by addition of acetylcholine,were tested in irradiated blood suspended in the same buffer. A striking decrease in rate of hemolysis, easily observed visually, was found in the irradiated blood to which acetylcholine had been added. Leakage was retarded in both irradiated and control blood by the addition of acetylcholine.

In view of the possibility that choline acetylase is inactivated or destroyed by radiation, it was decided to determine whether acetylcholine synthesis is blocked on irradiation. Some preliminary results were obtained on samples which were sent to Dr. Greig at Vanderbilt University where the acetylcholine content was determined. However, in order to obtain more complete data it was deemed necessary to set up our own apparatus for determination of acetylcholine. With assistance of Mr. Jack Davidson (Instrument Department) an apparatus was devised by which the contraction of a frog rectus abdominus muscle in response to the addition of acetylcholine, is measured by a strain gauge and recorded on a Brown recorder. By this method the acetylcholine content of small samples of blood can be determined.

Gamma-radiation sources. (Kirby-Smith, Darden) A recent mechanical failure in the large cobalt γ-ray source has required the complete rebuilding and reloading of the source capsule. The source has been reloaded with material of higher activity and greater uniformity and should result in a more satisfactory installation. The present γ-ray intensity is approximately two to three times the previous value.

The high intensity (150,000 r/hour) distributed source should be on hand in July. Completion of the shield is scheduled for the end of June and material for loading the source is expected by this time.

The design and construction of an intermediate distributed γ source planned by Conger (of Cytogenetics) is completed. Loading with approximately 10 curies of cobalt will be completed in the immediate future.

Effects of infrared in modifying aberration frequency. (Kirby-Smith) An increased chromosomal aberration frequency has been observed in the pollen tube division of *Tradescantia* due to infrared pretreatment of pollen grains before X or γ irradiation. These results are in qualitative agreement with the work of Swanson and Hollaender (Proc. Nat. Acad. Sci., 32: 295, 1946) on the effects of combined infrared and X radiation on *Tradescantia* microspore chromosomes. Further studies of the effects of infrared in modifying aberration rates are in progress. In the course of these experiments it has also been observed that the cobalt γ rays (1.1 and 1.3 mev) used in much of the work are approximately one-half as effective as 250 kv X rays in producing chromosomal aberrations.

Polonium—microsources. (Kirby-Smith, Darden) With the assistance of Rona (of ORINS), a successful attempt has been made to prepare a small polonium α source on the tip of a platinum wire by the distillation method of Rona and Schmidt (Z. Physik. 48: 784-788, 1928). An α counter has been set up in the counting room for the benefit of members of the Division working with α particles and a special jig is being fabricated to permit accurate counting of collimated microsources as well as sources of the flat surface type.

Radiological physics. (Darden) A program of studying the relative absorption of P^{32} β particles in tissue-like materials is in progress utilizing the extrapolation type β-ray chamber designed by Sheppard and Abele (ORNL-265, 1949). Polystyrene with a density of $\sim$ 1.05 (about the same as that of whole blood) has been the principal material used. A few measurements have been made on cellulose acetate. Preliminary experimental results, up to a thickness of $\sim$ 70 mg/sq cm, are shown by the individual plots in Fig. 7. The straight line represents the theoretical absorption of a β-particle emitter in terms of relative counts per minute rather than ionization. The slope is calculated according to a method given by Siri (Isotopic Tracers and Nuclear Radiations, McGraw-Hill Book Co., Inc., 1949) for an emitter having a spread of energies with a maximum value of 1.7 mev absorbed in material of unit density. The results are consistent with those made here earlier with different equipment (Raper, Zirkle, and Barnes, MDDC-661, 1945). It is planned to extend the data to thicknesses of several millimeters, i.e., on the order of the Feather range for P^{32} β particles.

Assistance has been given Operations Division in measurement of P^{32} plaque β-ray intensities with the surface exposure chamber and associated equipment transferred to them last summer. A 20 per cent discrepancy between their values and measurements on the same plaques made here with the extrapolation chamber has been resolved. The Operations Division is now able to supply plaques of much higher quality in respect to physical properties than those obtained in recent months.

Assistance is being given Dr. Gaulden's group in their neuroblast work to provide them with an especially accurate setup for β-ray dosimetry including calibration of the plaques used with our extrapolation chamber.

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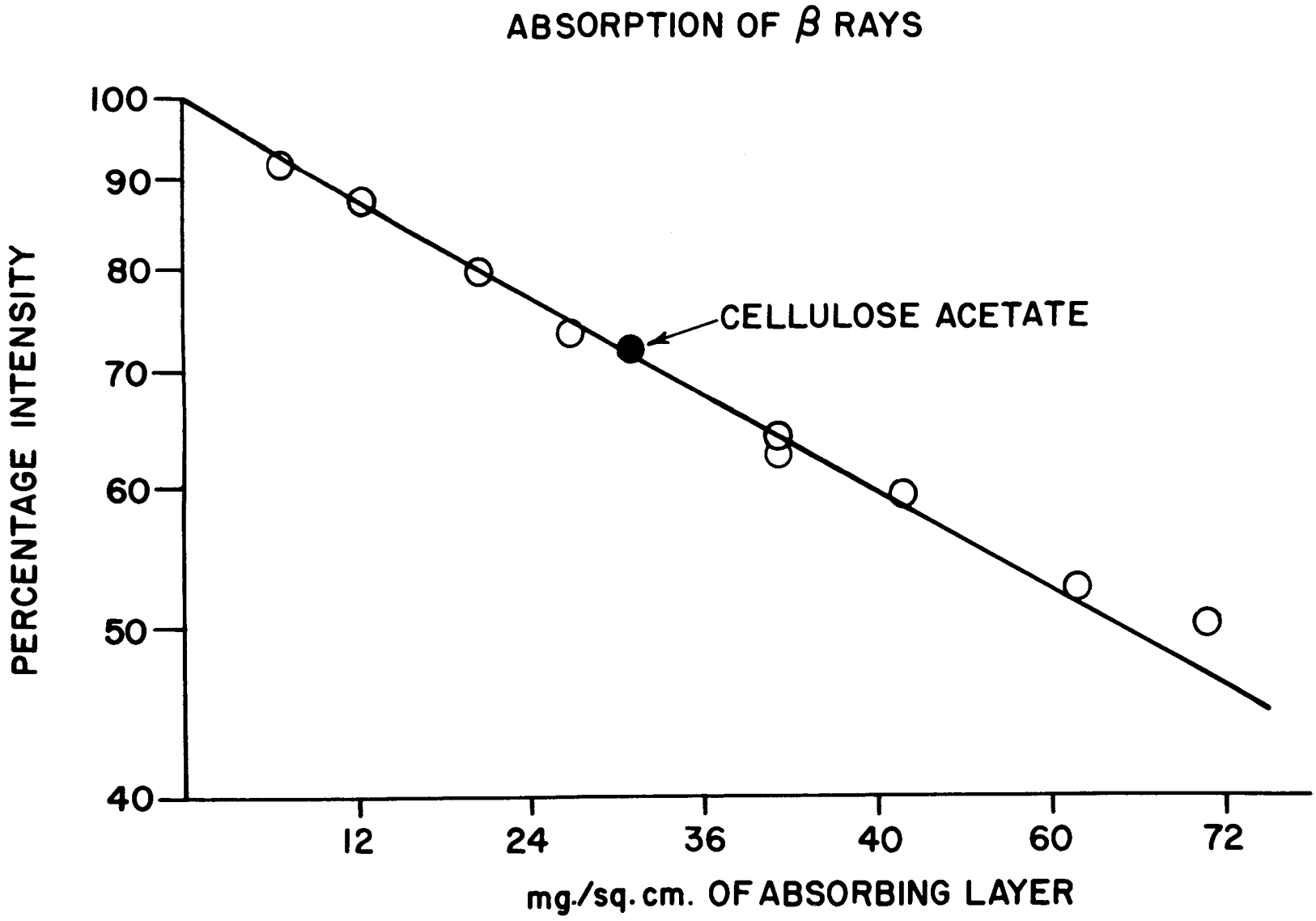

FIG. 7

The β-ray extrapolation chamber has been provided with adaptations designed to permit its use for calibration of 1-inch Ru^{106} plaques to be supplied in experimental form by the Operations Division. If accurate surface exposure measurements prove to be obtainable, this type of source having a half life of 1 year and emitting 3½ mev β particles (in comparison to 1.7 mev for P^{32}) should prove to be a useful radiobiological tool.

PLANT PHYSIOLOGY

G. R. Noggle (Leader)

L. P. Zill
J. H. Taylor*
J. S. Bangson**
G. M. Cheniae
Herbert Jonas***
M. Eleanor Schumacher

Plant biosynthesis: Amino acids. (Noggle) Carbon-14-labeled amino acids have been prepared by biosynthetic methods. After the introduction of carbon-14 into leaves (ORNL-787), sugars and soluble amino acids were extracted with 80 per cent ethyl alcohol. The alcohol was removed under reduced pressure and the aqueous extract was cleared with neutral lead acetate and oxalic acid. The protein-free extract was passed through a cation resin column (Duolite C-3) which retained the amino acids on the resin and allowed the sugars to pass on through. The amino acids were then eluted from the resin with dilute hydrochloric acid. The eluate was concentrated under reduced pressure to remove excess hydrochloric acid. Paper chromatography indicated the presence of about a dozen amino acids. Glycine, and glutamic and aspartic acids have been definitely identified and the others are being studied.

Glutamic and aspartic acids were removed from the amino acid mixture by passing the solution through an Amberlite IR-45 resin column maintained at *p*H 3.5. These acids were held on the column while the other amino acids passed through. They were then eluted with hydrochloric acid and the excess hydrochloric acid removed under reduced pressure. An attempt was made to separate the glutamic and aspartic acids by ion exchange but it was not possible to obtain radiochemically pure amino acids. Paper chromatography with phenol finally enabled the isolation of pure glutamic and aspartic acids. Additional work is being done on methods to isolate the amino acids by ion-exchange chromatography.

*Consultant.
**ORINS Research Participant.
***NRC-AEC Postdoctoral Fellow.

Glutamic acid degradation. (Mosbach, Phares, of Microbiology; Noggle) The carbon-14-labeled glutamic acid was degraded according to the method of Mosbach, Phares, and Long (Federation Proc., 10: 226-227, 1951). The specific activities of the carbon atoms in the glutamic acid are as follows.

$$\left.\begin{array}{ll} \text{COOH} & 0.196 \\ \text{HC-NH}_2 & 0.121 \\ \text{H-C-H} & 0.129 \\ \text{H-C-H} & 0.101 \\ \text{COOH} & 0.114 \end{array}\right\} \frac{0.661}{5} = 0.132 \text{ cts./sec./mg } BaCO_3$$

An aliquot of the glutamic acid was oxidized and the total activity of the sample was found to be 0.126 cts./sec./mg $BaCO_3$.

It is obvious that the glutamic acid is not uniformly labeled. The sample was obtained from plant material that had been in contact with $C^{14}O_2$ for at least 24 hours. Additional work is being done to investigate the distribution of tracer carbon in glutamic acid isolated from plants exposed to $C^{14}O_2$ for varying periods of time both in the light and in the dark.

Ascorbic acid metabolism of plants. (Noggle, Cheniae) The first phase of a newly initiated investigation of ascorbic acid metabolism in plants is concerned with normal variation of the acid concentration in plant tissue. Inasmuch as ascorbic acid is dependent upon light, temperature, and nutritional level and age of the plant, all these variables come in for scrutiny. Some work is also being done in identification of the precursors of ascorbic acid in plant tissue.

Physiology of Lilium longiflorum var. Floridii anthers. (Noggle, Schumacher) Preparation of a phosphatase enzyme source for use in phosphatase studies carried out with *Lilium longiflorum* var. Floridii was made as follows:

> Flower buds were removed from greenhouse plants and their bud length measured. Anthers were removed and their length recorded, after which the anthers (six from each single bud) were extracted with toluenated water in a glass homogenizer. The volume was made up to 10 ml and the extract centrifuged. The supernatant was decanted off and used as the source on a number of substrates.

Phosphatase activity was measured by the following method. A mixture of 8 ml of buffer, 1 ml of substrate, and 1 ml of enzyme solution was incubated for 6 hours at 30° C; inorganic phosphate was determined colorimetrically. Substrates used were —

0.1 *M* sodium β-glycerophosphate

0.01 *M* sodium pyrophosphate

0.01 *M* adenylic acid

100 mg/50 cc of sodium yeast nucleic acid (Schwartz).

Preliminary tests indicated acid phosphatase activity with a *p*H optimum of about 4.5 to 5.0. For all subsequent tests an acetate buffer at *p*H 5.0 was used.

Results of this experiment are shown in Fig. 8. Yeast nucleic acid was a poor substrate and the values for this material are not given. Also shown in the figure are data indicating the cytological condition of the sporogenous tissue of the anthers. These data are taken from Quarterly Report, ORNL-807, p. 65. Activity of adenylic acid and sodium pyrophosphate phosphatase decreased until the time of divisions I and II; there followed a slight increase in phosphatase activity coinciding with the time of tetrad formation. This, in turn, was followed by a decrease in the phosphatase activity. In the β-glycerophosphatase, there was a decrease in activity until the time of divisions I and II, followed by an increase in phosphatase activity at about the time of microspore formation. Additional studies for determination of the significance, if any, of these differences in phosphatase activity are under way.

Esterase, lipase, saccharase, and protease activities are also being investigated in the lily anther.

Radiation protection studies with rye seeds. (Bangson) Certified Rosen rye seed, being used in this study, is sown in flats of sand after treatment. In the majority of experiments X-ray dosages of 2500, 5000, 10,000, 20,000, and 40,000 r were administered, although some of the dry and some of the water-immersed controls were treated with 1250, 60,000, and 80,000 r. Seeds were treated in lots of 100. All experiments were done at room temperature.

To date, forty-three experiments, including control, have been undertaken. Part of the results of these experiments are summarized in Table 17. Additional experiments are under way employing different oxygen and nitrogen tensions at 2° C.

Figure 8

O - sodium β-glycerophosphate

▲ - sodium pyrophosphate

● - adenylic acid

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PHOSPHATASE ACTIVITY OF LILY ANTHERS

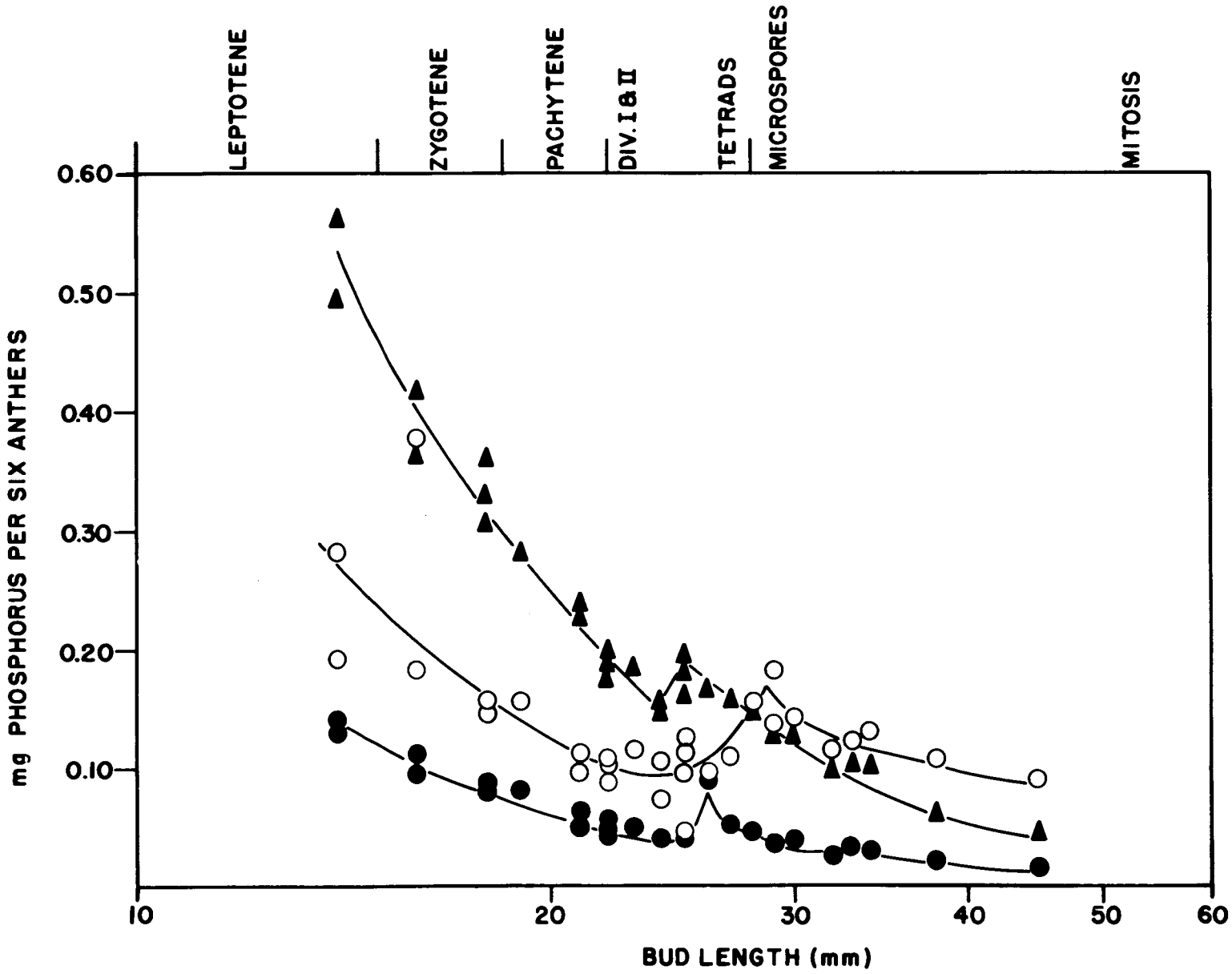

FIG. 8

TABLE 17

Per cent germination of seeds immersed in tapwater, ethyl alcohol, ethylene glycol, and BAL during irradiation

DOSAGE	DRY	SEEDS IMMERSED DURING IRRADIATION IN -										
		TAP-WATER	ETHYL ALCOHOL				ETHYLENE GLYCOL				BAL	
			5%	10%	15%	20%	5%	10%	15%	20%	0.004 *M*	0.04 *M*
1250	89	92	—	—	—	—	—	—	—	—	—	—
2500	97	92	92	91	93	98	87	91	90	95	92	97
5000	88	93	91	94	95	91	93	89	89	97	87	87
10,000	77	90	82	89	95	90	86	94	85	91	90	94
20,000	84	80	41	86	87	80	46	79	76	79	75	79
40,000	84	64	34	75	70	61	24	77	55	80	65	84
60,000	80	63	—	—	—	—	—	—	—	—	—	—
80,000	84	70	—	—	—	—	—	—	—	—	—	—