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# BIOLOGY DIVISION

# SEMIANNUAL PROGRESS REPORT

# FOR PERIOD ENDING FEBRUARY 15, 1958

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# BIOLOGY DIVISION

# SEMIANNUAL PROGRESS REPORT

**For Period Ending February 15, 1958**

Alexander Hollaender, Director

Stanley F. Carson, Assistant Director

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## CONTENTS


CYTOLOGY AND GENETICS ..... 1

Cytogenetic Effects of Radiation ..... 1

Postirradiation Modification of Ultraviolet-Induced Mutations by Streptomycin ..... 1

The Fate of Metaphase Aberrations ..... 2

Effect of Chloramphenicol on Chromosome Break Rejoining ..... 3

Somatic Variation of A Antigen in Human Erythrocytes ..... 4

Factors Influencing the Frequency of Recombination in the *ad-3* Region of *Neurospora* ..... 6

Quantitative Estimates of Radiation-Induced Forward-Mutation Rates at the *ad-3A* and *ad-3B* Loci in *Neurospora* ..... 8

Patterns of Interaction Between X Rays and Chemicals as Studied in Terms of Genetic and Toxic Effects in *Neurospora* ..... 10

Mutational Pattern of $c^m$, a Highly Mutable Gene in Maize ..... 11

Induced Mutation from Ultraviolet Irradiation of Bacteriophage ..... 12

Karyokinesis Without Cytokinesis in the Grasshopper ..... 13

Insect Cytology and Genetics ..... 13

Influence of the Nucleolus on Mitosis as Revealed by Ultraviolet Microbeam Irradiation ..... 13

Analysis of Chromosomal Translocations in *Habrobracon* ..... 15

*Drosophila* Genetics ..... 16

Sex-Linked Recessive Lethals in *Drosophila melanogaster* That Are Suppressed by the Y Chromosome ..... 16

New Combinations of the X and Y Chromosomes in *Drosophila melanogaster* ..... 18

Birth Order, Parental Ages, and Sex of Offspring ..... 18

Conference on Computing Methods ..... 19

MAMMALIAN GENETICS AND DEVELOPMENT ..... 21

Genetic and Developmental Effects of Radiation in Mice ..... 21

Effects of Chronic Gamma Irradiation on Male Fertility ..... 21

Influence of Dose Rate on the Sterilizing Effect of Radiation in Female Mice ..... 22

Effect of X Rays on the Mouse Ovary ..... 24

Pseudoallelic Recessive Lethals at the *Notch* Locus of *Drosophila melanogaster* ..... 26

MICROBIAL PROTECTION AND RECOVERY ..... 27

Radiation Protection and Recovery in Bacteria ..... 27

X-Ray Sensitivity of Synchronous Cultures of *Escherichia coli* ..... 27

An Aftereffect of Ionizing Radiation on a Mutant of *Escherichia coli* ..... 28

Oxygen Effect in X-Ray Inactivation of *Escherichia coli* $H_7$ ..... 29

MAMMALIAN RECOVERY ..... 31

Survival of Mouse-Grown Rat Erythrocytes ..... 31

Lymphatic Tissue Changes in Lethally Irradiated Mice Given Spleen Cells Intravenously ..... 32

Effect of High Protein Diet on the Foreign Bone Marrow Reaction ..... 33

Bone Marrow Cell Separation ..... 33

Effect of Fetal and Newborn Hematopoietic Tissue from Homologous and Heterologous Donors on Long-Term Survival of Lethally Irradiated Mice ..... 34

Effect of Delayed Injection of Foreign Adult Bone Marrow on Long-Term Survival of Lethally Irradiated Mice ..... 34
Method for the Determination of Rat Erythrocytes in the Mouse-Rat Chimera ..... 35

RADIATION IMMUNOLOGY ..... 37

Primary Antibody Response to a Distantly Related Heterologous Antigen During Maximum Depression Period Following Varying Doses of X Irradiation ..... 37
Antigens Responsible for Bone Marrow Transplantation Immunity ..... 37
Immune Status of Long-Term Survivors of X-Irradiated Mice Protected with Isologous, Homologous, or Heterologous Bone Marrow ..... 39
Erythrocyte Antigens as Markers for Repopulation by Homologous Erythropoietic Tissues in Irradiated Mice ..... 40

PATHOLOGY AND PHYSIOLOGY ..... 44

Long-Term Survival of Mice Protected with Isologous Bone Marrow and *S*,2-Aminoethylisothiuronium Bromide Hydrobromide ..... 44
Enhancing Action of Tissue Filtrates on Induction of Leukemia by X Rays ..... 45
Electrophoresis of Proteins of Irradiated Mouse Chimeras ..... 46
Paper Chromatography of Whole Tissue from Mice of Varying Ages ..... 48
Electrophysiological Changes in Irradiated Striated Muscle ..... 48
Relative Biological Effectiveness of X Rays, Gamma Rays, and Fast Neutrons in the Production of Weight Loss of the Small Intestine of the Mouse ..... 49
Strain Differences in Susceptibility of Mice to Radiation-Induced Leukemia ..... 51
Life Span of Rat Erythrocytes ..... 52

CELL PHYSIOLOGY ..... 53

Structure Formation by Liver Protein Solutions ..... 53
Amino Acids and Peptides of Rat Liver Microsomes ..... 53
Studies on the Metabolism of Radioiodine-Labeled Tobacco Mosaic Virus in the Mouse and Rat ..... 55

BIOCHEMISTRY ..... 58

Nucleic Acids, Enzymes, and Radiation-Protective Compounds ..... 58
Minor Constituents of Ribonucleic Acids ..... 58
RNA-Phosphorus Metabolism in *Escherichia coli* B ..... 58
RNA Turnover and DNA Synthesis in Bacteriophage-Infected Bacteria ..... 59
Structure and Activity in Radiation-Protective Sulfhydryl Compounds ..... 59
Hydrolysis of Carboxyalkylisothioureas by Transamidinase and Arginase ..... 60

ENZYMOLOGY AND PHOTOSYNTHESIS ..... 61

Purification of the Leucine-Activating Enzyme from *Escherichia coli* ..... 61
Glycine Activation by Extracts of *Photobacterium fischeri* ..... 61
Studies on the Synthesis of Ornithine Transcarbamylase by *Escherichia coli* ..... 62
Studies on the Relation Between Ribonucleic Acid and Protein Synthesis ..... 63
Catalysis of the Formation of Amino Acid Hydroxamates by Extracts of Beef Tissue ..... 63
Photosynthesis ..... 64

MICROBIOLOGY ..... 65
Tracer Studies on Intermediary Metabolism ..... 65
A Bacterial "Carbon Dioxide Enzyme" ..... 65
Properties of a Four-Electron Transfer DPNH Oxidase from *Clostridium perfringens* ..... 65

PLANT BIOCHEMISTRY ..... 67
Aging in Gamma-Irradiated Wheat Seedlings ..... 67
Serine Biosynthesis in Plants During Photosynthesis ..... 68
Effects of Gibberellic Acid, Kinetin, and Light on the Germination of Lettuce Seed ..... 68
Distribution of Radioactivity Among Compounds Extracted from Lettuce Seeds Germinated in the Presence of $HC^{14}O_3^-$, $S^{35}O_4^{--}$, or $H_2P^{32}O_4^-$ ..... 69

BIOPHYSICS ..... 71
Attempts to Photoreactivate Enzymes in Vitro ..... 71
Responses of Salamander Thyroids to TSH Administration ..... 71
Free Radicals Induced in Amino Acids by Radiation ..... 72

GLOSSARY ..... 74

PUBLICATIONS AND LECTURES ..... 75
Publications ..... 75
Scientific Society Lectures and Traveling Seminars ..... 79
Visiting Lecturers ..... 82
Speakers at Professional Meetings, Spring 1958 ..... 84
Eleventh Annual Biology Research Conference ..... 85
ORNL Advisory Committee for Biology ..... 86
Bone Marrow and AET Conference ..... 86
Education ..... 87

# BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

## CYTOLOGY AND GENETICS

### CYTOGENETIC EFFECTS OF RADIATION


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S. L. Scheinberg M. L. Patteson
S. M. Wilson


#### Postirradiation Modification of Ultraviolet-Induced Mutations by Streptomycin


R. F. Kimball N. Gaither
S. M. Wilson


**Introduction.** – It has been shown[3] that the number of recessive lethal mutations induced in *Paramecium aurelia* by alpha particles as well as by x rays is decreased by posttreatment with streptomycin. It was desirable to find out whether this same phenomenon would be found in the case of a nonionizing mutagenic radiation. For this purpose, 2537-Å ultraviolet radiation from a germicidal lamp was used. Early in the work it became obvious that ultraviolet dose curves would have to be obtained, because the streptomycin effect seemed to vary with dose. Dose curves were obtained with x rays for comparison.

The methods of x irradiation, posttreatment with streptomycin, and detection of recessive lethal mutations have been given in several previous semiannual reports. The ultraviolet source was a G-E 15-w germicidal lamp, and the energy incident on the small drops containing the paramecia was estimated to be approximately 40 $ergs \cdot mm^{-2} \cdot sec^{-1}$.

**Results.** – The data are plotted in Figs. 1 and 2. A transformation of the percentage frequency of normal clones was plotted because standard errors were readily available for the data in this form. The mean percentage frequencies are given in parentheses beside each point for comparison with previous reports. The x-irradiation data are consistent with the view that streptomycin simply reduces the effective dose, but the ultraviolet-irradiation data show more complex relations. It has been suggested[4] that streptomycin decreases mutation by allowing more time for spontaneous recovery from the radiation-initiated mutation process. Ultraviolet radiation, like streptomycin, slows growth and division, and it seems probable that it has a similar effect on mutation. X rays have very little effect on growth and division in the dose range used in mutation studies. Thus the complexity of the ultraviolet dose curves could be the result of two opposing actions of this radiation, mutagenic and recovery, whereas x rays act almost entirely as a mutagen and have little recovery action within the dose range used.

[1]On leave; Guggenheim Fellowship.

[2]Visiting investigator from abroad.

[3]R. F. Kimball, N. Gaither, and S. M. Wilson, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 19–20.

[4]R. F. Kimball, N. Gaither, and S. M. Wilson, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 13–14.

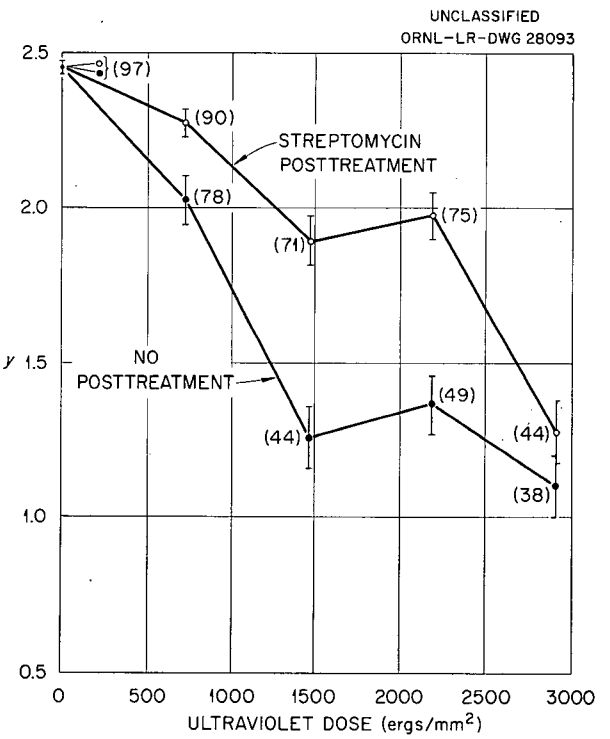


**Fig. 1. Dose Curves for Ultraviolet Irradiation With and Without Streptomycin Posttreatment.**

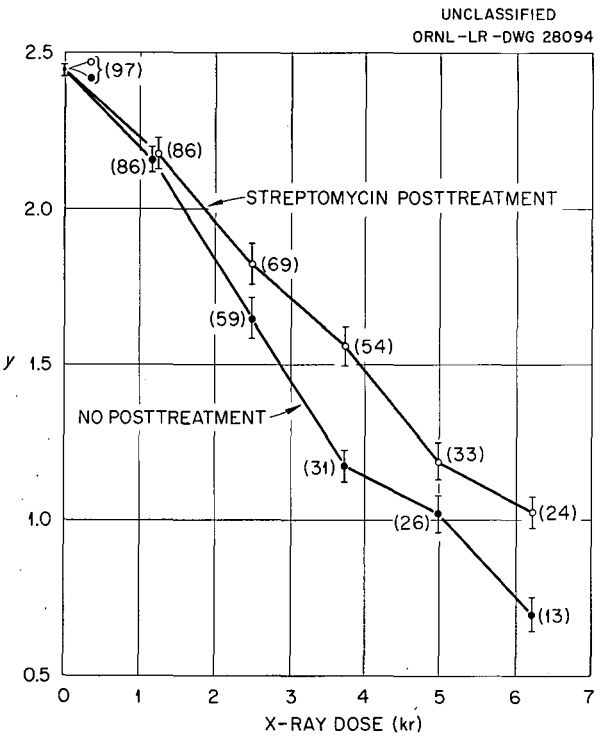


**Fig. 2. Dose Curves for X Irradiation With and Without Streptomycin Posttreatment.**

It has been known for many years[5] that the dose curve for ultraviolet-induced mutations reaches a maximum and even declines at higher doses, and it is possible that a similar explanation can be applied. In any event, it is obvious that the physiological effects of radiation must be taken into account in interpreting mutation data, since it has now been shown by several investigators[6] that the physiological state of the cell after irradiation can have important consequences for the amount of mutation produced.

### The Fate of Metaphase Aberrations

A. D. Conger H. J. Luippold

**Introduction.** – Radiation-induced chromosomal damage is commonly measured by two means. In favorable material, all aberrations can be observed directly at metaphase. In less favorable material where direct observation at metaphase is too difficult or impossible, bridges and fragments at anaphase are observed. The assumption is made that a constant proportion of the important exchange aberrations (dicentrics and rings) produce bridges at anaphase. Wolff[7] has recently shown that this is not true, at least in barley; he found that metaphase exchanges increased as the square of the dose, but that anaphase bridges increased linearly with dose, suggesting that the often-used observation of anaphase bridges is an unreliable indicator of the true level of chromosomal damage. The anaphase fate of exchanges is important, for bridge formation at anaphase by exchanges is believed to be one of the significant contributors to cell death from irradiation.

Metaphase exchanges (dicentrics and rings) could fail to make detectable bridges at anaphase by two mechanisms: (1) the exchanges divide in a "fall-free" manner, thus making no bridge, or (2) bridges are formed, but break. Either or both mechanisms could explain the discrepancy between metaphase exchanges and anaphase bridges, but in most materials it is impossible to analyze all exchanges in both metaphase and early anaphase and to tell the manner of separation. It can be done in *Tradescantia*, however.

**Results.** – *Tradescantia* was x-irradiated with a series of doses from 0 to 400 r, and in each slide, observations were made of cells in metaphase (all aberrations), early anaphase (all aberrations, and also the separation of exchanges in a fall-free, criss-cross, or interlock manner), and at middle, late, and very late anaphase (bridges and fragments). The critical observation was the one of the cells in early anaphase, where all exchanges could be observed, just as in metaphase, and also the proportion of them that separated so as to form bridges (criss-cross and interlock), as is observed in the customary analysis of "anaphase bridges."

Exchanges at metaphase and early anaphase, and bridges at early anaphase all increased as about the 1.5 power of the dose. About 90% as many exchanges were seen in early anaphase as at metaphase; the difference was probably due to a defect of detection, and not to a true loss. Of

[5] A. Hollaender and C. W. Emmons, *Cold Spring Harbor Symposia Quant. Biol.* 9, 179–86 (1941).

[6] A. Hollaender and G. E. Stapleton, *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955* 11, 311–14 (1956); A. Hollaender and R. F. Kimball, *Nature* 177, 726–30 (1956); E. M. Witkin, *Cold Spring Harbor Symposia Quant. Biol.* 21, 123–40 (1956).

[7] S. Wolff and H. E. Luippold, *Nature* 179, 208–9 (1957).

a total of 470 dicentric and ring exchanges observed at early anaphase, only 55% separated so as to form bridges; the others were fall-free, and this proportion was approximately constant at all doses. The frequency of bridges decreased at the progressively later stages of anaphase, and varied approximately linearly with dose. Data on bridges for the later stages of anaphase are not accurate enough to determine whether bridges increased linearly or somewhat more rapidly with dose, but at these later stages, bridges definitely did not increase as rapidly with dose as did exchanges and bridges at metaphase and early anaphase.

**Discussion.** – The observed discrepancy between metaphase exchanges, which increase about as the square of the dose, and anaphase bridges as commonly observed, which increase approximately linearly with dose, is thus not to be explained on the basis of an inconstant proportion of exchanges separating freely but is apparently due to the disappearance of bridges by breakage in the later stages of anaphase, the proportion breaking increasing with the dose. An association between restituted radiation-induced breaks and breakage of anaphase bridges could explain the results. Breaks increase proportionally to dose, and a large share of them show restitution. Hence, in every anaphase bridge, the number of restituted breaks is proportional to dose. If the restituted breakage points are weak points in the chromosome, then the breakage of anaphase bridges would increase proportionally to dose.

### Effect of Chloramphenicol on Chromosome Break Rejoining

S. Wolff H. E. Luippold

**Introduction.** – It has been observed in *Vicia* root tips[8] and in *Tradescantia* microspores[9] that the processes of chromosome breakage and rejoining are independent effects of x radiation. The postirradiation delay in rejoining has been found to be dose dependent[8] and also to be enhanced by treatments that inhibit the formation of ATP[10] (see Glossary). Since the doses of radiation that prevent rejoining are too low to have an appreciable effect on the oxidative metabolism of the cell, a radiation-sensitive "rejoining system" has been postulated.[11]

The present experiments were performed to investigate the effect of other metabolic inhibitors in an attempt to define the "rejoining system." The experimental procedure is similar to that utilized heretofore; that is, a dose of x radiation (600 r in vacuo) is administered to seeds of *Vicia faba* that have been soaked for 18 hr. This induces chromosome breaks that remain open for about one-half hour. Seventy-five minutes later a second dose of radiation (300 r in air) is administered to the same beans. If the breaks from the first dose have closed before the second irradiation, the two-hit aberration yield observed in the first mitotic cell division of the root tips will simply be the sum of those induced by the two doses. However, if immediately after the first dose a metabolic inhibitor that prevents restitution is applied, the two-hit aberration yield will be proportional to the square of the total dose.

**Results.** – In the experimental results presented in Table 1, it may be seen that 300 $\mu$g/ml of chloramphenicol prevents the rejoining of the breaks. In the present experiments, almost all the non-chloramphenicol-treated breaks rejoined before 75 min had elapsed. However, a few do remain capable of forming exchanges for at least this long. Thus the aberration yield for the nontreated beans is slightly higher than the additive amount. However, the application of chloramphenicol keeps all the breaks open so that the aberration yield reaches the value expected theoretically if no rejoining occurred within the 75-min period.

**Discussion.** – Chloramphenicol has been reported to inhibit protein synthesis in bacteria[12] and bacteriophage[13] systems. Until its effect has been checked in *Vicia* seeds, the present data constitute presumptive evidence that protein synthesis is necessary for chromosome-break rejoining. It is yet unknown if the "rejoining

[8] S. Wolff, *Nature* 173, 501 (1954).

[9] S. Wolff, *Genetics* (in press).

[10] S. Wolff and H. E. Luippold, *Science* 122, 231–32 (1955).

[11] S. Wolff and H. E. Luippold, *Progress in Radiobiology* (ed. by J. S. Mitchell, B. E. Holmes, and C. L. Smith), p 217–21, Oliver and Boyd, London, 1956.

[12] C. L. Wisseman, Jr., *et al.*, *J. Bacteriol.* 67, 662–73 (1954).

[13] L. Astrachan *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 76–77.

**Table 1. Effect of Chloramphenicol on Two-Hit Aberration Yield**

| Dose I (r, in vacuo) | Treatment During 75-min Interval | Dose II (r, in air) | Observed Aberration Yield (Number of 2-Hit Aberrations) / (Number of Cells Scored) | Observed Aberration Yield Percentage | Expected Aberration Yield With Rejoining | Expected Aberration Yield Without Rejoining |
|---|---|---|---|---|---|---|
| 600 | $H_2O$ | | 30/300 | 10.0 ± 1.8 | | |
| 600 | 300 μg/ml chloram | | 28/300 | 9.3 ± 1.8 | | |
| | $H_2O$ | 300 | 27/277 | 9.7 ± 1.9 | | |
| | 300 μg/ml chloram | 300 | 31/300 | 10.3 ± 1.9 | | |
| 600 | $H_2O$ | 300 | 112/432 | 25.9 ± 2.4 | 19.7 | 39.3 |
| 600 | 300 μg/ml chloram | 300 | 109/292 | 37.3 ± 3.6 | 19.7 | 39.3 |
| 600 | $H_2O$ | 300 | 82/295 | 27.8 ± 3.4 | 19.7 | 39.3 |
| 600 | 300 μg/ml chloram | 300 | 68/184 | 36.8 ± 4.5 | 19.7 | 39.3 |

system" itself is the system utilized for protein synthesis. If this were the case, it might mean that the chemical bonds formed during the rejoining of breaks are peptide bonds. An alternative explanation could be that protein synthesis is necessary to make enzymes that are used for the repair of the radiation-damaged "rejoining system."

### Somatic Variation of A Antigen in Human Erythrocytes

K. C. Atwood S. L. Scheinberg

**Introduction.** – The erythropoietic stem cells of the bone marrow form a sufficiently large population so that heterogeneity resulting from mutation is inevitable. Since mutant stem cells of certain genotypes would produce phenotypically exceptional erythrocytes, an examination of the peripheral blood for such exceptions has been undertaken. The first system examined is the human ABO locus, particularly with respect to loss of A antigen. Two phases of the problem are being studied: first, phenotypic characterization of the exceptional cells (EC) lacking an antigen possessed by the main population, and second, the estimation of the proportion of EC in different persons.

**Methods and Results. I.** – Cells from two $A_1B$ donors were isolated by means of their failure to agglutinate with lima bean anti-A lectin. The cells from 500 ml of blood were washed and resuspended in saline to a hematocrit of 50, then mixed with 125 ml of bean anti-A lectin in a large developing pan. After agglutination, the mixture was transferred to a cylinder, and the agglutinated material was separated by gravity. The supernatant was then concentrated by centrifugation in a Lucite cylinder spun on its axis and lined with Kel-F fluorocarbon oil while spinning. This procedure deposits the cells in a thin, friable layer, and is necessary to prevent entrapment of the EC in the cohesive pellets formed during ordinary centrifugation. As the cylinder comes to rest, fragmentation of the cell layer frees some of the agglutinable cells as well as the EC, so that entrapment is avoided at the cost of a somewhat less efficient separation. After gravity separation of the concentrated supernatant, agglutination was repeated in a smaller volume. Additional antibody was used, sufficient for strong agglutination at each stage, and the process was continued until a small volume of dilute cell suspension remained. The final removal of agglutinable cells was done over a layer of Kel-F in regular centrifuge tubes. The thin, disk-shaped pellets at this stage were easily broken into a mixture of free cells and small clumps, and separation was effected by a brief, low-speed centrifugation. After four to six such separations, the remaining cells were completely unagglutinable, even microscopically, when centrifuged from undiluted anti-A lectin. The final yield was a few milliliters of a 1% suspension. The EC were then tested against a variety of reagents

by a standard method (two drops of serum, plus one drop of cell suspension, centrifuged in a small test tube). The macroscopic score is obtained by tapping the tube in an effort to resuspend the pellet: pellet unbroken, 4; two or three pieces, 3; many fragments, 2; small fragments visible, 1; complete resuspension, 0. "Microscopic positive" denotes clumps present but not visible to the unaided eye. In Table 2 the reactions of the EC and the unselected cells are compared.

**Discussion. I.** – The strong reaction of EC with anti-B and with the unrelated MN reagents shows that EC differ only in allele specificity and do not exhibit an unspecific failure to agglutinate. The loss of A antigen is correlated with a gain in H, the antigen found predominantly on O cells, as shown by the reactions with *Ulex* anti-H lectin and with eel serum. If the EC are the result of somatic mutation, it is reasonable to suppose that their reactions would parallel those of OB individuals carrying a similar B allele. Several B's tested have reacted strongly with anti-H; hence the increase of H in EC is compatible with a mutational origin, A → O. The alteration of the A allele in the direction of O is often incomplete, as evidenced by the partial agglutination of EC by commercial anti-A typing serum. The proportion of EC showing this unexpected behavior is about 0.5 for one donor and appreciably less for the other. Evidently some of the EC react with an antibody which is present in commercial anti-A but absent in lima bean lectin. Since the lectin agglutinates $A_1$ and $A_2$ cells, while the commercial serum is negative with all O, the antibody in question is neither anti-$A_1$, anti-$A_2$, nor anti-H. If germinal mutation produces the same array of variants as that observed somatically, a population survey would be expected to

**Table 2. Comparison of Reactions of Exceptional and Unselected Cells**

| Antibody | Donor 1 – K.C.A. | | Donor 2 – J.O. | |
|---|---|---|---|---|
| | Exceptional Cells | Unselected Cells | Exceptional Cells | Unselected Cells |
| Lima bean anti-A | 0 (–) | 4 | 0 (–) | 4 |
| C.D.S. anti-A (commercial typing serum) | 0 (++) | 4 | 0 (+) | 4 |
| C.D.S. anti-$A_1$ | 0 (–) | 3 | 0 (–) | 1 |
| *Dolichos* lectin, anti-$A_1$ | 0 (–) | 4 | 0 (–) | 4 |
| B plasmas | | | | |
| E.D. | 0 (+) | 4 | 0 (+) | 4 |
| P.S. | 1 | 4 | 0 (+) | 3 |
| F.C. | 0 (+) | 3 | 0 (+) | 3 |
| O plasma (S.L.S.) absorbed with B cells from E.D. | 2 | 4 | 3 | 4 |
| O plasma as above, reabsorbed with P.S. cells | 2 | 4 | 0 (+) | 4 |
| O plasma as above, reabsorbed with $A_2$ cells | 1 | 3 | 0 (+) | 4 |
| Lima bean antibody eluted from $A_2$ cells | 1 | 4 | 0 (–) | 4 |
| C.D.S. anti-B | 4 | 4 | 4 | 4 |
| C.D.S. anti-M | 0 | 0 | 3 | 3 |
| *Vicia graminia* anti-N | 4 | 4 | 4 | 4 |
| *Ulex* anti-H | 3 | 0 (+) | 2 | 1 |
| Eel serum anti-H | 3 | 2 | 4 | 1 |
| K.C.A. plasma | 0 (–) | 0 (–) | 0 (–) | 0 (–) |
| J.O. plasma | 0 (–) | 0 (–) | 2 | 0 (–) |

reveal an A allele distinguished by failure to react with lima bean lectin, possibly one of the known rare A alleles. The cells showing the anomalous A reaction also react more strongly with anti-H than do the initial cells, a behavior consistent with that of known weak A alleles. A small proportion of EC from both donors failed to react with either commercial anti-A or *Ulex*. Possibly these cells are showing the phenotype of homozygous B, which might be expected to have less H substance than does the heterozygote. A most surprising result is the agglutination of EC from the second donor by her own plasma. The plasma was inactive on the initial populations and on the EC of the first donor, while the first donor's plasma was inactive in all combinations. This remarkable reaction is completely inhibited by the lima bean extract, even after all its anti-A activity has been absorbed out by the initial cells. The inhibition suggests a soluble antigen in the bean extract that resembles an antigen found only on the reactive EC, but the existence of such a system is inexplicable at present. In summary, the reactions of EC selected from $A_1B$ populations on the basis of failure to agglutinate with bean anti-A are consistent with the notion that the EC arise in part by mutation of A to O, in part by mutation to one or more suballeles of A, and to a much lesser extent by the production of homozygous B by mutation or mitotic crossing over.

**Methods and Results. II.** – The estimation of the proportion of EC in an individual is hindered by the strong dependence of the agglutination reaction on cell concentration. The settling of agglutinated cells depletes the supernatant so that the reaction will not go to completion unless the cells are packed by centrifugation. Efficient packing, however, leads to entrapment of the EC. These difficulties are avoided by an isotope dilution method. Several milliliters of cells are labeled with $Cr^{51}$ and agglutinated. After gravity settling, the supernatant is removed and restored to its original concentration by the addition of unlabeled cells. The agglutination is repeated, more unlabeled cells are added to the second supernatant, etc. Samples are removed at each stage for determination of the $Cr^{51}$ activity, and when the activity becomes constant, it is clear that the remaining labeled cells are not participating in the agglutinations. The ratio of the final to the initial activities, with dilutions and hemolysis taken into account, gives the proportion of EC. Two AB and five A bloods have been successfully scored by this method, and all had EC in the neighborhood of 0.001, with the exception of one A blood in which the final count was negligible.

**Discussion. II.** – Quantitative estimation of EC in a much larger number of individuals will be necessary before definite conclusions can be drawn, but an unexpectedly high proportion is already evident. If the EC arise by mutation, the proportions already found most likely represent an accumulation of mutant stem cells throughout the life of an individual. The relation of EC to age is therefore one of the most important lines of evidence bearing on their origin. A linear increase with age is expected on the mutation hypothesis, and the present fragmentary results can be explained by a mutation rate of about $3 \times 10^{-9}$ per hour. A second prediction of the mutation hypothesis is that the proportion of EC in homozygotes should be negligible in comparison with that in heterozygotes. Finally, mutagenic agents should increase the proportion of EC. The main alternative hypothesis for explaining the presence of EC is that the amount of antigen formed by cells of uniform genotype may have a large variance, the EC representing one extreme of the distribution. The criterion of age dependence offers the best chance of evaluating these alternatives when more data are available.

## Factors Influencing the Frequency of Recombination in the *ad-3* Region of *Neurospora*

F. J. de Serres

**Introduction.** – Data from crosses described previously[14] have shown that a group of 11 *ad-3A* mutants gave identical linkage relations in crosses with a strain carrying the closely linked markers *hist-2* and *nic-2*. However, when these same mutants were crossed to more closely linked markers at the *ad-3B* locus, a marked difference was observed in the linkage relations in the *ad-3A–ad-3B* region, depending on the *ad-3B* mutant used. The data indicated that the frequency of recombination in the *ad-3A–ad-3B*

[14] F. J. de Serres, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 26–27.

region obtained from crosses between *ad-3A* and *ad-3B* mutants derived from the same wild-type strains (*ad-3A* × *ad-3B* or *ad-3A′* × *ad-3B′*) was lower than that obtained from intercrosses between the two groups of mutants (*ad-3A′* × *ad-3B* or *ad-3A* × *ad-3B′*). The data suggested that there was less recombination in the *ad-3A–ad-3B* region when homologous chromosomes were derived from the same wild-type strain than when homologous chromosomes were derived from different wild-type strains. Since it seems reasonable to assume that there is some heterogeneity in the structure of homologous chromosomes in various wild-type strains, the working hypothesis developed from the data predicted less recombination between two markers when the structure of the intervening region on homologous chromosomes was homogeneous than when it was heterogeneous. To test this hypothesis, this same group of *ad-3A* mutants was crossed to another strain carrying closely linked markers related in origin to only one group of the 11 *ad-3A* mutants under study.

Of the *ad-3A* mutants used, A1, A2, A4, A5, A6, A7, A8, and A10 were derived from wild-type strain 74A; 38701 and 38709, from a cross of wild-type strains 1A and 25a; and 68306 from a cross of wild-type strains Abb 4A and 25a. Two of the markers, *ragged* (*rg*) and *hist-3* (located to the left of *ad-3A*), were derived from wild-type strain 74A, and the other marker *nic-2* from a cross of wild-type strains Abb 4A and Abb 12a. The same isolates of all 11 *ad-3A* mutants as used in previous experiments[14,15] were crossed to a strain carrying the markers *rg hist-3 nic-2*. Since two crossovers were known to have occurred in the *ad-3A–nic-2* region in the transfer of the *nic-2* marker from the *hist-2 nic-2* strain, described previously,[14] to the present strain, a portion of this region contains chromosomal material derived from wild-type strain 74A. If the working hypothesis were correct, it would be expected (1) that the *ad-3A* mutants derived from 74A would give less recombination with *rg* and *hist-3* than would mutants 38701, 38709, and 68306, and (2) that *ad-3A* mutants derived from 74A would give less than 3.0% recombination with *nic-2*, whereas the behavior of 38701, 38709, and 68306 for this same region was unpredictable.

**Results.** – The data from the plating analyses of the crosses described above are given in Table 3. Data on the frequency of recombination

[15] F. J. de Serres, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 19.

**Table 3. Recombination Frequencies in the *ragged–hist-3*, *hist-3–ad-3A*, and *ad-3A–nic-2* Regions Obtained from Crosses of *ad-3A* Mutants of Various Origins with a Strain Carrying the Markers *ragged hist-3 nic-2***

| Strain | Region | | |
|---|---|---|---|
| | *ragged–hist-3* | *hist-3–ad-3A* | *ad-3A–nic-2* |
| A1 | | 0.48 ± 0.07 | 2.66 ± 0.29 |
| A2 | 1.2 ± 0.3 | 0.46 ± 0.04 | 2.44 ± 0.17 |
| A4 | 1.4 ± 0.4 | 0.42 ± 0.05 | 2.50 ± 0.25 |
| A5 | | 0.47 ± 0.07 | 2.40 ± 0.29 |
| A6 | | 0.41 ± 0.06 | 2.24 ± 0.25 |
| A7 | | 0.33 ± 0.05 | 2.73 ± 0.24 |
| A8 | | 0.50 ± 0.06 | 2.62 ± 0.22 |
| A10 | 1.6 ± 0.5 | 0.50 ± 0.07 | 2.40 ± 0.26 |
| 38701 | | 1.18 ± 0.10 | 5.51 ± 0.38 |
| 38709 | 5.1 ± 0.8 | 1.09 ± 0.09 | 5.55 ± 0.39 |
| 68306 | | 0.51 ± 0.06 | 4.59 ± 0.34 |
| 74A | 1.9 ± 0.6 | | |

in the *rg–hist-3* region were obtained from only a portion of the crosses. The data from a cross of 74A to the marked strain are included for comparison with mutants A2, A4, and A10; 95% confidence limits are given for all recombination frequencies.

**Discussion.** – Although previous data from a cross of these 11 *ad-3A* mutants gave the same linkage relations with an unrelated *hist-2* marker (located 2.0 crossover units to the left of *ad-3A*), the present data indicate that *ad-3A* mutants derived from 74A show less recombination with other markers in this general region, which are related in origin, than do *ad-3A* mutants 38701 and 38709, which are unrelated in origin. Mutants A1 through A10 give a significant reduction in crossing over in the *ad-3A–nic-2* region. An average value of 3.04 ± 0.08 crossover units was obtained for this region from the crosses of these eight mutants to the *hist-2 nic-2* strain described above, whereas the data from the present crosses give an average value of 2.51 ± 0.08 crossover units. Mutants 38701, 38709, and 68306 gave a marked increase in recombination in this region; an average value of 2.90 ± 0.14 crossover units was obtained for this region in the crosses with the *hist-2 nic-2* strain. The marked difference in the estimates for this region in the two groups of crosses is most readily explained on the basis of the incorporation of 74A chromosomal material in the *ad-3A–nic-2* region in the synthesis of the *rg hist-3 nic-2* strain.

The response of *ad-3A* mutants of different origin in the present crosses is more striking if a comparison is made of the estimates of recombination in all regions at the same time. All mutants derived from 74A give the same estimates of linkage with each marker; mutants 38701 and 38709 give quite different estimates from the previous mutants but are identical with each other; the response of mutant 68306, however, provides a striking confirmation of the difference in the origin of this strain from either of the other groups of mutants.

These data and data from previous crosses[14,15] suggest that the linkage relations of closely linked markers are dependent upon factors other than the physical distance separating them on the chromosome. It seems quite unlikely that the physical distance between the markers is changed in these crosses, but rather that differences in the frequency of recombinants are due to a difference in the rate (or type) of exchange. From these data it appears that a greater number of chromosomes recombinant for a given region are recovered from a given cross when the homologous chromosomes are of diverse origin and are presumably heterogeneous in structure.

## Quantitative Estimates of Radiation-Induced Forward-Mutation Rates at the *ad-3A* and *ad-3B* Loci in *Neurospora*

F. J. de Serres H. G. Kølmark

**Introduction.** – The main difficulty in the estimation of forward-mutation (from wild type to mutant) rates at specific loci in microorganisms is the determination of the total number of specific mutations in a given population of cells after mutagenic treatment. In general, mutation rates are so low that the number of specific mutants cannot be readily determined by direct methods. An attempt has been made to determine forward-mutation rates at specific loci[16] by indirect methods, with the use of a technique which permits only a partial recovery of the induced mutants. An effort has been made to determine what proportion of the total mutants induced at each of these loci is recovered by means of such techniques in reconstruction experiments in which various mixtures of wild-type and mutant conidia are used. To circumvent the various assumptions which must be made in order to obtain estimates of the forward-mutation rates from the data obtained by such indirect methods, an experimental technique has been developed which permits the estimation of the forward-mutation rates of specific loci by direct methods.

The success of the present method depends upon the fact that certain mutants are morphologically distinct from wild type. In the case of *ad-3* mutants, a purple pigment is accumulated in the mycelium which readily distinguishes such mutants from wild type. In addition, the production of this pigment can be enhanced so that intermediate alleles, with only partial requirements for adenine, can also be recovered. Following mutagenic treatment, all conidia are grown on minimal medium supplemented with adenine, and

[16] V. W. Woodward, *Proc. Natl. Acad. Sci. U.S.* **42**, 752–58 (1956).

the purple mutants are recovered on the basis of the characteristic color of the mature colonies.

The fact that phenotypically identical *ad-3* mutants originate by mutation at two separate but closely linked loci[17] (individual mutants can be identified as being *ad-3A* or *ad-3B* by heterokaryon tests) permits a comparison of the mutation rates of two loci under the same conditions. The system also permits an investigation for specificity of mutagens, since a comparison can be made of the proportion of the two types of *ad-3* mutants after treatment of wild type with various types of mutagenic agents.

**Results.** – An experiment was performed in order to study the rates of forward mutation following various x-ray treatments by incubating five aliquots of a conidial suspension, one untreated and four irradiated with doses of 4.5, 18, 36, or 72 kr. The survival rates and the number of purple mutants obtained with each treatment of the five aliquots of conidia are given in Table 4. No mutants were recovered from untreated conidia, and 191 mutants were recovered from the four different x-ray treatments. The genotypes of individual purple mutants were determined in subsequent heterokaryon experiments by using *ad-3A* and *ad-3B* tester strains. Of the 191 purple mutants, 35 were *ad-3A* mutants and 156 were *ad-3B* mutants.

**Discussion.** – Since more purple mutants were recovered in this one experiment than in all other forward-mutation experiments combined,[18] it was of interest to determine whether all such mutants were either *ad-3A* mutants or *ad-3B* mutants. Since all the 191 mutants gave a positive heterokaryon test with one tester strain and a negative test with the other, all mutants were either *ad-3A* or *ad-3B*. No double *ad-3A ad-3B* mutants were recovered, and no evidence was obtained for the presence of other loci giving phenotypically purple mutants either in the *ad-3* region or elsewhere in the chromosome complement.

A comparison of the total numbers of *ad-3A* mutants and *ad-3B* mutants gives a ratio of 1:4.5. This difference in the recovery of the two types of mutants was not unexpected, since the combined data from numerous forward-mutation experiments with the "filtration-concentration" technique[18] indicated an excess of *ad-3B* mutants. The present experiment provides direct evidence for a greater sensitivity of the *ad-3B* locus to x radiation, as compared with the *ad-3A* locus. In Table 4 it can be seen that the induced rate of mutation of the *ad-3B* locus is about four to five times as high as that of the *ad-3A* locus at each dose level tested.

Although a microconidial strain was used in this experiment, the shape of the survival curve indicates that a proportion of the conidia was not uninucleate; thus the mutation rates for each locus were calculated on the basis of mutations per conidium, rather than in terms of mutations

[17] F. J. de Serres, *Genetics* 41, 668–76 (1956).

[18] F. J. de Serres, M. E. Case, N. J. Johnson, and N. H. Giles (unpublished).

**Table 4. Frequency of X-Ray-Induced Forward Mutations at the *ad-3A* and *ad-3B* Loci**

| Dose (kr) | Survival | | Total Purple Mutants | *ad-3A* Mutants | | | *ad-3B* Mutants | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of Conidia Analyzed | Percentage of Initial | | Number Found | Mutants per $10^6$ Conidia | | Number Found | Mutants per $10^6$ Conidia | |
| | | | | | Viable | Surviving | | Viable | Surviving |
| | $\times 10^6$ | | | | | | | | |
| None | 7.60 | 100.0 | 0 | 0 | | | 0 | | |
| 4.5 | 6.64 | 87.5 | 16 | 3 | 0.39 | 0.45 | 13 | 1.7 | 2.0 |
| 18 | 5.12 | 67.4 | 26 | 5 | 0.66 | 0.98 | 21 | 2.8 | 4.1 |
| 36 | 2.28 | 30.0 | 76 | 13 | 1.71 | 5.70 | 63 | 8.3 | 27.6 |
| 72 | 0.4 | 5.3 | 73 | 14 | 1.84 | 35.0 | 59 | 7.8 | 147.5 |

per nucleus or chromosome. By extension of the experimental points, the expected maximum forward-mutation rates with this strain at a dose of 55 kr are 9.7 and 2.1 per $10^6$ viable conidia treated for the *ad-3B* and *ad-3A* loci, respectively.

### Patterns of Interaction Between X Rays and Chemicals as Studied in Terms of Genetic and Toxic Effects in *Neurospora*

H. G. Kølmark

**Introduction.** – These experiments were initiated in order to study chemical protection against genetic and lethal effects of ionizing radiation in *Neurospora*. The strain of *Neurospora* used is derived from a strain, K 3/17, which was used earlier for the study of reverse mutations. It is macroconidiating and forms small colonies on plates. Further, it is adenineless (38701) and inositolless (37401). Both these loci can revert to a nonrequiring state. The number of reversions induced in the inositol locus by x rays is so low that significant results are difficult to obtain, and these data are not included in the present report. For a general outline of the *Neurospora* reverse mutation method, consult refs 19 and 20.

**Results.** – In recent years a considerable interest has been focused on AET (see Glossary) and chemically related compounds for use as protective agents. For recent work, consult refs 21, 22, and 23.

The following treatment conditions were used: MEG (see Glossary) 0.01–0.09 *M*, pH 7, with and without x rays, 60 kr. When AET is adjusted to pH 6 to 8, it is rapidly and quantitatively rearranged to form MEG.[23] Under these conditions a strong toxic effect was found. When conidia had been pretreated with MEG for 45 min and then irradiated with 60 kr, an increased mutagenic effect was found. Under these conditions, 0.09 *M* MEG plus 60 kr gave 13.2 mutations per $10^6$ survivors, as compared with 2.8 for x rays alone.

In all other conditions under which AET·Br·HBr has been tested, a considerably less toxic and less mutagenic effect was found if x rays had also been employed. In no case has AET or MEG shown any mutagenic effect when given alone.

Other conditions employed were as follows: chemical present as AET or MEG at pH 3, time of pretreatment varied, pretreatment performed under growth conditions. In general it can be said that hardly any significant protection has been observed under the conditions tested. Irradiation of MEG before adding it to the conidia did not result in any alterations of toxic or mutagenic properties.

A few other chemicals have been tested for possible protection against x rays:

*Sodium Hydrosulfite*, $Na_2S_2O_4$. – In concentrations from 0.004 to 0.064 *M*, this compound was found to be nontoxic and nonmutagenic. In combination with x rays, no increase in toxicity nor any protection was found, but there was possibly a slight increase in the mutagenic effect, as compared to x rays alone.

*Cysteamine*, $NH_2CH_2CH_2SH$. – With the chemical alone, in concentrations from 0.001 to 0.064 *M*, a toxic effect was found which increased with concentration. Also a slight mutagenic effect was noted. Combination with x rays resulted in a decreased survival and a rather strongly increased mutation rate.

*Chloroethyl Methanesulfonate*, $ClCH_2CH_2OSO_2CH_3$ *(Cl·EMS)*. – This chemical is highly active as an inducer of reverse mutations in the adenine locus 38701. There is, however, a lag period of several hours before any appreciable amount of mutations is induced. As the mutagenic effect in the adenine locus seems to be highly specific, it was thought that perhaps the chemical would have a tendency to occupy some critical point on the gene and thereby protect against x-ray-induced mutations. The experiment showed that the chemical gave no protection against x-ray-induced mutations during the lag period lasting about 7 hr. If the mutagenic effects of the two treatments, x ray and chemical, are independent of each other, it is to be expected that the combined treatment with these two agents would result in a mutation rate equal to the sum of the two single treatments. When irradiation is performed during the period in which the chemical is active in inducing mutations, that is, after the lag period, a decrease in mutation rate is found,

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[19] G. Kølmark and M. Westergaard, *Hereditas* 35, 490–506 (1949).

[20] K. A. Jensen, *Cold Spring Harbor Symposia Quant. Biol.* 16, 245–61 (1951).

[21] A. W. Kimball, W. T. Burnett, Jr., and D. G. Doherty, *Radiation Research* 7, 1–12 (1957).

[22] D. G. Doherty, W. T. Burnett, Jr., and R. Shapira, *Radiation Research* 7, 13–21 (1957).

[23] R. Shapira, D. G. Doherty, and W. T. Burnett, Jr., *Radiation Research* 7, 22–34 (1957).

as compared with the rate found for the chemical alone. Thus for the three experimental points at 13, 19, and 25 hr, the following is derived:

| | Hours of Treatment with Chloroethyl Methanesulfonate | | |
|---|---|---|---|
| | 13 | 19 | 25 |
| Number of reversions per $10^6$ survivors expected if mutagenic effects of chemical and x rays are additive | 37 | 79 | 147 |
| Number of reversions per $10^6$ survivors found | 18 | 23 | 33 |

X rays, thus, very markedly have the effect of decreasing the number of mutations induced by the chemical treatment.

**Discussion.** – It appears that different patterns of interaction between chemicals and x rays can be studied with the present system. The following represent some of these patterns:

1. A nonmutagenic chemical such as AET or sodium hydrosulfite becomes slightly mutagenic when combined with x rays.

2. A slightly mutagenic chemical, cysteamine, became strongly mutagenic in combination with x rays.

3. The mutagenic effect of x rays is decreased by a chemical. This decreasing effect has been demonstrated so far only to a very small extent in the present system. In a few instances a small decrease in toxic and mutagenic effect of x rays has been noticed when the conidia had been treated with a 0.01 *M* concentration of AET. This "protection" amounts to only a few per cent and is thus not very significant. It is becoming evident that protection with these chemicals gives widely varying results from one organism to another.

4. The strong mutagenic effect of a chemical, such as chloroethyl methanesulfonate, is decreased when combined with x rays. Apparently the decrease is not due to chemical protection against x-ray-induced mutations. This can be seen from the fact that the x-ray-induced mutations form the base line in the mutation curve (Fig. 3). On the contrary, it seemed that the opposite phenomenon was being dealt with: an x-ray interference with the mutagenic effect of the chemical. At present the observed phenomenon might be explained in either of two ways: (*a*) x rays are differentially killing the mutations newly induced by the chemical; (*b*) x rays are preventing or canceling the mutations by some kind of interaction during a nascent or intermediary state of the mutation process; this interaction might take place by interfering with a complex of the chemical and the gene, or by pushing the gene back into an original state during an intermediate unstable or excited period before the mutation has been established.

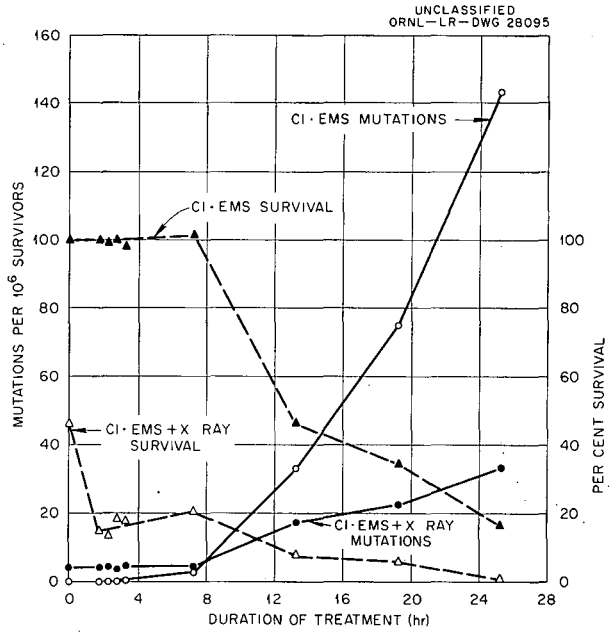


**Fig. 3. Alteration of the Mutagenic Effect of 0.1 *M* Cl·EMS by 45 kr of X Rays.** Time of pretreatment varied; 66 × $10^6$ viable conidia treated at each point on curve.

## Mutational Pattern of $c^m$, a Highly Mutable Gene in Maize

D. Schwartz M. L. Patteson

The previous semiannual report[24] contains a description of studies on $c^m$, a highly mutable gene in maize at the locus which governs the formation of anthocyanin pigmentation in the aleurone layer of the endosperm. The mutations to the stable dominant (*C*) condition and to the stable

[24] D. Schwartz and M. Patteson, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 30.

recessive ($c$) can occur in the sporophyte and gametophyte as well as in the endosperm. The mutational pattern of the $c^m$ gene is the subject of this paper.

In crosses of $c^m/c \times c/c^{♂}$, three distinct states of the mutable gene are found:

1. a late state in which almost 100% of the mutations occur only after fertilization;
2. an intermediate state in which up to about 10% of the kernels in the colored class show germinal mutations, appearing as self-colored kernels; these occur singly on the ears and represent mutations which occurred before fertilization; the remainder are variegated, with the mutations occurring after fertilization;
3. an early state in which the majority of the colored kernels show germinal mutations and appear in sectors on the ears; both singly occurring, self-colored, and variegated kernels are also found.

The variegated kernels are indicative of late postfertilization mutations. However, their behavior in crosses is quite divergent and is dependent upon the state of the ears from which they were picked. For the most part, the variegated seeds produce ears which show the mutational pattern exhibited by the parental ears. It is thus possible to select kernels showing only late mutations but which produce early mutating plants.

The three states of the mutable gene reflect the stage in ontogeny at which the gene begins to mutate. The $c^m$ gene is stable in the zygote, and no mutations occur here. This stable condition persists until some time during development when the gene becomes unstable and is free to mutate. At fertilization, those genes in the zygotes which have not yet mutated to either $C$ or to the stable $c$ revert to the stable condition, and the cycle is repeated. However, the unstable condition persists in the endosperm after fertilization, resulting in frequent somatic changes.

Preliminary experiments have indicated that by means of a high-temperature shock it is possible to knock the $c^m$ gene out of its stable state into the unstable condition where it is free to mutate. About 4% (13/300) of the variegated kernels from the intermediate ($c^m$ stable until about meiosis) class, heterozygous for a stable recessive $c$, produce ears that are either completely colorless or half self-colored and half colorless. These result from mutations to either $c$ or $C$ in the egg prior to fertilization so that there is a lack of correspondence between the genetic constitution of the embryo and the endosperm. The seeds which received the high-temperature shock gave 15% completely mutated ears (6/40). The difference is significant at the 1% level, with a chi-square value of 7.6. The temperature shock was administered by dropping the seeds through a quartz tube which was heated to approximately 1800°F. Similar experiments with infrared exposure of pollen grains showed no effect.

### Induced Mutation from Ultraviolet Irradiation of Bacteriophage

D. R. Krieg

**Introduction.** – An increased frequency of genetic variants, presumably induced mutants, has been found among the progeny of bacteriophage which were irradiated with ultraviolet light prior to infection of untreated bacteria. Other investigators using different systems have reported increased frequencies of variant phage after irradiation of infected bacteria, after growth of irradiated phage in separately irradiated bacteria, and even after growth of untreated phage in irradiated bacteria. Where irradiated phage were grown in unirradiated bacteria, either no mutations were induced[25,26] or only a small and possibly spurious increase was found.[27] It has not been established whether the variant phage originate by induced mutation or by some kind of recombination or interaction between the phage and the host bacterium. The system described here, in which phage-host recombination probably cannot occur, was designed to permit high doses to phage and to test whether irradiation of the host cell was necessary for induced mutation.

**Results.** – In each experiment a stock of an *r*II mutant of bacteriophage T4 was irradiated and then used to infect *Escherichia coli*, strain B, at a ratio of more than one phage particle per bacterium. Viable phage were produced (by multiplicity reactivation) from the inactivated phage; this progeny population was assayed for the frequency of $r^+$ phage, and the frequency of back-mutants was compared with that in the stock prior to

[25] J. J. Weigle, *Proc. Natl. Acad. Sci. U.S.* **39**, 628 (1953).

[26] R. Latarjet, *Acta Unio Intern. contra Cancrum* **10**, 136 (1954).

[27] E. S. Tessman, *Virology* **2**, 679 (1956).

treatment. In a set of experiments in which the frequency of back-mutants from *r*59 was increased fiftyfold by the treatment, evidence was obtained that growth of pre-existing $r^+$ phage is not especially favored under these conditions. The frequency of $r^+$ phage increased by only 20% in a similarly treated mixture of *r*59 and wild type, and among the progeny of unirradiated but otherwise similarly treated *r*59 phage the frequency rose by only 50%. In another set of parallel experiments for comparing back-mutation from six different mutants, the frequency of back-mutants from *r*59 was increased about tenfold to 27 per $10^8$. No induced mutants (fewer than 1 per $10^8$) were found from three similarly treated stocks of mutants which have never been known to spontaneously back-mutate. The frequency of $r^+$ phage from two other mutants rose by about 50% from 2 and 3 per $10^6$ in the untreated stock.

**Discussion.** – These preliminary experiments indicate that irradiation of extracellular phage particles can induce mutations. This has been most clearly demonstrated by back-mutation from an *r* mutant with a very low spontaneous reversion frequency. Mutants which are apparently incapable of spontaneous back-mutation (small deficiencies?) may also be insensitive to induced back-mutation. Materials and technics have been prepared to test these working hypotheses and to investigate the role of multiplicity reactivation in this system.

### Karyokinesis Without Cytokinesis in the Grasshopper

M. Mota

**Introduction and Results.** – Preparations of living grasshopper spermatocytes of the species *Melanoplus differentialis* and *Chortophaga viridifasciata* (De Geer) were made in order to provide material for an experimental study of the anaphase movement. Among these preparations many cells were found in which cytokinesis had not followed karyokinesis; this resulted in binucleate cells from both primary and secondary spermatocytes.

In some of these cells a clearly visible ring of jelly-like substance, brighter under the phase microscope than the surrounding cytoplasm, could be seen constricting the middle of the bunch of mitochondria that outline the spindle remnants, very much in the same way that the cleavage furrow does in a normal cytokinesis. The ring did not last under observation more than 15 min, breaking and fading away after that period of time or earlier.

**Discussion.** – The origin of this aberration is unknown but cannot be attributed to the technique, since it has been seen immediately after the preparations were made. The very few minutes taken between the excision of the testis and the observations would not be time enough to cause such a change. The constriction of the middle of the spindle remnants (even in cases where the ring was no longer visible) in perfectly round cells makes it impossible to explain the aberration as being caused by mechanical damage at the time of making the preparation.

The existence of such a ring gives support to the theories that explain cell cleavage, in which the existence of a contracting ring located on or immediately under the cell membrane at the equator is assumed. The existence of such a ring had been inferred from indirect evidence, but it seems evident that in these cases the ring not only contracted but separated from the cell membrane.

## INSECT CYTOLOGY AND GENETICS

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### Influence of the Nucleolus on Mitosis as Revealed by Ultraviolet Microbeam Irradiation

M. E. Gaulden R. P. Perry

**Introduction.** – The nucleolus is a structure found in most plant and animal nuclei. Although its morphological characteristics and cyclic changes during mitosis have been much studied and its biochemical properties explored, its function has remained a mystery. Incidental observations by several workers have suggested a relationship between nucleoli and mitotic activity of cells. An excellent tool for determining whether such a relationship does in fact exist is the ultraviolet microbeam, which makes possible selective irradiation of cell structures. A microbeam was constructed and was used to irradiate the nucleolus and nonnucleolar portions of the nucleus of the

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grasshopper neuroblast in vitro. This cell is large (about 25 $\mu$ in diameter) and has two nucleoli, approximately 3 $\mu$ in diameter, that rarely fuse. Irradiated and unirradiated cells were individually followed by microscopic observation to determine mitotic activity subsequent to irradiation.

**Methods.** – Neuroblasts were separated from the 14-day grasshopper embryo by treatment with trypsin-hyaluronidase.[31] Culture preparations were made by spreading thinly on a quartz cover glass a small drop of cells and tissue fragments suspended in Shaw's culture medium.[32] This medium promotes good growth for several days. The cover glass was sealed over a flat-bottomed depression slide.

The microbeam used has been described by Uretz and Perry.[33] A reflecting objective (0.56 N.A.) serves for viewing the specimen and for simultaneously focusing onto a particular part of the cell a demagnified image of a pinhole on which there is a real image of an ultraviolet source. The source was a G-E AH6 lamp, filtered to remove most of the radiation below 225 and above 330 m$\mu$. The energy flux of biologically effective radiation per unit area was approximately 0.01 erg$\cdot\mu^{-2}\cdot$sec$^{-1}$ at the focal point of the microbeam. The beam at this point was a little less than 3 $\mu$ in diameter.

Cells were irradiated and studied at room temperature (24 ± 2°C). Three cells fairly close to one another and in the same stage of division were selected: in one cell one nucleolus was irradiated; in another cell a nonnucleolar portion of the nucleus was irradiated; and the third cell served as control. The duration of exposure of a cell to the ultraviolet microbeam was 3 sec. The irradiated cells were observed until they reached mid-mitosis or for at least 24 hr; observation on some cells extended for as long as 3 or 4 days.

**Results.** – Data were collected on 81 cells (Table 5) irradiated mainly in middle, late, and very late prophase (designated MP, LP, and VLP, respectively). A few cells were irradiated in stages from late telophase through early prophase (pre-MP).

Ultraviolet microbeam irradiation of one nucleolus for 3 sec is sufficient to prevent further division of practically all the neuroblasts irradiated in

[31] G. S. St. Amand and S. R. Tipton, *Science* 119, 93–94 (1954).

[32] E. I. Shaw, *Exptl. Cell Research* 11, 580–86 (1956).

[33] R. B. Uretz and R. P. Perry, *Rev. Sci. Instr.* 28, 861–68 (1957).

**Table 5. Effect of Ultraviolet Microbeam Irradiation on Ability of Cell to Divide**

| Mitotic Stage | Nuclear Part Irradiated* | | | | Number of Cells |
|---|---|---|---|---|---|
| | One Nucleolus | | Nonnucleolar Region | | |
| | Cells Divided | Cells That Did Not Divide | Cells Divided | Cells That Did Not Divide | |
| Pre-middle prophase (pre-MP) | 0 | 4 | 2 | 1 | 7 |
| Middle prophase | | | | | |
| MPa | 1 | 5 | 5 | 1 | 12 |
| MPb | 3 | 3 | 5 | 3 | 14 |
| MPc | 3 | 4 | 4 | 1 | 12 |
| Late prophase | | | | | |
| LPa | 5 | 1 | 6 | 0 | 12 |
| LPb | 5 | 0 | 7 | 0 | 12 |
| Very late prophase (VLP) | 1 | 0 | 11 | 0 | 12 |
| | | | | Total | 81 |

*Cells exposed for 3 sec except for several very late prophase cells that were subjected to greater exposures.

stages from late telophase through MPa and of about half the cells irradiated in MPb and MPc (Table 5). Since nonnucleolar irradiation of any mitotic stage for the most part does not prevent division, failure of nucleolar-irradiated cells to divide can be attributed to an effect on the nucleolus even though less intense parts of the beam are obviously going through some of the nucleus and cytoplasm. Irradiation of one or both nucleoli in LP and VLP cells does not usually prevent division of the cell.

These data can best be interpreted to mean: (1) in stages from late telophase through MPa the nucleolus is directly involved in the mitotic process – this period represents about 58% of the total mitotic cycle including interphase; (2) there is a transition period during MPb and MPc in which the nucleolus is losing its ability to influence mitosis – thus some of the cells irradiated in this period divide and some do not; and (3) by LP the nucleolus is no longer functioning with respect to the mitotic process.

Six cells irradiated in a nonnucleolar region at stages pre-MP and MP did not divide (Table 5). Failure of division may possibly have been caused by proximity of the microspot to the nucleolus – in two of the cells it is definitely known that the microbeam was focused in such a way that the edge of the microspot was about 3 $\mu$ from one nucleolus.

**Discussion.** – Although the present study does not indicate the specific role of the nucleolus, it does demonstrate that this structure is directly involved in the mitotic process. The neuroblast requires extracellular materials for completion of the major part of the prophase processes.[34] That the nucleolus may be involved in the utilization of such materials by the cell is suggested by autoradiographs of neuroblasts cultured with tritiated cytidine[35] which indicate that the nucleolus can incorporate this substance at mitotic stages from late telophase through MPb. The nucleolus appears, therefore, to be metabolically active in those mitotic stages in which it is susceptible to ultraviolet radiation. That the synthesizing capabilities of the nucleolus are requisites for the mitotic process can be inferred from the present work.

[34] M. E. Gaulden and K. L. Kokomoor, *Proc. Soc. Exptl. Biol. Med.* 90, 309–14 (1955).

[35] The authors are indebted to J. H. Taylor, Columbia University, for the cytidine.

### Analysis of Chromosomal Translocations in *Habrobracon*

R. C. von Borstel

**Introduction.** – Stocks of *Habrobracon* have been established that show inherited partial sterility when outcrossed.[36] These stocks are regarded as being homozygous for chromosomal translocations, since the genetic behavior of these lines resembles known translocations in other species. Two of the translocation stocks demonstrated characteristics that merited especial attention. This report will deal with these two stocks only.

**Results.** – It has not been possible to make one of the translocations (T1) homozygous. Females thought to be homozygous for T1 are sterile. This translocation is constantly maintained in the heterozygous condition, and considerable progeny testing is required merely to keep the translocation for analysis. The T1 translocation has been maintained for two reasons: (1) the theoretical minimum for egg hatchability of a female heterozygous for a translocation is 33% (T1 heterozygotes have hatchabilities approaching this percentage); and (2) the egg hatchability of T1 heterozygotes varied appreciably from test to test.

Since the egg hatchability so closely approached the minimum theoretical value, an experiment was undertaken to determine whether T1 was in actuality two translocations, each approaching the maximum value for hatchability, 50%. Ninety-six males were collected from heterozygous females. Each of the males was mated with a female of normal chromosome constitution, $F_1$ daughters were collected, and one from each cross was tested. If the T1 stock had been made up of two translocations, then the hatchabilities of the $F_1$ daughters would have been expressed in a 1:2:1 ratio where $\frac{1}{4}$ of the $F_1$ daughters would have 25% or less hatchability, $\frac{1}{2}$ would have between 33 and 50% hatchability, and $\frac{1}{4}$ would have 100% hatchability. If only one translocation were present, two hatchability modes would be obtained: $\frac{1}{2}$ of the $F_1$ daughters would have a hatchability between 33 and 50%, and $\frac{1}{2}$ would have hatchabilities of

[36] R. C. von Borstel and M. L. P. Rekemeyer, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 32–33.

100%. In the actual test of 96 matings, 60 of the daughters had approximately 100% hatchability and 36 had an average of about 39% hatchability. It appears that T1 is a single translocation.

A simple test was set up in order to study the variability of hatchability data of T1 heterozygotes. Six heterozygous females were set and moved twice daily. Eggs were counted and hatchability was recorded for each laying period. These females were used for 30 continuous days of laying. The data are summarized in Table 6. As the females grew older a gradual decline in average hatchability was observed. Such a decline has never been observed in wild-type stocks or in other translocation stocks. This may have contributed to the variability between experiments on the T1 stock.

**Table 6. Egg Hatchability of Females Heterozygous for the T1 Translocation**

| Day | Viable/Total | Hatchability |
|---|---|---|
| 1–5 | 179/453 | 0.395 |
| 6–10 | 142/695 | 0.204 |
| 11–15 | 87/417 | 0.209 |
| 16–20 | 67/417 | 0.161 |
| 20–25 | 26/182 | 0.143 |
| 25–30 | 10/121 | 0.083 |

The other translocation stock investigated was T4,5, a stock thought to be made up of two translocations, since the T4,5 heterozygous females had exceptionally low hatchability (approaching 25%). A progeny test like that used on T1 was employed in order to separate the translocations, and, surprisingly, a 1:1 ratio of high hatchability to low hatchability females was obtained. This indicated that the T4,5 stock has in fact only one translocation.

**Discussion.** – These two stocks both appear to have one translocation, and both expressed lower hatchability in the heterozygous state than is usually observed either in *Habrobracon* or other species; T1 is close to the theoretical minimum, but T4,5 is below this minimum. In either case the low hatchabilities can be explained by (1) assuming involvement of more than two chromosomes which have breaks that are closely linked to one another, or (2) assuming that there is an extremely large difference in size between two members of the translocation. Since the expression of these two translocations is unexpected, further genetic, statistical, and cytological analyses are in progress.

## *DROSOPHILA* GENETICS

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### Sex-Linked Recessive Lethals in *Drosophila melanogaster* That Are Suppressed by the Y Chromosome

D. L. Lindsley C. W. Edington
E. S. Von Halle

**Introduction.** – A method has been devised for screening irradiated gametes for sex-linked recessive lethals and semilethals whose expression is suppressed by the presence of the Y chromosome. It has been found that for every four lethals that are recorded by using the orthodox Muller-5 test there is one that is missed because XY males are viable.

**Results.** – A series of 32 of these lethals has been studied, and the results of the studies are presented in Table 7. The lethals are divided into two groups on the basis of whether or not they are deficient for the *bb* region; the 28 lethals which are not *bb* deficient may be further subdivided on the basis of the fertility of $y\ 1/Y$ males. The three groups defined by this classification seem to be fairly consistent within themselves with respect to the other data collected. For example, the male sterile lethals showed a high rate of nondisjunction from $Ins(1)sc^{4L},S,sc^{8R}$, $y\ sc^4\ B\ w^a\ sc^8$, whereas in the other groups nondisjunction was very low. Furthermore, the male sterile lethals tend to be associated with translocations and tend not to reduce total recombination in the X chromosome to an extremely low value. Also they tend to be located proximally on the chromosome. The male fertile non-*bb* deficient lethals, on the other hand, tend not to be

[37]American Cancer Society Fellow.
[38]ICA-NRC Fellow.
[39]Consultant.

Table 7. Summary of the Data Obtained from Sex-Linked Lethals Whose Expression Is Suppressed by the Y Chromosome

| Lethal No. | Bobbed Region Present | X/Y Male Fertility | Primary Nondisjunction in Females | Translocation | Total Recombination in X Chromosome | Location | Relative Viability as | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | X/O Male | X/Y Male |
| 3a | – | + | 0 | | 0.47 | f-sfa | 0 | 1.03 |
| 74 | – | + | 0 | | 0.45 | f-sfa | 0 | 1.17 |
| 158 | – | + | 0 | | 0.48 | f-sfa | 0 | 1.11 |
| 452 | – | + | 0 | | | | 0 | 1.15 |
| 456 | – | + | | | 0.52 | f-sfa | 0 | 1.15 |
| 481 | – | + | 0 | | 0.28 | f-sfa | 0 | 0.94 |
| 11 | + | + | 0 | 1;4 | 0.67 | f-sfa | 0.04 | 0.76 |
| 306 | + | + | 0 | | 0.49 | y | 0 | 0.78 |
| 47 | + | + | 0.01 | | 0.44 | v-f | 0 | 0.35 |
| 59 | + | + | 0 | | 0.05 | y-cv | 0 | 0.63 |
| 132 | + | + | 0.01 | | 0.02 | cv-v | 0.01 | 0.82 |
| 146 | + | + | 0 | | 0.01 | | 0 | 0.38 |
| 227 | + | + | 0 | | 0.06 | f-sfa | 0 | 0.47 |
| 231 | + | + | 0 | | 0.09 | f-sfa | 0 | 1.13 |
| 459 | + | + | 0.02 | 1;3 | 0.09 | cv | 0 | 0.78 |
| 223 | + | – | 0.01 | | 0.45 | f-sfa | 0 | 0.40 |
| 451 | + | – | 0.01 | | 0.40 | f-sfa | 0.03 | 0.65 |
| 3 | + | – | 0.31 | 1;3 | 0.24 | cv | 0 | 0.74 |
| 25 | + | – | 0.39 | | 0.41 | f-sfa | 0 | 1.05 |
| 75 | + | – | 0.29 | 1;2 | | | 0 | 0.25 |
| 129 | + | – | 0.37 | | 0.36 | f-sfa | 0.26 | 0.90 |
| 150 | + | – | 0.36 | 1;2 | 0.18 | f-sfa | 0 | 0.20 |
| 163 | + | – | 0.34 | 1;3 | 0.26 | f-sfa | 0.01 | 0.14 |
| 216 | + | – | 0.28 | 1;2;3 | 0.06 | cv | 0 | 0.13 |
| 219 | + | – | 0.30 | 1;2 | 0.13 | v | 0 | 1.11 |
| 220 | + | – | 0.41 | 1;2;3 | 0.37 | f-sfa | 0.25 | 0.34 |
| 252 | + | – | 0.30 | 1;3 | 0.24 | y | 0.02 | 0.99 |
| 361 | + | – | 0.42 | 1;3 | 0.46 | f-sfa | 0 | 1.04 |
| 453 | + | – | 0.37 | 1;3 | 0.44 | f-sfa | 0 | 0.96 |
| 454 | + | – | 0.24 | 1;2;3;4 | 0.13 | f-sfa | 0 | 0.49 |
| 455 | + | – | | | 0.36 | y | | |
| 463 | + | – | 0.35 | 1;3 | 0.55 | f-sfa | 0.15 | 0.49 |

associated with translocations, drastically reduce total recombination in the X chromosome in the majority of cases, and are distributed more or less randomly on the X chromosome. The *bb* deficient lethals behave quite normally. They are not associated with translocations nor do they reduce recombination, and, of course, they are all located proximally on the X chromosome. Some of them do not disjoin regularly from a Y chromosome during spermatogenesis. The relative viability figures compare the viability of *y l*/Y or O with that of *y*/Y or O males derived from similar crosses. They illustrate the extent to which the Y chromosome can suppress the effect of a lethal or semilethal change on the X chromosome.

**Discussion.** – There are several surprising facts about these data: One is the infrequent involvement of the *bb* region in the lethals studied; another is the curious correlation between male sterility, translocation, and primary nondisjunction in the female (actually 3–1 disjunction from a translocation heterozygote). Still another is the lower average frequency of recombination in the male sterile lethals which are generally not associated with a rearrangement (at least an interchromosomal one), compared with the frequency of recombination observed for the male sterile lethals which are mostly associated with translocations.

A final interesting point is that the male sterility of the third class of lethals seems to be caused by a failure of spermiogenesis, more specifically, the failure of sperm-head elongation. The severity of this failure varies among lethals but is constant among males of the same lethal stock.

### New Combinations of the X and Y Chromosomes in *Drosophila melanogaster*

D. L. Lindsley E. Novitski

**Introduction.** – The sex chromosomes (the X and the Y chromosomes) of *Drosophila melanogaster* provide one of the most versatile tools for studies of chromosome behavior. Over the past eight years the authors have been able to synthesize a large number of chromosomes carrying different combinations of elements derived from the X and Y chromosomes. Briefly, these elements are the X, by which is meant primarily the euchromatic portion of the X chromosome, $Y^s$, which is the short arm of the Y chromosome and is necessary for male fertility, and $Y^L$, which is the long arm of the Y chromosome, also required for male fertility.

**Results.** – The majority of these new chromosomes fall under the classification of attached XY (= XY) chromosomes, that is, chromosomes which have attached to a single centromere (designated by a center point) all the material of the X and Y chromosomes necessary for male fertility and viability. Most XY chromosomes that have been synthesized are of the constitution $Y^SX{\cdot}Y^L$, with the X chromosome element either in normal sequence, singly inverted, or doubly inverted. Parker has produced an $XY^S{\cdot}Y^L$ and an $XY^L{\cdot}Y^S$, and from these $Y^SXY^L{\cdot}Y^S$ and $Y^SXY^S{\cdot}Y^L$ have been derived. A rod-shaped XY of constitution $Y^SXY^L$ is also available, and currently the construction of a ring-shaped XY chromosome is being attempted. In addition to XY chromosomes, there are available an XY4 of constitution $Y^SX{\cdot}Y^L4$ and the following compound chromosomes carrying two X chromosomes in addition to one or more Y elements: $XY^L{\cdot}X$, $XY^L{\cdot}Y^LX$, and $Y^SXX{\cdot}Y^L$.

**Discussion.** – These new chromosomes have found many uses in research here and are widely used in other laboratories throughout the country. They are useful in balancing other chromosomes for stock maintenance; they permit manipulation of the Y chromosomes and other X homologs in studies of chromosome behavior, and they are useful in studying the effects of Y chromosome constitution on gene action.

### Birth Order, Parental Ages, and Sex of Offspring

E. Novitski A. W. Kimball[40]

Variations in the proportion of male births (i.e., the secondary sex ratio) have been analyzed many times as a function of those characteristics of the parents, the sibship, or the environment for which available data lend themselves to statistical treatment. Among the interesting relationships discovered previously is the observation that the age of the mother apparently has no, or very little, effect on these changes, whereas the increasing age of the father is correlated with a decreasing secondary sex ratio.[41]

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[40] Mathematics Panel.

[41] E. Novitski, *Science* 117, 531 (1953).

This is a most puzzling result, since there are a number of ways in which it can be imagined that some factor related to maternal age might be responsible, as, for instance, differential mortality of the two sexes before birth. However, a system related to the age of the father (and relatively independent of the mother's age) is not so easily conceived.

An attempt to assess the relative contributions of the parental ages and birth order showed that there was no simple unifying interpretation of data that gave, on the one hand, the parental ages and sex ratio of offspring and, on the other hand, the maternal age, birth order, and sex ratio.[42] It was therefore suggested that a more informative interpretation would depend on the analysis of data that gave, for each birth, the sex of the child, both of the parental ages, and the birth order simultaneously. Such data for the year 1955 have been made available by the National Office of Vital Statistics.

**Results.** – The analysis of these data differs from previous analyses in several important respects. Because the Oracle was available for use in this analysis, a fit of a quadratic regression surface was made on all three variables simultaneously. As was suggested by earlier work, the direct contribution of the age of the mother to the variations in sex ratio is not significant. Birth order, age of the father, and an interaction term involving these two factors prove to be significant.

**Discussion.** – Since it is known that changes in birth order and parental age are both related to changes in secondary sex ratio, it is clear that birth data must not be analyzed without taking these into account. For this reason the authors would hesitate to place any great reliance on an apparent influence of the maternal age based on a simple breakdown by parental ages only, and would be inclined to question the revelance of such analyses to a biological interpretation of the causes of variations in sex ratio. Specifically, Lejeune and Turpin[43,44] have analyzed data from the Public Health Service giving the sex ratio of live births as a function of the parental ages only. On the basis of an apparent effect of the maternal age, they hypothesize that the change in sex ratio results from the increased incidence of sex-linked lethals in older mothers, and by equating the regression coefficient on the age of the mother with a spontaneous mutation rate, they arrive at a "doubling dose" for humans. In view of the preceding discussion, it seems clear that they are working with a spurious correlation, and that an analysis of the type presented here is the more appropriate one.

[42] E. Novitski and L. Sandler, *Ann. Human Genet.* 21, 123 (1956).

[43] J. Lejeune and R. Turpin, *Compt. rend.* 244, 1833 (1957).

[44] J. Lejeune and R. Turpin, *Compt. rend* 244, 2425 (1957).

### Conference on Computing Methods

E. Novitski

During the week of February 3–8, 1958, the Biology Division sponsored a conference on the application of automatic computers to biological problems. The participants consisted of a small group of biologists who had expressed an interest in advancing the analysis of biological problems in this particular way. Those from outside the Biology Division were as follows:

N. A. Barricelli, University of Rochester, Rochester

C. C. Cockerham, University of North Carolina, Raleigh

E. R. Dempster, University of California, Berkeley

R. Lewontin, University of North Carolina, Raleigh

H. Papazian, New Haven

The participants from the Division included W. Arnold, D. R. Krieg, D. L. Lindsley, and E. Novitski, as well as A. W. Kimball of the Mathematics Panel. During the sessions, lectures on the programing methods for automatic computers were presented by E. C. Long of the Mathematics Panel, J. Cornelison of International Business Machines, Chattanooga, and E. Novitski of the Biology Division. Two demonstrations of such equipment were given: one of the Oracle, operated by the Mathematics Panel of the Oak Ridge National Laboratory, and one of the IBM 704, operated by the Numerical Analysis Section at K-25.

Subsequent to the more formal lectures on programing methods, a smaller group of the participants spent some time writing actual

programs for specific biological problems. It was the consensus of the group that the usefulness of computers in biology fell primarily into two distinctly different areas: one that involved the statistical evaluation of data from biological experiments; and another primarily concerned with the solution of problems arising in natural or artificial populations where the number of variables is so great that a simple arithmetic or algebraic solution is impossible, and in the empirical solution of which the computer is programed to mimic the biological behavior of the population. R. Lewontin discussed the relevance of Monte Carlo methods to problems of this type in which the populations involved might be biological organisms ranging from whole animals, as in a typical evolutionary problem, to single cells, as in a tissue transplant.

# MAMMALIAN GENETICS AND DEVELOPMENT

## GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell, Section Chief

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M. K. Freeman
M. S. Hawkins
J. R. Inman
E. M. Kelly
S. A. Kennedy
M. M. Larsen
E. J. McCrone
S. C. Maddux
E. L. Phipps
E. A. Smith

### Effects of Chronic Gamma Irradiation on Male Fertility

W. L. Russell E. M. Kelly

Each of several experiments now being conducted to measure mutation rates at specific loci in mice exposed to a $Cs^{137}$ source has yielded some new information about the effects of chronic gamma irradiation on the fertility of males.

In the first two experiments, vigorous $F_1$ hybrid males from a cross of inbred 101 strain females with C3H strain males were exposed to total doses of 1000 and 600 r, respectively, at a rate of approximately 100 r/week continuous irradiation. These males were sterile when removed from the radiation field, but regained their fertility later. The first fertile matings occurred at 71 days after irradiation in the 1000-r experiment and at 59 days after irradiation in the 600-r experiment. These results may be compared with those of Deringer *et al.*[2] obtained with $LAF_1$ hybrid mice exposed to a radium source. No temporary sterility was found in males given a total dose of 1100 r at the rate of either 30.8 r/week continuous irradiation or 61.6 r/week administered in daily 8-hr exposures. However, temporary sterility lasting about 59 days was found after a total dose of 1760 r given at the 61.6-r/week rate. Unless the two hybrids are markedly different in their sensitivity, it would appear that the larger effect, presumably due to a greater depletion of spermatogonia, in our experiments, as compared with those of Deringer *et al.*, resulted from the higher dose rate. It is noteworthy that in spite of the greater damage in our experiments, a dose of 1000 r did not induce permanent sterility.

The above conclusion, that higher dose rate produces more effect, is in contrast to the conclusion of Carter *et al.*,[3] who stated that the results of their experiments on inbred males exposed to a radium source showed that "decrease in dose-rate, or increase in time of exposure, increased the damage." However, the comparison which these authors made of the effects of different dose rates was not a valid one. They stated that 200 r given at 8.0 r/week produced significant effects on fertility, but that the same total dose given at 33.3 r/week produced no obvious effect. What makes this comparison invalid is the time interval over which fertility was tested, namely, only the seven days immediately following removal from the radiation field. It takes at least five weeks for a type B spermatogonium to mature and reach the ejaculate. Thus, in the 33.3-r/week experiment, males tested in the week following the six weeks in which they had received their total dose of 200 r would be using sperm which had received most or all of this dose in postgonial stages, which are known to be insensitive so far as induction of sterility is concerned. It is, therefore, not valid to compare animals tested in this way with those treated at the 8.0-r/week dose rate, for, at the time of testing in the week following the accumulation of 200 r, the latter group would be using sperm that had received perhaps three-fourths of the total dose in spermatogonial stages. Testing the 33.3-r/week group at a time when the sperm used had accumulated a comparable dose in spermatogonial stages did show marked effects on fertility. Thus, despite the conclusion of the authors, the results of Carter *et al.* are not actually in conflict with our conclusion that higher dose rates are more damaging.

Another feature of the results from our 600-r experiment is worth especial note. This is the fact that the males in this experiment had already entered their sterile period at six weeks after the start of the irradiation. One would have expected mature sperm to be present at the time of mating

[1]Consultant.

[2]M. K. Deringer, W. E. Heston, and E. Lorenz, *Natl. Nuclear Energy Ser. Div. IV* **22B**, 149–68 (1954).

[3]T. C. Carter, M. F. Lyon, and R. J. S. Phillips, *Brit. J. Radiol.* **27**, 418–22 (1954).

because, as was pointed out above, most of the dose would have been received in postspermatogonial stages. Perhaps an explanation for this early onset of sterility, much earlier than that found in any of the experiments of the other investigators cited, can be found in the very sensitive period of about four days in which the cells are in the type B, intermediate and perhaps late type A spermatogonial stages. This hypothesis rests on the assumption that a dose rate of 100 r/week is sufficient to destroy enough type B, intermediate and late type A gonia to produce sterility, an assumption which is not out of line with the sensitivity of these stages as determined by Oakberg.[4]

In another experiment, inbred 101 strain males were given a total dose of 600 r at 100 r/week. These also were sterile on removal from the radiation field. The first fertile mating did not occur until 94 days after irradiation. Thus, these animals were more affected than were the hybrids after the latter received a total dose of 1000 r. Lorenz and Heston[5] found the fertility of inbred C3H animals to be more easily damaged than that of $LAF_1$ hybrids.

The 101 strain was used in another experiment in which the males received a total dose of 300 r at 100 r/week. These animals were fertile when removed from the radiation field. They then became temporarily sterile, and fertility later returned, in the earliest instance at 74 days after removal from the field. Noteworthy here is the low total dose at which temporary sterility was induced.

A comparison of these results with the effects of single acute x-ray exposures[6] indicates that, so far as the induction of temporary sterility is concerned, chronic irradiation at the dose rate used here causes at least as much damage as does the acute irradiation. This is in striking contrast with the effects on female fertility presented later in this report (see "Influence of Dose Rate on the Sterilizing Effect of Radiation in Female Mice").

[4]E. F. Oakberg, *J. Exptl. Zool.* **134**, 343–56 (1957).

[5]E. Lorenz and W. E. Heston, *Natl. Nuclear Energy Ser. Div. IV* **22B**, 1–11 (1954).

[6]W. L. Russell, *Radiation Biology*, (ed. by A. Hollaender), vol 1, Part II, chap. 12, McGraw-Hill, New York, 1954.

## Influence of Dose Rate on the Sterilizing Effect of Radiation in Female Mice

L. B. Russell M. K. Freeman

**Introduction.** – Radiation effects on the fertility of female mice are being studied for two purposes: (1) to augment the histological investigations on the ovary[7] and the data obtained in connection with dominant lethal studies[8] in providing information on the sensitivity of oogenic stages; and (2) to determine the conditions of radiation that will minimize the reduction in number of offspring when genetic effects of radiation in females are to be investigated. At the time of the last report[9] it was shown that fractionated doses of x rays have considerably less effect on female productivity than do single doses. Also, since more young were produced when a given dose was divided into 10-r fractions than when it was divided into 25-r fractions, it seemed probable that accumulation of the same dose by chronic exposure of females would have even less effect on fertility. The present report shows that this has indeed proved to be the case. Since the females in the experiment have neared, but not yet reached, the end of their reproductive life span, the data presented at this time are total number of young per female, without breakdown into more detailed parameters.

**Method.** – Female $(101 \times C3H)F_1$ mice were exposed to gamma rays from a 5-curie $Cs^{137}$ source. Dose rate was regulated by distance. The exposure was essentially uninterrupted until the required dose had been accumulated, following which the females were removed from the gamma facility and mated singly to $(101 \times C3H)F_1$ males for the remainder of their life. The 12 exposure groups (of 12 females each) and the control group (18 females) were all run synchronously, as far as possible. The 162 females have, to date, produced 11,054 young.

**Results and Discussion.** – Results are shown in Fig. 4, which also includes, for comparison, the

[7]"Effect of X Rays on the Mouse Ovary," this report.

[8]L. B. Russell and W. L. Russell, *Progress in Radiobiology* (ed. by J. S. Mitchell, B. E. Holmes, and C. L. Smith), p 187–92, Oliver and Boyd, Edinburgh, 1956.

[9]L. B. Russell and M. K. Freeman, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 40.

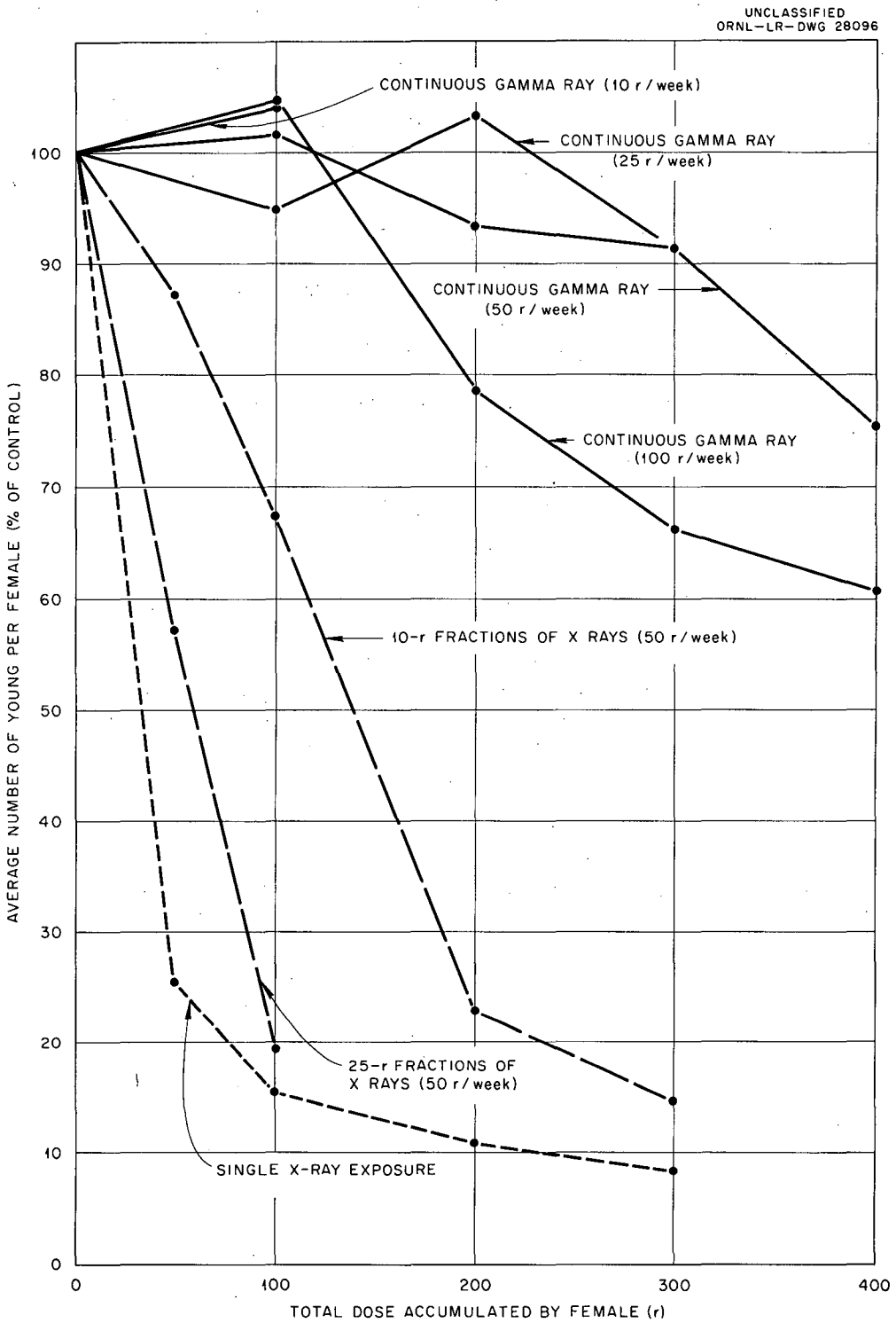


**Fig. 4. Effect of Dose Rate on Number of Young Produced Subsequent to Irradiation of Female Mice.** It is estimated that the final difference between the gamma-ray experiment (solid lines), which is not yet quite complete, and the x-ray experiment will be slightly less than that shown on the present graph.

data from 144 x-irradiated females reported earlier.[9] These data, as already discussed, indicated a lessening of the sterilizing effect with increasing fractionation of x-ray doses. Within the new data on continuously gamma-irradiated females there is also a clear effect of dose rate on total young subsequently produced, at least for total accumulated doses of over 100 r. In view of the intensity effect being thus demonstrated within each of the two experiments, it is highly probable that the difference *between* the two experiments is also largely due to the same factor, although exact comparison is not possible because the RBE of x rays and $Cs^{137}$ gamma rays is not known for sterilizing effects in females. Although the RBE may contribute part of the difference between the two sets of data, it would require an RBE greatly different from unity to contribute all of it.

The cause for the great dependence of the radiation effect on dose rate is puzzling, since the adult ovary apparently contains no oogonia that have to undergo mitotic divisions, but only oocytes – all of which (except for those very few which have entered the first meiotic division just a few hours prior to ovulation) are in the same stage, the so-called "dictyate."[7] Thus, the death of oocytes in young follicles that has been observed shortly after irradiation[7] is not due to chromosome imbalance following cell division, and an intensity effect cannot be explained in terms of chromosome rearrangements. The intensity effect suggests that the sensitive mechanism, whatever it is, is capable of repair.

### Effect of X Rays on the Mouse Ovary

E. F. Oakberg

After an acute dose of 50 r, female mice usually have a greatly reduced number of litters in comparison with controls,[9] then become sterile for the remainder of their life. This is in marked contrast with male mice, where regeneration of the seminiferous epithelium occurs from a few surviving type A spermatogonia; and after a temporary sterile period, fertility is regained even after doses of 1000 r or more. In order to explore the basis for the different response of the ovary, adult female hybrid (101 × C3H) mice were exposed to 50 r of x rays and killed at intervals ranging from 3 hr to 70 days after irradiation. Both ovaries were fixed in Zenker-formol, and 7-$\mu$ serial sections of the entire tissue were stained in hematoxylin–azure II–eosin. All oocytes were counted in five sections selected from the center of each series. Cells were scored as normal or necrotic on the basis of morphology and staining reaction of the nucleus. Primary oocytes were classified on the basis of follicle development, as given in Table 8.

The rapid and complete degeneration of primary oocytes that are associated with only a few follicle cells and in follicles consisting of a single layer of flattened cells, together with the severe reduction in number of normal primary oocytes in follicles with a single layer of cuboidal cells (Table 8), eventually results in depletion of mature follicles. No initial effect on primary oocytes was observed in follicles with an antrum, but as a result of destruction of earlier stages and failure of replacement, mature follicles had almost completely disappeared by 70 days. A few rare follicles at younger stages also were observed at this time, but their frequency was too low to be significant.

No new formation of primary oocytes occurred, which supports the conclusion of others that no cell comparable to an oogonium persists in the adult mouse ovary. This is in agreement with the observation that all oocytes in the mouse ovary have reached the dictyate condition by 72 to 96 hr after birth.[10] In the ovary of the adult, all oocytes except those in follicles about to rupture remain in the dictyate condition. This particular stage appears to be extremely sensitive to acute radiation exposure, with a rapid degeneration of damaged oocytes in all but the older follicles (Table 8). Once destroyed, no replacement can occur, since no cell comparable to the type A spermatogonium of the testis exists in the ovary.

From these results it is clear that experiments dealing with radiation-induced genetic effects in female mice will be limited primarily to the response of the dictyate nucleus, and, with carefully timed irradiation, to oocytes in meiotic division.[11] Thus the results of genetic effects on

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[10] F. W. R. Brambell, *Proc. Roy. Soc.(London)* B101, 391–408 (1927).

[11] L. B. Russell and W. L. Russell, *Progress in Radiobiology* (ed. by J. S. Mitchell, B. E. Holmes, and C. L. Smith), p 187–92, Oliver and Boyd, Edinburgh, 1956.

Table 8. Relative Frequencies of "Normal" and Necrotic Oocytes at Various Intervals After Exposure to 50 r of X Rays

| Dose (r) | Time After Irradiation | "Normal" Oocytes, Experimental/Control Ratios* | | | | | Degenerating Oocytes, Necrotic Cells/Total Cells | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Stage of Follicular Development | | | | | Stage of Follicular Development | | | | |
| | | A Few Cells | Single Layer, Flattened Cells | Single Layer, Cuboidal Cells | Two or More Layers | Antrum Present | A Few Cells | Single Layer, Flattened Cells | Single Layer, Cuboidal Cells | Two or More Layers | Antrum Present |
| 0 | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.007 | 0.027 | 0.000 | 0.083 | 0.053 |
| 50 | 3 hr | 1.209 | 0.986 | 0.761 | 1.023 | 1.194 | 0.074 | 0.034 | 0.018 | 0.022 | 0.044 |
| 50 | 6 hr | 0.255 | 0.462 | 0.662 | 0.955 | 1.222 | 0.708 | 0.460 | 0.130 | 0.023 | 0.228 |
| 50 | 9 hr | 0.129 | 0.359 | 0.577 | 1.159 | 1.056 | 0.840 | 0.540 | 0.255 | 0.038 | 0.073 |
| 50 | 12 hr | 0.051 | 0.145 | 0.423 | 1.295 | 1.139 | 0.939 | 0.832 | 0.508 | 0.081 | 0.000 |
| 50 | 15 hr | 0.058 | 0.117 | 0.493 | 1.205 | 1.028 | 0.926 | 0.851 | 0.540 | 0.172 | 0.196 |
| 50 | 18 hr | 0.000 | 0.048 | 0.225 | 0.909 | 0.972 | 1.000 | 0.873 | 0.556 | 0.111 | 0.205 |
| 50 | 24 hr | 0.005 | 0.028 | 0.211 | 0.909 | 1.389 | 0.976 | 0.897 | 0.500 | 0.259 | 0.123 |
| 50 | 30 hr | 0.005 | 0.028 | 0.070 | 0.568 | 1.194 | 0.962 | 0.846 | 0.737 | 0.546 | 0.189 |
| 50 | 36 hr | 0.000 | 0.021 | 0.155 | 0.614 | 1.028 | 1.000 | 0.786 | 0.577 | 0.509 | 0.213 |
| 50 | 48 hr | 0.000 | 0.000 | 0.070 | 0.318 | 0.861 | | 1.000 | 0.737 | 0.667 | 0.296 |
| 50 | 72 hr | 0.000 | 0.000 | 0.099 | 0.205 | 0.889 | | 1.000 | 0.708 | 0.796 | 0.333 |
| 50 | 10 days | 0.000 | 0.000 | 0.070 | 0.227 | 0.861 | | 1.000 | 0.546 | 0.600 | 0.225 |
| 50 | 20 days | 0.000 | 0.007 | 0.014 | 0.068 | 0.611 | | 0.000 | 0.000 | 0.864 | 0.290 |
| 50 | 45 days | 0.000 | 0.000 | 0.000 | 0.000 | 0.083 | | 1.000 | 1.000 | 1.000 | 0.250 |
| 50 | 70 days | 0.000 | 0.000 | 0.014 | 0.045 | 0.056 | | 0.000 | 0.000 | 0.333 | 0.600 |

*Four mice per group.

female mice may differ considerably, both from those in the male mouse, where all stages from spermatogonia to mature sperm are present, and in the females of species where oogonia and all subsequent oocyte stages occur in the adult.

### Pseudoallelic Recessive Lethals at the *Notch* Locus of *Drosophila melanogaster*

W. J. Welshons

In an earlier report[12] the linear arrangement from left to right on the x chromosome of three recessive visible mutants at the *Notch* locus was established as follows: *facet* (fa), *facet-notchoid* ($fa^{no}$), and *split* (spl). An additional study[13] has indicated that the dominant *Notch* (N) mutants plus the recessive visible mutants constitute a pseudoallelic array similar to the *Star-asteroid* and the *Ultrabithorax-bithoraxoid* series described by Lewis.[14] In each of these three different series, one member of the pseudoallelic array is a dominant mutant that is lethal in the homozygous condition. Additional studies have indicated that the *Notch* pseudoallelic array differs from the other two in one important respect: there are at least three different sites capable of mutating to a dominant *Notch*. Since *Notches* are sex-linked recessive lethals, there is at this complex locus a pseudoallelic recessive lethal series in addition to a recessive visible pseudoallelic array. The pseudoallelism of the various *Notch* mutants was established on the basis of the experiments outlined below.

Females of the $w^a$ $N^{Co}$+/+ spl dm genetic constitution yielded the following exceptional types of chromosomes which were neither *Notch* nor *split*: 8 – $w^a$ + dm, 1 – $w^a$ + +, 1 – + + dm. The last two exceptions, which arose without recombination between $w^a$ and dm, were assumed to be reversions, or to have come about by a rare double exchange. The 8 – $w^a$ + dm chromosomes were assumed to be the result of a single exchange between $N^{Co}$ and spl. If this assumption is correct, $N^{Co}$ must be localized to the right of spl. The reciprocal product of recombination carrying both spl and $N^{Co}$ would probably not be detectable.[14]

Females of the $w^a$ $N^{264\text{-}40}$+/+ spl dm genetic constitution yielded 11 – + + + chromosomes and 1 – + + dm. It was assumed that the 11 – + + + chromosomes resulted from an exchange between $N^{264\text{-}40}$ and spl. This would place $N^{264\text{-}40}$ to the left of spl and $N^{Co}$.

If these interpretations are correct, then females which are y $w^a$ $N^{264\text{-}40}$+ +/+ $w^a$ + $N^{Co}$ rb and which carry a small duplication for the loci of *white* and *Notch* on one of their autosomes (the duplication covers the lethality of the *Notch* combination) should yield, as the consequence of a single exchange between $N^{264\text{-}40}$ and $N^{Co}$, chromosomes which are + $w^a$+ + +. Approximately 75,000 chromosomes were tested, of which 13 were of the expected type.

These experiments demonstrate that one *Notch* mutant is localized to the left of *split* and another to the right of *split*. Additional experiments, which will not be detailed here, indicate that there are at least several *Notches* that are at the *split* locus or very close to it. Therefore there must be at least three genetic sites capable of mutating to *Notch*. Since the mutations at these sites are separable by crossing over, they constitute a pseudoallelic recessive lethal series.

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[12] W. J. Welshons, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 42–43.

[13] W. J. Welshons, *Proc. Natl. Acad. Sci. U.S.* (in press).

[14] E. B. Lewis, *Cold Spring Harbor Symposia Quant. Biol.* 16, 159 (1951).

# MICROBIAL PROTECTION AND RECOVERY

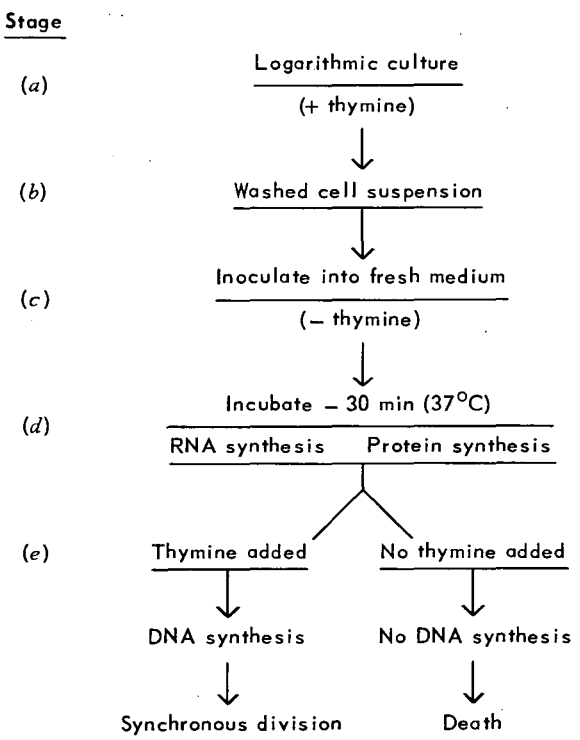
## RADIATION PROTECTION AND RECOVERY IN BACTERIA

A. Hollaender H. I. Adler
G. E. Stapleton N. A. Sicard[1]
A. S. Angel

### X-Ray Sensitivity of Synchronous Cultures of *Escherichia coli*

G. E. Stapleton N. A. Sicard

**Introduction.** – In a previous report[2] it was indicated that synchronized cell division as well as synthesis of DNA (see Glossary) could be obtained with a thymineless mutant strain ($15_{T-}$) of *E. coli*. Moreover, synchronized cultures showed large changes in x-ray sensitivity as a function of incubation time in the first division cycle, the greatest sensitivity being correlated in time with the onset of DNA synthesis and the division of the cells. The typical behavior of this strain can be described by the following scheme:




Since it was possible to have cultures treated identically through stage (*d*) in the scheme, but thereafter very differently, depending on whether or not thymine was added, it seemed important to compare the sensitivity changes of both types of cells in order to attempt any meaningful correlation of sensitivity and biochemical activity.

**Results.** – Cultures were grown and were treated identically through stage (*d*) in the scheme presented above. At stage (*e*), thymine was added to one culture and left out of the other. (1) As reported earlier, cultures to which thymine was added show almost immediate and rapid synthesis of DNA, and about 20 min after the addition of thymine a large increase in viable cells is measured. As shown in an earlier report,[2] a sizable decrease in the number of survivors per unit dose is obtained during the period when rapid DNA synthesis is occurring. This period is followed by a return to normal sensitivity, and shortly thereafter a second peak of sensitivity is measurable, which is correlated in time with the synchronous cell division. (2) Cultures to which thymine is *not* added show similar alterations in sensitivity at the same time, an unexpected result, since obviously from the scheme these cells show no net synthesis of DNA and die at a rate of about 90% per 40-min incubation period. It is of interest to note that occasionally, even though conditions are presented to the cells for synchronous division, normal random division occurs. Cultures dividing at the random rate show no such variations in sensitivity.

**Discussion.** – It is obvious from the results obtained in the experiments described that although remarkable time correlations can be made between synthetic processes and cell sensitivity to ionizing radiation, a causal relationship can hardly be made. It may be important to note that the same processes which make synchronization of division of these cells possible (i.e., a period of "unbalanced growth"[3]) could be responsible for their alteration in sensitivity. The peculiar nature of this strain of *E. coli* in which the biochemical requirement precludes synthesis of its genetic material may very well have made it a poor but interesting choice for such studies. Since there

[1] Visiting investigator from abroad.

[2] G. E. Stapleton and N. A. Sicard, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 45.

[3] S. S. Cohen and H. D. Barner, *Proc. Natl. Acad. Sci. U.S.* 40, 885 (1954).

are now available other methods for obtaining synchronous cultures, similar studies are under way in which the different techniques are being used.

### An Aftereffect of Ionizing Radiation on a Mutant of *Escherichia coli*

H. I. Adler G. E. Stapleton

**Introduction.** – The hemin-requiring mutant ($H_7$) of *E. coli* has been found to be a useful bacterium for radiation studies, in that this organism, when grown in the presence of hemin, can elaborate porphyrin-containing enzymes and can form no measurable porphyrins when grown in the absence of its requirement. Adler[4] has shown that the cells grown in the absence of hemin ($H_{7-}$), when irradiated with x rays in water or $\frac{1}{15}$ *M* phosphate buffer, show an inactivation that continues for some hours after irradiation, a phenomenon not displayed by the organism grown in the presence of hemin ($H_{7+}$). It is also clear that the agent which produces the aftereffect is produced by the radiation in the medium surrounding the cell and can be substituted for quantitatively by $H_2O_2$ in the concentration produced in the medium by a particular dose of x rays. This study is concerned with defining conditions which influence the aftereffect.

**Results.** – The magnitude of the aftereffect is measured, unless otherwise specified, as the rate of continued inactivation of x-irradiated $H_{7-}$ cells incubated at 25°C in an aqueous solution of known $H_2O_2$ concentration in equilibrium with air. For convenience the conditions are divisible into several classes on the basis of whether they are presented to the cells before, during, or after the actual irradiation.

1. Among the preirradiation conditions investigated were the atmosphere (i.e., whether cultures were aerated or not during the growth period) and the stage of the growth cycle from which the culture was harvested. The results of these studies can be simply summed up by stating that vigorously aerated cultures and nonaerated cultures showed approximately the same aftereffect. No significant difference in the aftereffect could be found for cells harvested from logarithmic or stationary phase cultures of $H_{7-}$.

2. The conditions imposed on the cell suspensions during the irradiation markedly influence the aftereffect:

*Dose.* – The inactivation of $H_{7-}$ with 250-kvp x rays at 8700 r/min is shown in Fig. 5. This graph represents the rate of inactivation under the beam, indicated as part *A* of the graph, and the rate of inactivation that continues with time following irradiation in $2 \times 10^{-5}$ *M* $H_2O_2$ in $\frac{1}{15}$ *M* phosphate buffer (part *B*). The 1-, 2-, and 3-min points represent the prompt inactivation with 8700, 17,400, and 26,100 r, respectively. The slope of the delayed inactivation curve (aftereffect) is taken as a measure of the amount of sensitization that results from the dose of radiation received by the cells surviving prompt inactivation. Sensitization, as measured by this technique, at least over the dose range used, appears to be a linear function of dose. More data at higher doses are being obtained at present to determine whether this relationship holds over a wide dose range.

*Oxygen Effect.* – The removal of oxygen from suspensions prior to and during irradiation by use of bubbling nitrogen has a marked effect on the prompt and delayed inactivation. As is shown

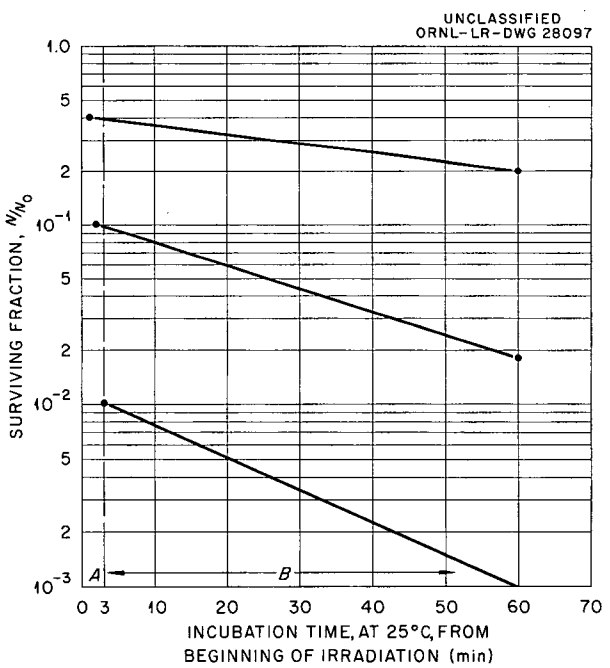



**Fig. 5. Prompt and Delayed Inactivation of *Escherichia coli* ($H_{7-}$).**

[4]H. I. Adler, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 46.

(see "Oxygen Effect in X-Ray Inactivation of *Escherichia coli* $H_7$," this report), prompt killing per unit dose is affected by the oxygen tension in a similar manner to that previously described.[4,5] Also, the aftereffect depends to some extent on the oxygen tension during irradiation. The rate of delayed inactivation, which is assumed to be a measure of sensitization, per unit dose is also dependent on the presence of oxygen during irradiation but not to the same extent as is prompt killing. This finding suggests that sensitization may be brought about by both direct and indirect action. More experiments are needed to elucidate the mechanism of sensitization.

*Temperature.* – Preliminary experiments, concerned with the effect of temperature (4 to 37°C) during irradiation, have been carried out, and the data indicate that sensitization and prompt inactivation are probably temperature independent.

3. The effects of temperature, $H_2O_2$ concentration, and cell concentration on the delayed killing have been studied. Cell concentration in the reaction mixture of irradiated cells and $H_2O_2$ has no effect on the rate at which the aftereffect proceeds. On the other hand, temperature and $H_2O_2$ concentration have a marked effect on the rate of delayed inactivation. Over the temperature range (12 to 37°C), the rate of death in a $2 \times 10^{-5}$ *M* $H_2O_2$ solution is more than doubled for each 10 deg of temperature increase. Several experiments indicate that the rate of delayed inactivation is doubled when the $H_2O_2$ concentration is increased from $2 \times 10^{-5}$ to $10^{-4}$ *M*. Both types of experiments indicate that the rate of delayed death is dependent on the rate of diffusion of $H_2O_2$ to "sensitized material."

**Discussion.** – The phenomenon of sensitization to one agent induced by another is probably a general one. The whole idea of sensitization in the experiments described results from the finding that the catalase-negative $H_{7-}$ strain shows an inactivation that continues for a period of hours after the completion of irradiation. Unirradiated cells of the same strain can be inactivated by $H_2O_2$, but of the order of 1000 times the concentration of peroxide is required to kill at the same rate. Moreover, the $H_{7+}$ cells require the high concentration of peroxide whether irradiated or nonirradiated. The aftereffect therefore is probably restricted to cells which lack an enzyme system for decomposing $H_2O_2$. One possible question is whether the prompt killing results from interaction of sensitized material and $H_2O_2$ produced at or near the site. Experiments are under way to test this possibility, and also to determine whether or not sensitization can be induced without loss of viability. This has not been possible to demonstrate in any experiment to date.

[5]A. Hollaender, G. E. Stapleton, and F. L. Martin, *Nature* 167, 103 (1951).

## Oxygen Effect in X-Ray Inactivation of *Escherichia coli* $H_7$

H. I. Adler G. E. Stapleton

**Introduction.** – The unusual growth requirement of *E. coli* ($H_7$) makes possible a unique study of the role of respiratory enzymes in the oxygen effect in radiobiology. It has frequently been considered that the mechanism by which oxygen enhances the lethal effect of ionizing radiation may involve the alteration, by reaction with oxygen, of some cellular components existing before irradiation. These altered components might result in an increased sensitivity of the entire cell. One class of compounds considered very likely to be involved are the porphyrin enzymes, particularly the cytochromes. In fact, the cytochromes have been investigated from this point of view.[6,7] The porphyrin enzymes, as a class, are certainly very responsive to oxygen within the cell.

The bacterium $H_7$, which can be grown with or without these enzymes, makes it possible to study their role in living cells without the use of chemical inhibitors. When grown aerobically without hemin, $H_7$ completely lacks the characteristic absorption bands of the cytochromes and is unable to oxidize a wide variety of common substrates. It is also catalase negative. If grown with hemin, these substrates can be oxidized, and the cells are catalase positive.[8]

For convenience, two oxygen effects may be defined for bacteria. The first of these is the effect of oxygen present during cell division and

[6]W. D. Bellamy and E. J. Lawton, *Ann. N.Y. Acad. Sci.* 59, 595–603 (1955).

[7]H. Laser, *Nature* 174, 753 (1954).

[8]M. Beljanski, *Compt. rend.* 240, 374 (1955).

growth of the culture.[9] This phenomenon is being studied in *E. coli* ($H_7$), but only preliminary data are available. This effect may well involve an influence of oxygen on the chemical and structural makeup of the final cell population. The second, and the one which is of primary concern in this report, is the role of oxygen in contact with resting cells immediately before and during the period of irradiation.

**Results and Conclusions.** – Aerobic cultures of $H_7$ were grown with ($H_7+$) and without ($H_7-$) hemin, and the magnitude of the oxygen effect was investigated in order to decide whether porphyrin-involved enzymes played an important role. The experiments were performed in such a way as to minimize the hydrogen peroxide aftereffect previously described for $H_7-$ (ref 10). It can be seen from Fig. 6 that an oxygen effect occurs, the magnitude of which is independent of the presence of porphyrin-involved enzymes. Similar data have been obtained in several experiments in which sodium hydrosulfite was used for oxygen removal. As previously reported for *E. coli* B/r, survival[11] in sodium hydrosulfite is slightly better than in nitrogen.

It is also obvious from Fig. 6 that the shape and position of survival curves are influenced by growth in hemin. Cells grown in hemin yield a more nearly exponential survival curve than those grown in its absence. Microscopic observation does not indicate that there are any significant differences in size or in chain lengths. In both cases the cells exist almost entirely as individual units. Cell wall stains show no evidence of significant multicellularity. Preliminary deoxyribonucleic acid and ribonucleic acid determinations do not indicate any differences in the nucleic acid content per cell for aerobically grown $H_7+$ and $H_7-$.

Although the influence of growth in hemin on the shape and position of $H_7$ curves cannot yet be explained, the main purpose of this study has been fulfilled. It is clear that an oxygen effect exists for this organism and that the magnitude of this effect is independent of respiratory enzymes present in the cell at the time of irradiation.

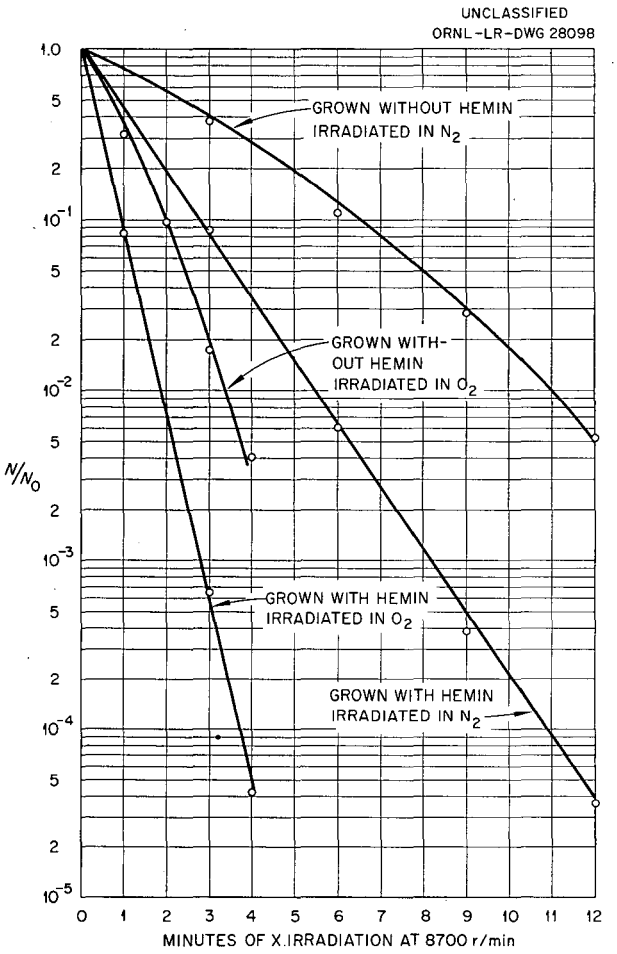



**Fig. 6. Oxygen Effect in X-Ray Inactivation of *Escherichia coli* $H_7$.**

[9] A. Hollaender, G. E. Stapleton, and F. L. Martin, *Nature* 167, 103 (1951).

[10] H. I. Adler, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 46.

[11] W. T. Burnett *et al.*, *Proc. Soc. Exptl. Biol. Med.* 77, 636 (1951).

# MAMMALIAN RECOVERY

C. C. Congdon

L. H. Smith P. Urso[2]
J. W. Goodman I. S. Urso
J. C. Tohá[1] D. B. Duda
A. P. Henley

## Survival of Mouse-Grown Rat Erythrocytes

L. H. Smith J. C. Tohá

**Introduction.** – A study of the survival time of $Cr^{51}$-labeled mouse-grown rat erythrocytes [from x-irradiated (950 r) mice given rat bone marrow] has continued.[3]

[1]Visiting investigator from abroad.

[2]Graduate student, the University of Tennessee.

[3]J. Tohá and L. H. Smith, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 56–57.

**Results.** – The survival times of mouse-grown rat erythrocytes and of normal mouse and rat erythrocytes in the rat have been measured. The curves representing these survival times are presented in Fig. 7. The survival curves for mouse-grown rat erythrocytes and for normal rat erythrocytes were very similar, and the 5% survival time, that is, the time when 5% of the initial activity remained, was approximately 51 days for both types of erythrocytes. The 5% survival time for normal mouse erythrocytes in the rat, however, was approximately three days. When the survival of

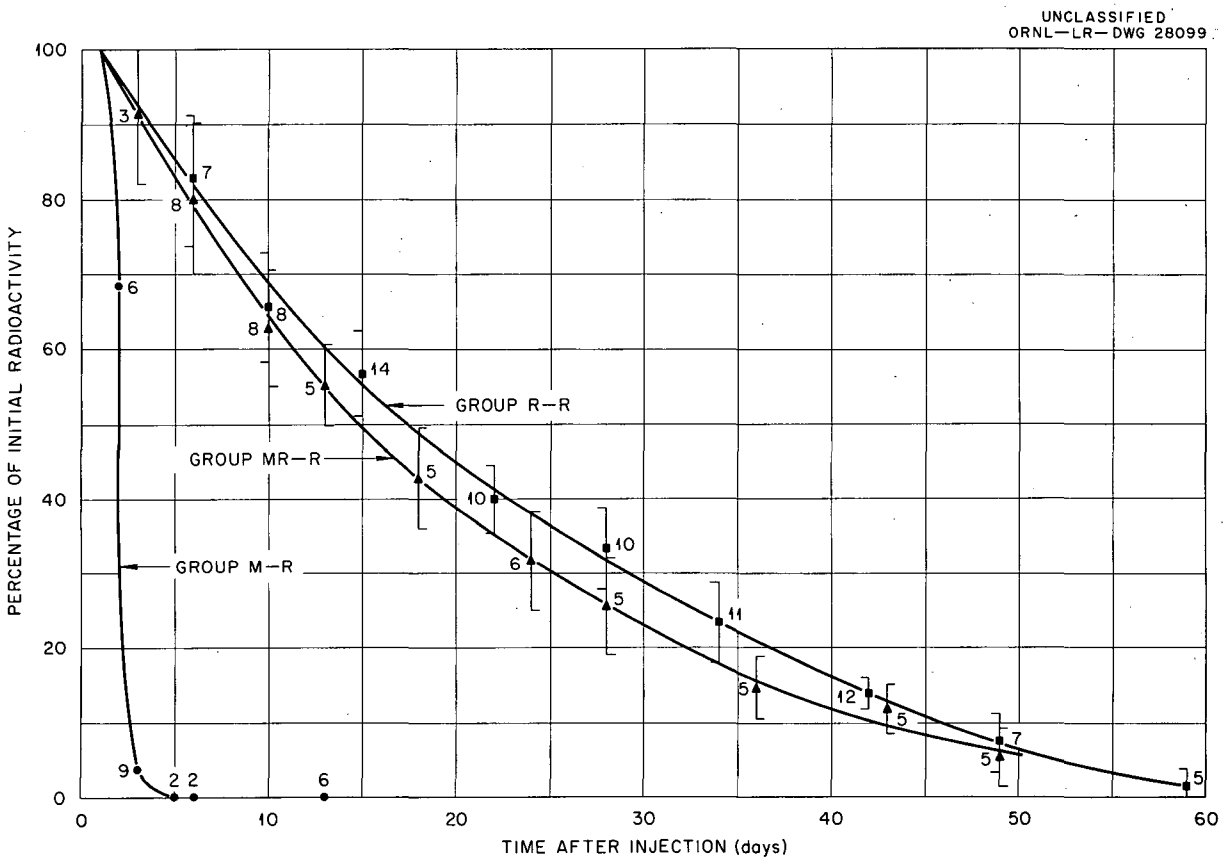



**Fig. 7. Survival of Normal Rat, Mouse-Grown Rat, and Normal Mouse Erythrocytes in the Rat, as Determined by $Cr^{51}$ Labeling.** R-R = rat RBC in rat; MR-R = mouse-grown rat RBC in rat; M-R = mouse RBC in rat. Vertical bars show ± one standard deviation. Figures beside the points indicate the number of animals.

these three types of erythrocytes was studied in the mouse (Fig. 8), the mouse-grown rat erythrocytes and the normal rat erythrocytes reach the 5% survival level on about day 3. The 5% survival time of normal mouse erythrocytes in the mouse was approximately 49 days.

**Conclusion.** – The data show that by one physiological criterion erythrocytes of x-irradiated (950 r) mice given rat bone marrow are of rat origin because (1) their survival time in the rat was essentially the same as the survival time of normal rat erythrocytes in the rat, and (2) they were not compatible with a normal mouse environment, as evidenced by a 5% survival time of about three days.

## Lymphatic Tissue Changes in Lethally Irradiated Mice Given Spleen Cells Intravenously

C. C. Congdon

**Introduction.** – Spleen treatment after irradiation injury was compared with bone marrow treatment.

**Results and Conclusions.** – The treatment of irradiated mice with isologous spleen caused quicker regeneration of splenic white pulp and of lymph nodes than that seen in treatment with isologous bone marrow. Nests of "large lymphocytic cells" accumulated in special locations in the spleen and lymph nodes of lethally irradiated mice within 24 hr after injection of isologous spleen. These cells required nearly two weeks to

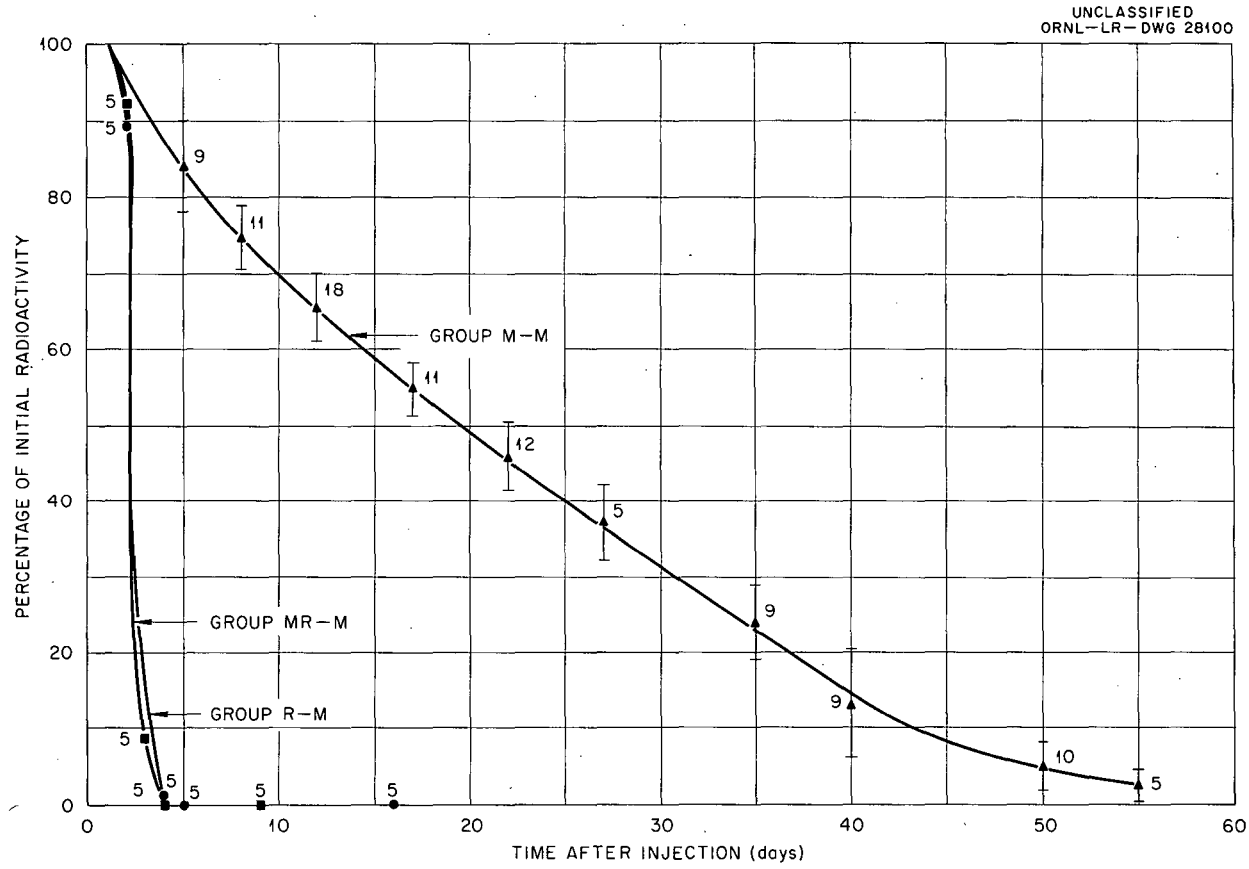



**Fig. 8. Survival of Normal Mouse, Mouse-Grown Rat, and Normal Rat Erythrocytes in the Mouse, as Determined by $Cr^{51}$ Labeling.** M-M = mouse RBC in mouse; MR-M = mouse-grown rat RBC in mouse; R-M = rat RBC in mouse. Vertical bars show ± one standard deviation. Figures beside the points indicate the number of animals.

appear after treatment with isologous bone marrow. Homologous spleen injection in lethally irradiated mice had a detrimental effect.[4] Violent reactive changes occurred in lymph nodes and spleen, and parenchymous degeneration was noted in several organs. Accumulation of large lymphocytic cells in special locations in spleen and lymph nodes preceded the reactive changes. Homologous bone marrow injection in lethally irradiated mice eventually gave somewhat similar reactive changes in spleen and lymph nodes without prior accumulation of large lymphocytic cells. Injection of thymocyte suspension did not cause the accumulation of large lymphocytic cells in spleen and lymph nodes. Rat and guinea pig spleen also failed to produce the large lymphocyte accumulation effect in lethally irradiated mice under these conditions.

### Effect of High Protein Diet on the Foreign Bone Marrow Reaction

D. B. Duda

**Introduction.** – Irradiated mice treated with foreign bone marrow lose weight and do not grow new hair. These manifestations of the delayed foreign bone marrow reaction suggest a defect in protein metabolism. To investigate this, irradiated mice treated with rat bone marrow were placed on a high protein diet and followed for long-term survival.

**Results.** – Table 9 shows the failure of the high protein (64%) diet to significantly influence the long-term survival of irradiated mice treated with rat bone marrow. No effect was observed of the high protein on normal mice fed the diet for the same length of time as the experimental group. Untreated irradiated mice fed a high protein diet died at approximately the same time as those fed a normal protein (27%) diet.

**Conclusions.** – These results are in agreement with those observed by J. Mayer and C. C. Congdon (unpublished data) on irradiated mice fed a high protein diet after homologous bone marrow treatment. Mayer and Congdon also noted that a high fat diet was detrimental to survival after treatment of irradiated mice with homologous bone marrow.

[4]T. Makinodan, N. Gengozian, and I. Shekarchi, *J. Natl. Cancer Inst.* (in press).

**Table 9. Effect of Level of Dietary Protein on Survival of Irradiated Mice (900 r) Treated with Rat Bone Marrow**

| Diet | Number of Mice | Percentage Mortality at | |
|---|---|---|---|
| | | 30 Days | 53–60 Days |
| High protein | 124 | 40 | 78 |
| Normal protein | 53 | 45 | 65 |

### Bone Marrow Cell Separation

J. W. Goodman

**Introduction.** – The continuous density-gradient technique previously described[5] was used in further isologous studies on (C57BL × 101)$F_1$ normal bone marrow cell suspensions, and several methods were tried in vivo to alter donor bone marrow.

**Results.** – Additional data were obtained to confirm the earlier finding[6] that none of the layers separated by this method were more effective than normal whole bone marrow in promoting survival of lethally irradiated mice. When the bottom two layers, equal in effectiveness to whole bone marrow, were resuspended and carried through the very same separation procedure a second time, similar layers were found and tested. The lower layers again were as effective and the upper layers not so effective as whole bone marrow, although the upper layers appeared to have been improved somewhat.

Attempts to alter the cell layers by changing donor bone marrow in vivo included use of starvation, cortisone, ACTH, cobalt, and RBC (see Glossary) plethora. Results of initial experiments indicate that no striking changes, measurable by this bioassay, have occurred, either in whole bone marrow or cell layers, with respect to the effective cell.

**Discussion.** – The improvement observed in the upper layers on the second centrifugation might be expected as a closer approximation of the theoretical separation possible by this sedimentation method. It is also possible that cells which appear the second time in the upper layers are

[5]N. G. Anderson, T. Makinodan, and I. C. Shekarchi, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 59–61.

[6]J. W. Goodman, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 53–54.

more resistant to damage (more "viable" in a sense) than were their counterparts in the first layers.

### Effect of Fetal and Newborn Hematopoietic Tissue from Homologous and Heterologous Donors on Long-Term Survival of Lethally Irradiated Mice

I. S. Urso

**Introduction.** – Preliminary experiments on the long-term survival of irradiated mice treated with foreign fetal or newborn blood-forming tissues were reported earlier.[7] Additional data have been obtained.

**Results.** – Survival is shown in Table 10. Homologous fetal liver showed greater long-term survival among the irradiated recipients than did heterologous fetal liver. Of the newborn tissues injected, neither spleen nor liver showed reduction in the occurrence of delayed deaths. Isologous $F_2$ fetal liver gave 100% survival in irradiated $F_1$ mice at 30 days.

**Discussion.** – The use of the $(101 \times C3H)F_2$ generation for homologous fetal liver introduces genetic variation into the donor material. The significance of this variation has not been evaluated.

[7] I. S. Urso, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 57.

### Effect of Delayed Injection of Foreign Adult Bone Marrow on Long-Term Survival of Lethally Irradiated Mice

P. Urso

**Introduction.** – Preliminary studies[8] suggested that waiting an interval of one or more days after irradiation to administer homologous bone marrow gave greater long-term survival than when the homologous bone marrow was given on day 0. Additional studies with homologous and heterologous bone marrow were carried out.

**Results and Conclusions.** – Table 11 shows the results of these experiments. Delayed injection was not superior to injection on day 0 when large groups of mice were studied. $LAF_1$ bone marrow given to irradiated $(C57BL \times 101)F_1$ mice gave 20% better survival at 90 days on delayed injection than when given on day 0. A difference of this magnitude is probably not significant.

[8] C. C. Congdon and I. S. Urso, *Am. J. Pathol.* **33**, 749–67 (1957).

**Table 10. Effect of Fetal and Newborn Blood-Forming Tissues on the Long-Term Survival of Irradiated Mice**

| Donor Tissue | Donor Age (days) | Cell Dose | Number of Mice | Sex | Survival (%) at Day | | |
|---|---|---|---|---|---|---|---|
| | | | | | 30 | 60 | 90 |
| Homologous[a] | | $\times 10^6$ | | | | | |
| Fetal liver | 12–20 | 20–116 | 160 | F | 72 | 65 | 62 |
| Newborn liver | 1–5 | 30–56 | 55 | MF | 69 | 42 | |
| Newborn spleen | 1–5 | 10–22 | 68 | F | 59 | 38 | 37 |
| Adult bone marrow $(101 \times C3H)F_1$ | 120 | 8–26 | 125 | MF | 59 | 27 | 20 |
| Heterologous[b] | | | | | | | |
| Fetal liver | 18–21 | 80–135 | 49 | F | 57 | 39 | |
| Newborn spleen | 1–6 | 10–24 | 21 | F | 29 | 24 | 19 |
| Adult bone marrow | 10–140 | 54–120 | 33 | F | 58 | 30 | 27 |
| Isologous[c] fetal liver | 14–17 | 38 | 20 | F | 100 | | |

[a] Homologous donors were $(101 \times C3H)F_2$ or $(C3H \times 101)F_2$ mice. The recipients were $LAF_1$ mice given 900 r.
[b] Heterologous donors were Sprague-Dawley rats. The recipients were $(101 \times C3H)F_1$ mice given 950 r.
[c] Isologous donors were $(L \times A)F_2$. The recipients were $LAF_1$ mice given 900 r.

Table 11. Effect of Delayed Bone Marrow Injection on Long-Term Survival of Lethally Irradiated Mice

| X-Ray Dose (r) | Donor | Recipient | Number of Mice* | Day of Injection | Survival (%) at Day | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 30 | 60 | 90 | 120 | 150 | 180 |
| 900 | (101 × C3H)$F_1$** | $LAF_1$ | 69 | 0 | 72 | 45 | 35 | 33 | 33 | 33 |
| | | | 187 | 1–4 | 73 | 39 | 33 | 30 | 29 | 28 |
| 900 or 950 | $LAF_1$ | (C57BL × 101)$F_1$ | 70 | 0 | 63 | 53 | 47 | | | |
| | | | 218 | 1–4 | 76 | 71 | 67 | | | |
| 900 or 950 | Rat | (C57BL × 101)$F_1$ | 45 | 0 | 49 | 24 | 18 | | | |
| | | | 208 | 1–4 | 54 | 29 | 19 | | | |
| 900 | (C57BL × 101)$F_1$ | (C57BL× 101)$F_1$ | 20 | 0 | 90 | 90 | 90 | | | |
| | | | 80 | 1–4 | 89 | 89 | 89 | | | |

*In each case both sexes were used.

**Sometimes (C3H × 101)$F_1$ were used.

## Method for the Determination of Rat Erythrocytes in the Mouse-Rat Chimera

W. St. Amand[9] L. H. Smith

**Introduction.** – A qualitative species difference in erythrocytes is being investigated as a basis for the determination of ratios of mouse and rat erythrocytes in mixtures in vitro and in mouse-rat chimeras. In a solution of mannitol ($\frac{1}{6}$ *M* in 0.45% NaCl), mouse erythrocytes hemolyze, but rat erythrocytes do not.[10] The optical density of a suspension of erythrocytes (initial reading) when plotted against the optical density of the supernatant fluid after standing at room temperature for 3 hr and centrifugation at 1200 rpm for 15 min (final reading) generates a linear function whose slope is related to the composition of the blood sample. The initial reading provides a measure of the total number of cells and obviates the necessity of using measured volumes of blood. The final reading is a measure of the amount of hemoglobin released by the hemolyzed mouse cells in the sample. A chart of the family of curves derived from known mixtures of mouse and rat blood in vitro was constructed.

**Results.** – In Fig. 9, estimates of the proportion of circulating erythrocytes that are of rat origin in

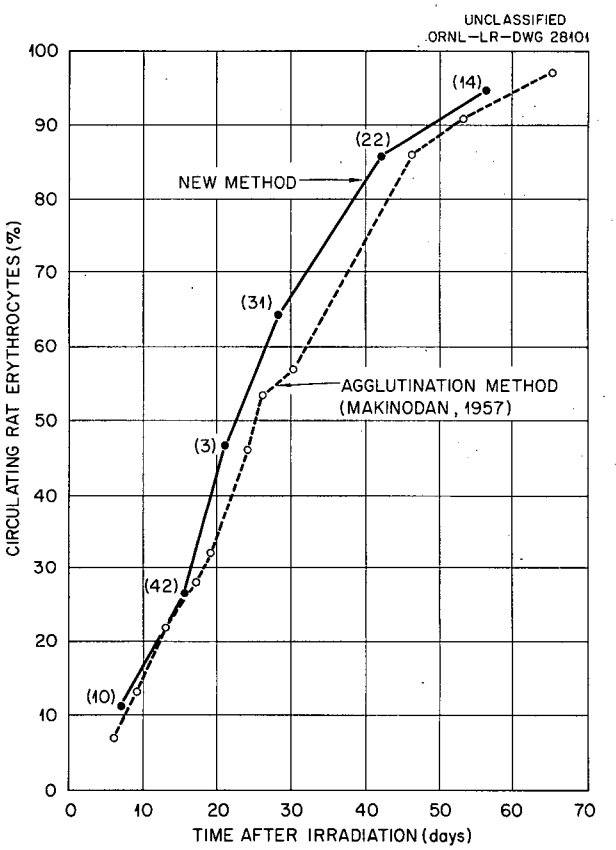



Fig. 9. Appearance of Circulating Rat Erythrocytes in Irradiated Mice (950 r) Given Rat Bone Marrow. Numbers in parentheses refer to the number of mice tested.

[9]Mammalian Genetics and Development Section.

[10]H. Ulrich, *Arch. ges. Physiol., Pflüger's* **234**, 42–50 (1934).

(101 × C3H)$F_1$ mice irradiated with 950 r of x rays and given rat bone marrow are compared with the results obtained by an agglutination method.[11]

[11] T. Makinodan, *J. Cellular Comp. Physiol.* **50** (supp), 157–61 (1957).

**Discussion.** – Although the average values obtained with the new method and those found by the agglutination technique show good correspondence, variation was encountered. Preliminary experiments suggest that the variability in the hemolytic method can be reduced, and attempts to effect this are being made.

# RADIATION IMMUNOLOGY

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## Primary Antibody Response to a Distantly Related Heterologous Antigen During Maximum Depression Period Following Varying Doses of X Irradiation

T. Makinodan N. Gengozian

**Introduction.** – Recently an investigation of the effects of the temporal relation of x irradiation and antigen injection on antibody production in (C3H × 101)$F_1$ mice was completed.[2] It was found that maximum immunologic depression occurred in mice receiving antigen 5 min to 1 day after x irradiation. This report presents data on the primary antibody response to a distantly related heterologous antigen, sheep RBC (see Glossary), in these mice receiving antigen one day after varying doses of x radiation. The immune status of these mice was based on the following criteria: (1) induction period, (2) primary rate of appearance of antibodies in the circulation, (3) mean peak titer, and (4) mean total titer.

**Result.** – The 30-day $LD_{50}$ dose for this strain of mice was found to be 756.3 r, with a 95% confidence interval of 726.1 and 786.5 r. The minimum 30-day $LD_{100}$ dose was found to be ~875 r. No difference in mean peak titer was found among groups receiving 100, 300, and 500 r, and the titers were only slightly lower than those of the group receiving 0 r, but significantly higher than those of the group receiving 710 r. In contrast, a definite radiation dose dependency was apparent in terms of the induction period, primary rate of appearance of antibodies in the circulation, and mean total titer. With increase in radiation dose there was a proportional increase in the induction period and a decrease in the primary rate of appearance of antibodies in the circulation and in the mean total titer.

IBM (see Glossary) was found to have no influence in altering the induction period. At both the 600- and 710-r level, one set of mice was treated with IBM, while the second set was not; and in both sets, identical induction periods were obtained. At higher x-irradiation dose levels, it was prohibitive to irradiate mice without IBM treatment because of the high percentage of mortality. By treatment with IBM, it was possible to determine the induction period even after 790 to 1100 r. The results are summarized in Table 12.

**Discussion.** – The data presented here show that the mean peak titer is the least sensitive of the four criteria employed in evaluating the immune status of irradiated mice. In terms of simplicity, economy, and accuracy, the induction period appears to be the most suitable index that can be used to determine the degree of radiation injury in the immune mechanism, especially after a nonkilling dose of x radiation. In confirmation of previous findings,[3–6] it was found that IBM did not enhance the immune mechanism of x-irradiated mice immediately after irradiation. Thus a determination could be made of the immune status in terms of the induction period of mice receiving x radiation doses as high as 1100 r.

In view of these results, the apparent lack of a precise relation between radiation dose and antibody-response relationship in rabbits[7,8] can be best explained in terms of individual variability.

## Antigens Responsible for Bone Marrow Transplantation Immunity

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**Introduction.** – Based on morphologic, electrophoretic, and immunologic changes,[10] it was concluded that the "foreign bone marrow reaction" is

[1]Research participant.

[2]N. Gengozian and T. Makinodan, *J. Immunol.* (in press).

[3]T. Makinodan, N. Gengozian, and C. C. Congdon, *J. Immunol.* **77**, 250–56 (1956).

[4]N. Gengozian and T. Makinodan, *J. Immunol.* **77**, 430–36 (1956).

[5]T. Makinodan, I. C. Shekarchi, and C. C. Congdon, *J. Immunol.* **79**, 281–87 (1957).

[6]N. Gengozian and T. Makinodan, *Cancer Research* **17**, 970–75 (1957).

[7]F. J. Dixon, D. W. Talmage, and P. H. Maurer, *J. Immunol.* **68**, 693–700 (1952).

[8]W. H. Taliaferro and L. G. Taliaferro, *J. Infectious Diseases* **95**, 117–33 (1954).

[9]Cell Physiology Group.

[10]T. Makinodan, *J. Cellular Comp. Physiol.* **50**, supp 1, 327–51 (1957).

Table 12. Antibody Response After Varying Doses of X Radiation

| X-Ray Dose (r) | IBM Treatment | Induction Period (days) | Rate of Appearance of Antibodies in the Circulation ($\log_2$ titer/day) | Mean Peak Titer | Mean Total Titer |
|---|---|---|---|---|---|
| 0 | – | 2 | 2.2 | 8.7 | 8.01 |
| 100 | – | 3 | 1.7 | 7.8 | 7.41 |
| 300 | – | 4 | 1.1 | 8.0 | 6.97 |
| 400 | – | 5 | | | |
| 500 | – | 6 | 0.5 | 7.8 | 5.73 |
| 600 | – | 8 | | | |
| 600 | + | 8 | | | |
| 710 | – | 11 | 0.2 | 3.6 | 2.33 |
| 710 | + | 11 | | | |
| 790 | + | 15 | | | |
| 850 | + | 16 | | | |
| 950 | + | 17 | | | |
| 1100 | + | 19 | | | |

caused by an in vivo antigen-antibody reaction. It was further deduced that this reaction involved the host immune mechanism, which is recovering from the radiation effect, against the antigens of the proliferating foreign bone marrow transplant. Obviously, if the host were sensitized to the foreign bone marrow antigens prior to the irradiation–bone marrow treatment, an earlier and greater proportion of death would be expected. This has been found in the preliminary studies. The present report is concerned with the localization and nature of such antigens. Mice were sensitized to rat cells and cell fractions 6 or 2 weeks before the 950-r and RBM (see Glossary) treatment (950 r–RBM). Indicative of the sensitizing antigens was the increased mortality 15 and 30 days after 950 r–RBM, in comparison with the mortality of the nonsensitized 950 r–RBM mice.

**Result.** – Studies on the localization of antigens showed that the antigens were absent in the serum and the red blood cells, but were present in the nucleus, mitochondria, and microsomes in a reasonable concentration, and present in the supernatant fraction in a relatively low concentration. Results of the enzyme treatment studies are shown in Table 13. It can be seen that the antigens in the mitochondrial and microsomal fractions were resistant to nuclease and protease treatment. The nuclear antigens were resistant to RNase (see Glossary) and papain treatment and were slightly susceptible to trypsin. The antigenicity of DNase-treated nuclei was variable. A salt-fractionated DNA-protein-like material was also found to be antigenic.

**Discussion.** – That a minimum of two classes of antigens responsible for bone marrow transplantation immunity are present is indicated by these results. One class of antigens is localized in the mitochondrial and microsomal fractions, which are resistant to nuclease and protease treatment. These materials were found to be antigenic at a concentration as low as 0.6 mg of nitrogen. Such findings suggest that the antigens are either simple or complex polysaccharide-like materials.

The second class of antigens is localized in the nuclei which are resistant to RNase and papain but which are slightly susceptible to trypsin. One preparation of nuclei was slightly susceptible to DNase treatment, while another was resistant. The DNA-protein materials fractionated from the

**Table 13. Enzyme Treatment of Transplantation Antigens**

| Sensitizing Antigens | Enzymes | Number of Mice | Mortality (%) from 950 r and RBM at Indicated Times After Treatment | |
|---|---|---|---|---|
| | | | 15 Days | 30 Days |
| Supernatant (0.6 mg of N per mouse) | None | 20 | 10 | 20 |
| | DNase | 20 | 0 | 35 |
| | RNase | 20 | 5 | 15 |
| | Trypsin | 20 | 5 | 10 |
| Microsomes (0.6 mg of N per mouse) | None | 20 | 65 | 85 |
| | DNase | 20 | 90 | 100 |
| | RNase | 20 | 65 | 65 |
| | Trypsin | 20 | 85 | 85 |
| Mitochondria (0.6 mg of N per mouse) | None | 20 | 80 | 85 |
| | RNase | 20 | 60 | 80 |
| | Trypsin | 20 | 65 | 85 |
| Nuclei | | | | |
| $1 \times 10^5$ | None | 40 | 12 | 15 |
| | DNase | 20 | 25 | 25 |
| $1 \times 10^6$ | None | 40 | 68 | 70 |
| $2 \times 10^6$ | None | 20 | 100 | |
| | DNase | 20 | 95 | 95 |
| | RNase | 20 | 90 | 95 |
| | Papain | 19 | 100 | |
| | Trypsin | 19 | 63 | 74 |

third preparation of nuclei were also active in their sensitizing effect. These results suggest that the antigens residing in the nucleus are labile DNA-protein-like materials. Billingham, Brent, and Medawar[11] suggested that the antigenic materials responsible for skin homograft immunity in mice are also labile DNA-protein-like substances.

[11] R. E. Billingham, L. Brent, and P. B. Medawar, *Nature* 178, 514–19 (1956).

## Immune Status of Long-Term Survivors of X-Irradiated Mice Protected with Isologous, Homologous, or Heterologous Bone Marrow

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R. D. Owen

**Introduction.** – Excellent protection in terms of survival and recovery of body weight is afforded

[12] Mammalian Recovery Group.

lethally irradiated mice by the injection of IBM (see Glossary) after irradiation. Lethally irradiated mice injected with HBM or heterologous RBM, although protected from radiation death, show an incomplete recovery as expressed by a marked secondary body weight loss and high incidence of mortality occurring from the third through the sixth weeks after treatment. Mice surviving this critical period or "foreign bone marrow reaction" show a gradual return to normal body weight by about the 60th to the 70th day after treatment, and in gross appearance may compare favorably to the isologously treated mice. That this comparison was not valid physiologically was revealed in subsequent immunologic studies which showed a great divergence in the ability of these isologously and heterologously treated mice to produce antibodies to foreign RBC antigens. The 950 r–IBM mice showed a normal immune response to sheep or rat RBC when the antigens were injected 60 days after treatment.[13] The 950 r–RBM mice showed a subnormal response to sheep RBC with antigen injection 30 or 60 days after treatment.[14] In order to establish the temporary or permanent nature of this lowered immune response among the foreign-marrow-protected mice, a study of the immune status of long-term survivors (200 to 300 days after treatment) of lethally irradiated mice treated with isologous, homologous, or heterologous bone marrow was undertaken.

**Results.** – At intervals of 200 to 300 days after 950 r and bone marrow treatment (IBM, HBM, or RBM), each mouse received, intraperitoneally, 1 ml of a 1% suspension of sheep or rat RBC. Animals were bled 18 days after injection, and the serum was titrated for agglutinating antibodies. The results are shown in Table 14. The 950 r–IBM mice showed a normal response to sheep and to rat RBC. Relative to the 950 r–IBM group, the 950 r–HBM mice showed a significantly lower response to both the sheep and the rat RBC, and within this group the response to sheep RBC was found to be significantly greater than to the rat RBC. With the 950 r–RBM mice a considerable depression in the immune response to sheep RBC was observed. The mean sheep-agglutinin titer for this group was also found to be significantly lower than that obtained with the 950 r–HBM mice.

All foreign-bone-marrow-protected mice used in the present study showed 100% donor-type RBC in the peripheral blood at the time of antigen injection. Two additional 950 r–RBM mice found to have 100% host (mouse) type RBC in their circulation were also injected with sheep RBC. The agglutinin $\log_2$ titer of these two was 7.0, which is comparable with the normal group.

Preliminary autopsy examinations of the 950 r–HBM mice showed a marked atrophy of lymphatic tissues and fibrous replacement of most of the peripheral lymph nodes, while the hematopoietic and lymphatic systems of the 950 r–IBM mice appeared normal. Mice in the 950 r–RBM group were not autopsied.

**Discussion.** – The results of this study demonstrate the subnormal immune status of the lethally irradiated foreign-marrow-protected mice and, in conjunction with the previous study, suggest this impairment to be of a permanent nature provided that the foreign transplant persists in these animals. These findings necessitate a re-evaluation of the concept of tolerance as applied to these animals, and two postulates are considered in the interpretation of the subnormal immune mechanism: (1) the lowered immune response is a quantitative expression of the functional antibody tissue remaining in these mice after recovery from the foreign bone marrow reaction, or (2) an in vivo antigen-antibody reaction, reflecting the incompatibility of host and graft, persists in these animals and consequently affects their ability to respond normally to other unrelated antigens.

## Erythrocyte Antigens as Markers for Repopulation by Homologous Erythropoietic Tissues in Irradiated Mice

R. D. Owen

**Introduction.** – Serological specificities of red cells derived from implanted erythropoietic tissues have previously been used to establish the fact of natural transplantation in cattle,[15] and to follow the course of experimental repopulation, both in

[13] T. Makinodan, N. Genozian, and C. C. Congdon, *J. Immunol.* 77, 250–56 (1956).

[14] N. Gengozian and T. Makinodan, *J. Immunol.* 77, 430–36 (1956).

[15] R. D. Owen, *Science* 102, 400 (1945).

**Table 14. Agglutinin Response to Sheep and Rat RBC Antigens in Lethally X-Irradiated Mice Treated with Isologous, Homologous, or Heterologous RBM**

| Antigen (1 ml 1%, Injected 200–300 Days After Treatment) | Agglutinin Response ($log_2$ titer) | | | |
|---|---|---|---|---|
| | Not Treated (Age Control) | Treated with 950 r Plus IBM | Treated with 950 r Plus HBM | Treated with 950 r Plus Heterologous RBM |
| Sheep RBC | 8 | 7 | 5 | 0 |
| | 9 | 6 | 5 | 0 |
| | 8 | 8 | 5 | 3 |
| | 8 | 7 | 4 | 6 |
| | 8 | 7 | 7 | 6 |
| | 8 | 7 | 5 | 0 |
| | 8 | 7 | 5 | 2 |
| | 8 | 8 | 4 | 2 |
| | 7 | 7 | 6 | 3 |
| | 8 | | 2 | 3 |
| | | | 5 | 1 |
| | | | 4 | 1 |
| Mean titer | 8.0 | 7.1 | 4.8 | 2.3 |
| Rat RBC | 8 | 7 | 1 | |
| | 8 | 7 | 4 | |
| | 7 | 8 | 1 | |
| | 8 | 5 | 2 | |
| | 8 | 8 | 2 | |
| | 9 | 7 | 3 | |
| | 9 | 8 | 4 | |
| | 8 | 7 | 1 | |
| | 9 | 7 | 1 | |
| | 9 | 7 | 4 | |
| | | | 2 | |
| | | | 4 | |
| Mean titer | 8.3 | 7.1 | 2.4 | |

the homologous system with rats[16] and in the heterologous system, rat into mouse.[17] Such markers have not been applied to studies of homologous systems in mice, although the mouse has been used extensively for "homologous bone marrow" experiments, and segregating antigens are known in this species.[18] In the present study, antisera were prepared by injecting tissues of particular inbred or hybrid strains into others; then erythrocyte suspensions from irradiated mice injected with homologous erythropoietic tissues were tested with these antisera. Two techniques were used in the tests: (1) agglutination in a human serum–dextran medium,[18] and (2) hemolysis with rabbit complement in saline medium reinforced with magnesium.[19]

**Results.** – The agglutination system proved to be capable of qualitative identification of specific cell types in chimeras, but the loose aggregates in the dextran–human serum medium have provided only a rough basis for quantitative evaluation of the degree of intermixture of cell types. A modification of the lytic system was easily put on a quantitative basis, by reading hemoglobin spectrophotometrically in the test supernatants. Figure 10 illustrates the results of two experiments: one (exp OR4, dotted lines) in which the reaction is shown at weekly intervals for ten weeks after 60 C3H × 101 female mice given 950 r were injected with male ($LAF_1$) bone marrow; and another similar experiment (OR9, solid line) with 40 recipients, bled and tested at more closely spaced intervals for five weeks. Each point in the figure represents an average of 5 to 14 mice, which were samples of the surviving populations. Experiment OR4 included treatments of the mice that will not be considered here, because these treatments did not affect the course of "repopulation," the subject of the present report.

The present donor-specific reagents lyse only a fraction of the cells of normal donors, ranging, for example, from 22 to 34% for one reagent and from

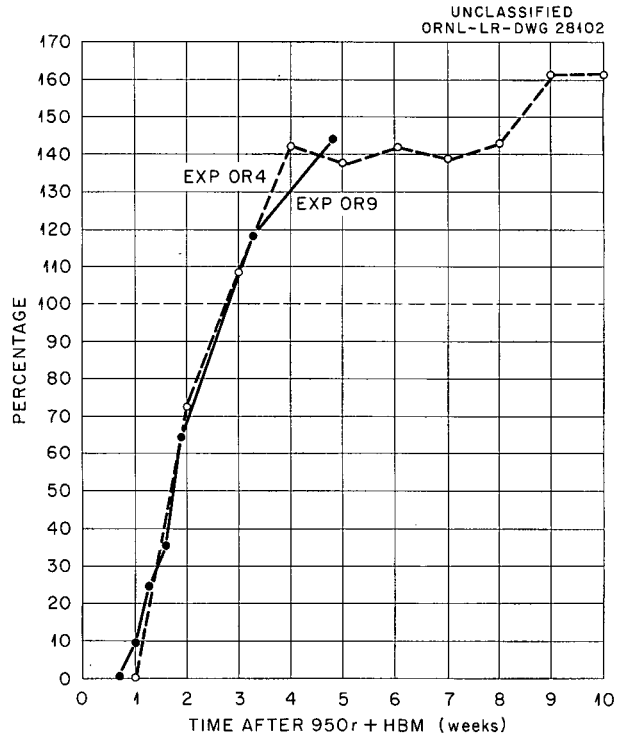


**Fig. 10. Lysis by Donor-Specific Reagents of Cell Suspension from Irradiated Recipients of Homologous Bone Marrow.** Ordinate: percentage relative to normal donors, taken as 100%.

59 to 70% for another used in these tests, depending on the complement used, the conditions of incubation, and other factors. It is therefore the specifically "lysable fraction," rather than the total cell population, that is evaluated by the hemolytic technique. The figure shows that specifically lysable cells of donor type increase rapidly in the recipient's circulation after the seventh day, reaching values characteristic of normal *donors* before the end of the third week. Obviously, this is not necessarily complete repopulation by cells of donor type, because nonlysable cells of host type escape detection by this technique. In fact, the cell populations of these recipients continue to display, for long periods of time, a donor type "lysable cell fraction" in considerable excess of that found in normal donors. Agglutination reactions are also much stronger in these animals.

**Discussion.** – Serological markers provide convenient tags for studies of repopulation by homologous erythropoietic tissues, and several experiments are in progress in which these markers are

[16] D. L. Lindsley, T. T. Odell, Jr., and F. G. Tausche, *Proc. Soc. Exptl. Biol. Med.* **90**, 512–15 (1955).

[17] T. Makinodan, *Proc. Soc. Exptl. Biol. Med.* **92**, 174–79 (1956).

[18] P. A. Gorer and Z. B. Mikulska, *Cancer Research* **14**, 651–55 (1954).

[19] W. H. Hildemann, *Transplantation Bull.* **4**, 148–49 (1957).

used. Failure of the quantitative, specific hemolytic technique to account for all cells requires caution in extending values for the lysable fraction to the total cell population, particularly while the basis for the division of this population into lysable and nonlysable fractions remains unknown. Thus, the apparently quicker repopulation by homologous rather than by heterologous marrow in the mouse[17] and by homologous marrow in the mouse rather than in the rat[16] may be more a reflection of the techniques employed than of true differences in these systems. On the other hand, recognition of an excessive lysable fraction in the homologous mouse chimeras, over long periods of time, provides some compensating merits for the hemolytic technique. The technique reveals, for example, that the donor marrow is not "at home," as a normal cell population, in these heavily irradiated homologous recipients.

## PATHOLOGY AND PHYSIOLOGY

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### Long-Term Survival of Mice Protected with Isologous Bone Marrow and S,2-Aminoethylisothiuronium Bromide Hydrobromide

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**Introduction.** – Female (101 × C3H)$F_1$ mice, protected against a single lethal dose of x radiation with AET (see Glossary), IBM, or AET and IBM together, are being observed throughout life to determine the extent of protection afforded against delayed effects of whole-body irradiation.

**Results.** – The results to date of exposure to approximately the $LD_{50}$/30 days of 250-kvp x rays, as modified by the type of protection used, are presented in Table 15 and Figs. 11–13.

**Discussion.** – The longevity of the protected mice was more closely associated with their 30-day survival (Fig. 12) than with their radiation dose (Fig. 13), if the results are compared with those obtained with mice having a similar life span. This suggests that the degree of protection afforded against the life-shortening action of radiation paralleled that afforded against acute lethality. It appears noteworthy that the induction of thymic lymphoma was inhibited by administration of either AET or bone marrow. Nephrosclerosis was much more prevalent in the mice exposed to doses above 1000 r than in those exposed to 700 to 800 r, indicating that AET did not protect the kidney to the same extent that it did the hemopoietic tissues and the intestine. The data on tumor incidence are as yet of a preliminary nature and require further analysis before interpretation (90% of the controls are still alive); however, it is of interest that tumors of the

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Table 15. Incidence of Delayed Radiation Effects in Mice Protected with AET and Bone Marrow

| X-Ray Dose (r) | Protective Treatment | Number of Mice Exposed | Mice Surviving 30 Days | | Thymic Lymphoma (%[a]) | Nephrosclerosis (%[a]) | Tumors (%[a]) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Number | % | | | Ovary | Lung | Total[b] |
| 0 | | 110 | 110 | 100 | 0.9 | 0 | 2.7 | 0 | 7.2 |
| 700–800 | | 199 | 116 | 58 | 5.2 | 14 | 40 | 14 | 77 |
| 1100–1200 | IBM[c] | 239 | 113 | 47 | 0 | 50 | 27 | 6 | 44 |
| 1200–1400 | AET[d] | 230 | 134 | 52 | 0.7 | 50 | 32 | 10 | 64 |
| 1600–1800 | AET,[d] IBM[c] | 255 | 110 | 43 | 0 | 62 | 8 | 2.7 | 16 |

[a]Percentage of those alive 30 days after irradiation.
[b]Includes tumors of breast, intestine, adrenal, kidney, liver, skin, uterus, bone, soft tissue, and orbit.
[c]Isologous bone marrow cells, 1 femur-equivalent, injected intravenously immediately after irradiation.
[d]Aminoethylisothiuronium, 9.0 mg, injected intraperitoneally a few minutes before irradiation.

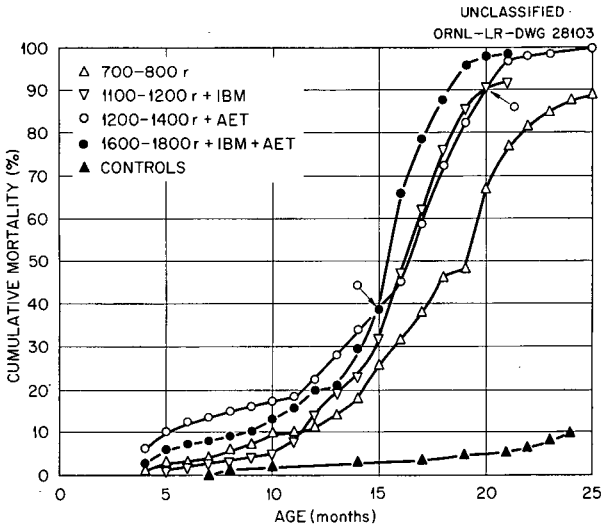


**Fig. 11. Cumulative Mortality of (101 × C3H)$F_1$ Female Mice in Relation to Type of Protection and Dose of X Rays. All mice were irradiated at 12–14 weeks of age.**

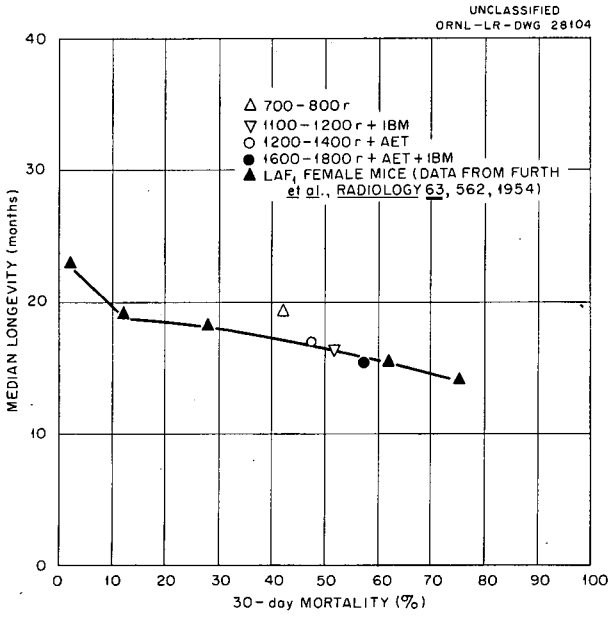


**Fig. 12. Median Longevity of Lethally Irradiated Survivors in Relation to 30-day Mortality. (C3H × 101)$F_1$ mice unless otherwise specified.**

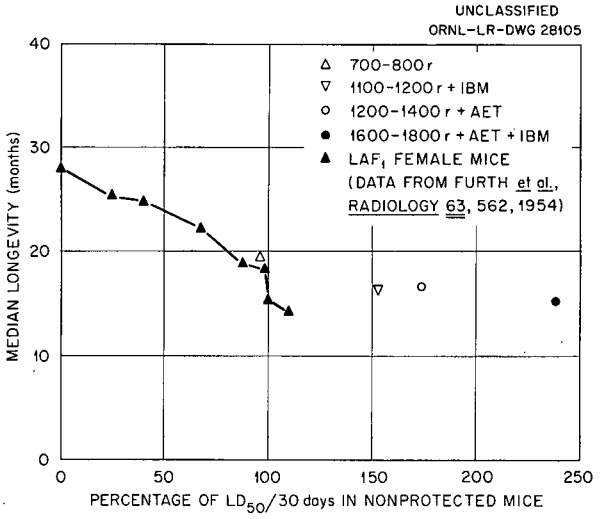


**Fig. 13. Median Longevity in Relation to Radiation Dose. (C3H × 101)$F_1$ mice unless otherwise specified.**

ovary and lung appear to be less common in protected mice than in those not protected against radiation.

### Enhancing Action of Tissue Filtrates on Induction of Leukemia by X Rays

F. F. Wolff W. D. Gude

**Introduction.** – Previous studies have shown that leukemia may be induced in leukemia-resistant mice by injection of cell-free filtrates of leukemic tissue shortly after birth. These experiments were undertaken to determine whether the administration of such filtrates would enhance the induction of leukemia by x radiation in adult mice.

**Results.** – Injection of bone marrow from nonleukemic C3H and AKR mice and of filtrates of neoplasm and brain tissue from leukemic AKR mice enhanced the induction of thymic lymphomas in irradiated RF male recipients (Table 16).

**Discussion.** – The leukemogenic action of filtrates of leukemic AKR tissues, previously demonstrated in the newborn mouse, is also demonstrable in the irradiated but not in the nonirradiated adult recipient. These results are consistent with the hypothesis that the agent is of viral nature and is resisted by the immune

Table 16. Enhancement of Lymphoma Induction in Irradiated RF Mice by Tissue and Tissue Filtrates from AKR Mice

| X-Ray Dose (r)[a] | Material Injected[b] | Leukemia Incidence (%) | | |
|---|---|---|---|---|
| | | Myeloid | Lymphoid, Thymic | Other |
| 0 | Tyrode's solution | 4 | 2 | 40 |
| 0 | Nonleukemic AKR bone marrow[c] | 4 | 4 | 45 |
| 450 | Tyrode's solution | 33 | 5 | 23 |
| 450 | Nonleukemic AKR bone marrow[c] | 25 | 15 | 33 |
| 450 | Nonleukemic C3H bone marrow[c] | 22 | 14 | 25 |
| 450 | Filtrate of AKR lymphoma[d] | 23 | 14 | 36 |
| 450 | Filtrate of leukemic AKR brain[e] | 14 | 24 | 38 |

[a]Single exposure to 250-kvp x rays administered when the mice were ten weeks old (70 to 100 mice per treatment group).

[b]All materials injected intravenously on day after irradiation.

[c]Suspension of marrow cells in Tyrode's solution (equivalent to one femur per recipient) obtained from normal donors five to ten weeks of age.

[d]Selas filtrate (cell-free) of pooled leukemic lymphoid and brain tissue obtained from AKR mice bearing a transplanted lymphoma.

[e]Selas filtrate (cell-free) of pooled brain tissue obtained from AKR mice bearing a transplanted lymphoma.

mechanism of the normal adult but not by that of the newborn or irradiated adult recipient. It is noteworthy that filtrates from lymphomatous mice did not enhance the development of myeloid leukemia. Chemical and biological characterization of the active agent is in progress. The coleukemogenic action of bone marrow from nonleukemic C3H and AKR mice may represent the presence of a filterable agent in these tissues, but this cannot be inferred without further investigation.

## Electrophoresis of Proteins of Irradiated Mouse Chimeras

R. A. Popp L. H. Smith[8]

A comparative study is being made of the water-soluble proteins in spleen, lymph nodes, bone marrow, thymus, serum, liver, duodenum, and kidney of Sprague-Dawley rats, $(101 \times C3H)F_1$ mice, and rat-mouse chimeras $[(101 \times C3H)F_1$ mice given 950 r of x rays followed by injection of Sprague-Dawley RBM]. Protein fractions are characterized according to electrophoretic migration in starch gel[9] and to selective staining reactions as being of rat or mouse type.

**Results.** – Proteins of both rat and mouse types have been found in hematopoietic tissues of the chimeras. The serum and tissue albumins are of mouse type. The serum globulins are apparently exclusively mouse type; however, the levels of alpha and gamma globulin are ordinarily increased, and the level of beta globulin is reduced. The relative quantities of rat- and mouse-type gamma globulin in spleen, lymph nodes, thymus, and bone marrow of chimeras are presented in Fig. 14. Similar results have been obtained in these tissues for the relative quantities of rat- and mouse-type alpha globulins. Proteins of rat type have not been found in the liver, doudenum, and kidney.

**Discussion.** – The relative quantities of rat- and mouse-type proteins at the sites of RBM transplantation in irradiated mice have been determined during the establishment of chimerism. The results are consistent with other types of evidence for the transplantation of rat cells into

[8]Mammalian Recovery Group.

[9]O. Smithies, *Biochem. J.* 61, 629–41 (1955).

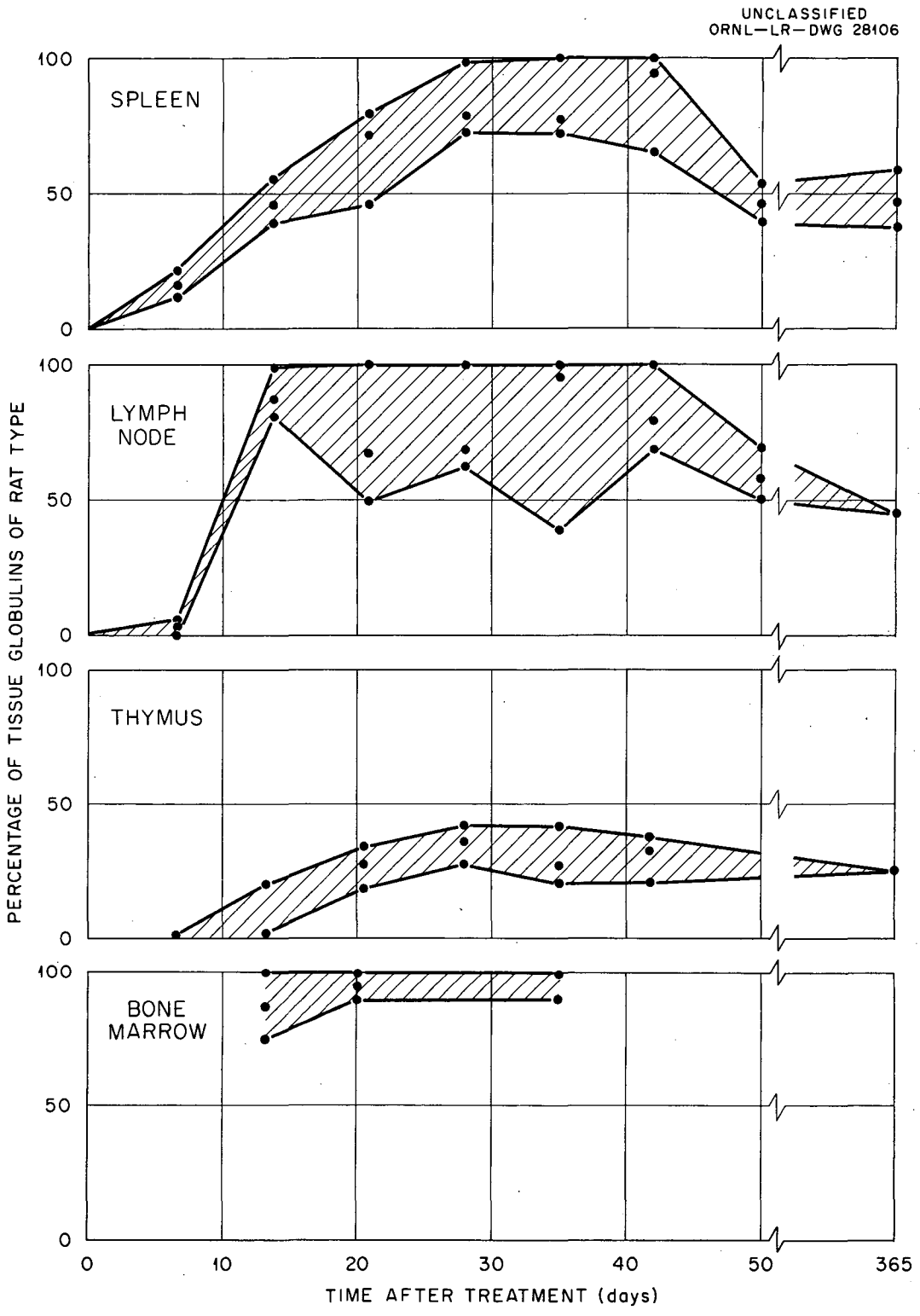


**Fig. 14. Relative Quantities of Mouse and Rat Gamma Globulins in Extracts of Tissue from Chimeras. Each point represents a single animal.**

the hematopoietic tissues of irradiated mice. By use of the technique of zone electrophoresis coupled with histological examination, the physiological status of these tissues is being correlated with their cellular architecture.

### Paper Chromatography of Whole Tissue from Mice of Varying Ages

T. T. Odell, Jr. B. Anderson
J. A. Rutherford

**Introduction.** – Rogers and Berton[10] observed that chromatography of pieces of whole tissue revealed chemical differences between one-day-old and young adult rabbits. Since it appeared that their method might be adaptable to the study of aging, a similar comparison of tissues from immature, young adult, and old mice was undertaken.

**Results.** – The liver and inguinal lymph nodes of three immature (3 weeks old), three young adult (10 weeks old), and three old (21½ months old) mice were chromatographed as follows: Twelve pieces of liver from each mouse, approximately 1 to 2 $mm^3$ in size, were placed in boiling water for about 1 min and were then pressed onto Whatman No. 1 chromatographic paper. Two sets of papers were prepared, one set being run in $NaH_2PO_4$–isoamyl alcohol solvent and the other set in BuOH:AcOH:$H_2O$ (4:1:5). The first set was examined under ultraviolet light (2537 and 3660 Å) for nucleotides, and the second set was observed with ultraviolet light and then sprayed with aniline hydrogen phthlate for detection of sugars and uronic acids. The lymph nodes were removed, similarly boiled, and individually pressed on chromatography paper, and the papers were run in BuOH:AcOH:$H_2O$ (4:1:5), examined under ultraviolet light (2537 and 3660 Å), and sprayed with ninhydrin. No consistent differences were seen among the tissues of the three age groups under any of the conditions studied, except that a stationary yellow fluorescent spot was uniformly present at the origin of the liver samples from immature mice when the papers were run in BuOH:AcOH:$H_2O$ (4:1:5) and viewed with ultraviolet light of 3660-Å wavelength.

**Conclusions:** – Although satisfactory chromatographs of liver and lymph node were obtained with the procedures described, no quantitative or qualitative differences between animals of different age groups were found. Hence the results of the specific methods used in this experiment are of only negative significance in the study of aging in mice.

[10] S. Rogers and W. M. Berton, *Lab. Invest.* 6, 310 (1957).

### Electrophysiological Changes in Irradiated Striated Muscle[11]

E. B. Darden, Jr.

**Introduction.** – In rat muscle locally exposed *in situ* to 75 kr of x rays, no clear evidence of direct radiation-induced injury, as investigated by a variety of methods, was detected in the fibers themselves.[12,13] In an attempt to differentiate between direct and indirect changes in muscle tissue produced by ionizing radiation, the effect of x rays on isolated frog muscle preparations was studied by electrophysiological means. Electrical and electrolyte disturbances were detected which, in connection with subsequently observed histological alterations, constitute a pattern of deterioration of possibly some general significance.

**Results.** – Each sartorius muscle, isolated under magnification in order to minimize fiber injury, was supported, inside surface up, in phosphate Ringer's solution containing 2.5 mmoles of potassium. Individual fiber transmembrane potentials were surveyed transversely across the belly of the muscle with a glass ultramicroelectrode (tip diameter = 0.5 $\mu$) filled with 118 m*M* (isotonic) KCl. One muscle from each animal was exposed to 250-kvp x rays or to $Co^{60}$ gamma rays, and its mate served as the control. Surveys of potentials made periodically after exposure showed a decline from normal (75 to 80 mv), with wide variations in time of onset and rate of fall in individual fibers (Fig. 15). Median potentials of the control and the irradiated groups were compared and tested for significant differences by

[11] This work was begun while the author was on an ORINS predoctoral fellowship in the Departments of Zoology and Entomology of the University of Tennessee through facilities made available at the Biology Division, ORNL.

[12] W. S. Wilde and C. W. Sheppard, *Proc. Soc. Exptl. Biol. Med.* 88, 249 (1955).

[13] E. B. Darden, Jr., and C. W. Sheppard, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 97–98.

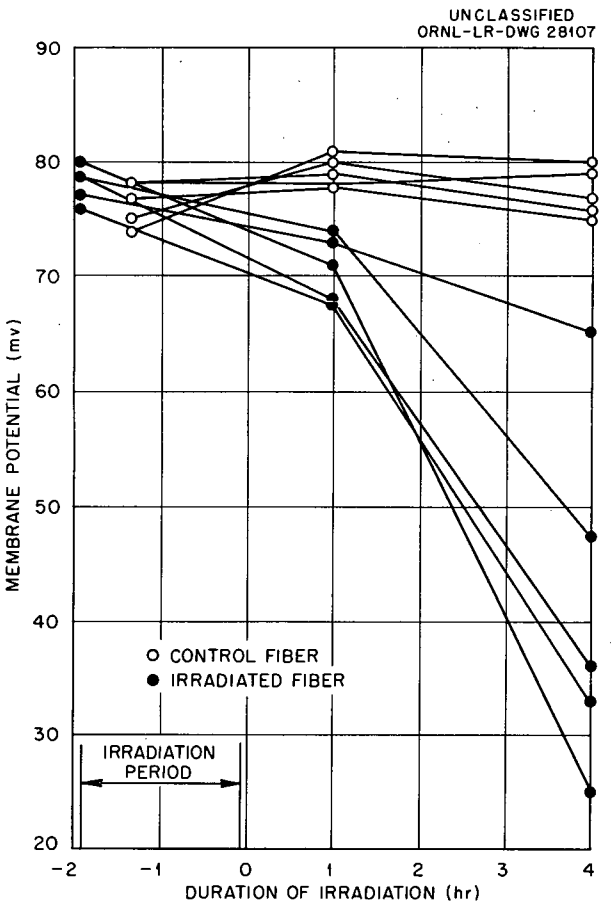


**Fig. 15. Membrane Potentials of Individual Muscle Fibers at Intervals After Exposure to 130 kr of X Rays.**

a nonparametric statistical method, a difference at or below the 5% significance level being considered meaningful.[14] The results showed that roughly 100 kr was required to produce a significant effect within a few hours if the tissue was kept at room temperature, or within about 24 hr if the tissue was stored in the cold. The latent period declined with increasing exposure dose, until at around 200 kr a significant effect was found immediately after irradiation. Changes in potassium content caused by irradiation were determined by flame photometric analyses of both the muscle ash and the Ringer bath at intervals after irradiation. Below 150 kr the relative loss of potassium was very small or undetectable; after higher doses the rate of loss increased with exposure and with time after irradiation (Table 17). Comparisons of the transmembrane potential along treated fibers with their microscopic appearance (50X, water immersion objective) indicated a correlation between regions of low or minimum potential and sites of latent injury, exhibited by a characteristic alteration in striated structure preceding localized retraction and clotting of fiber contents.

**Conculsions.** – It is suggested that radiation injury in the excised muscle develops in a fairly characteristic pattern, with early evidence of injury reflected in an increase in membrane permeability at scattered foci. The unaffected regions of the fiber surfaces are progressively involved, and eventually the loss of potassium, presumably reflected at first in the decline in transmembrane potentials, becomes great enough to be detected by gross analysis. Subsequently, the ability to maintain normal fiber structure is lost, and irreversible degenerative changes occur.

### Relative Biological Effectiveness of X Rays, Gamma Rays, and Fast Neutrons in the Production of Weight Loss of the Small Intestine of the Mouse

M. A. Patetta-Queirolo J. A. Sproul, Jr.
M. L. Randolph[15] M. Kastenbaum[16]

**Introduction.** – Since intestinal weight loss has been considered to be a quantitative index of intestinal injury and since the earlier mortality of neutron-irradiated mice, as compared with gamma-irradiated mice, has been ascribed to "intestinal death," this experiment was performed to determine the RBE of 14-Mev neutrons for intestinal weight loss in mice.

**Results.** – In ten-week-old female RF mice exposed at a rate of 3 to 5 rad/min to 250-kvp x rays, $Co^{60}$ gamma rays, and 14-Mev neutrons (produced by the *d*-T reaction in a Cockcroft-Walton accelerator), the intestinal weight, as determined by a modification of the methods of Conard[17] and Storer *et al.*,[18] declined during

[14]The advice of A. W. Kimball and M. Kastenbaum of the Mathematics Panel in the statistical procedures is acknowledged.

[15]Biophysics Group.

[16]Mathematics Panel.

[17]R. A. Conard, *Proc. Soc. Exptl. Biol. Med.* **82**, 333 (1953).

[18]J. B. Storer *et al.*, *Radiation Research* **6**, 188 (1957).

the first 48 hr after exposure, and returned to normal levels during the ensuing three to five days after doses below 300 rad. Linear regressions of weight with dose, fitted by the method of least squares, gave the intercepts and slopes shown in Table 18. Comparison of the percentage of weight loss per unit dose (or slope) of the various radiations yielded the RBE values shown in Table 19. The LET was calculated on the basis of track average.

**Discussion.** – It is noteworthy that the RBE of 14-Mev neutrons for causing intestinal weight

**Table 17. Terminal Potassium Content of Muscle as Affected by Radiation Dose and Temperature**

| Exposure (kr) | Time After Irradiation (hr) | Potassium Concentration (mmoles per kg of wet weight) | | Percentage of Control |
|---|---|---|---|---|
| | | Irradiated | Control | |
| | | **Room Temperature (25°C)** | | |
| 97 | 9 | 88.5 | 83.8 | 105.5 |
| 140 | 8.5 | 77.5 | 83.5 | 93.0 |
| 150 | 8.0 | 66.0 | 79.0 | 83.5 |
| 150 | 10.0 | 46.0 | 70.0 | 66.0 |
| 183 | 4.8 | 68.0 | 85.0 | 80.0 |
| 195 | 10.0 | 13.4 | 79.0 | 17.0 |
| 207 | 10.8 | 9.2 | 85.0 | 10.8 |
| | | **Cold Temperature (2–3°C)** | | |
| 90 | 68 | 87.0 | 87.0 | 100 |
| 92 | 54 | 83.7 | 86.0 | 97.5 |
| 160 | 66 | 23 | 77.0 | 30.0 |
| 190 | 30 | 51.8 | 79.3 | 65.5 |
| 200 | 27 | 18.6 | 82.6 | 22.5 |
| 202 | 7 | 65.5 | 93.0 | 70.5 |
| 203 | 20 | 60.2 | 88.3 | 68.0 |

**Table 18. Linear Regression Coefficients of Intestinal Weight with Dose**

| Radiations | Approximate Track Average LET (kev/$\mu$) | 95% Confidence Intervals | |
|---|---|---|---|
| | | Intercept (g) | Slope (g/$10^4$ rad) |
| $Co^{60}$ gamma rays | 0.27[a] | 1.26 ± 0.07 | 3.61 ± 1.65 |
| 250-kvp x rays | 3.0[b] | 1.36 ± 0.07 | 5.83 ± 2.01 |
| 14-Mev neutrons | 12.0[c] | 1.14 ± 0.07 | 6.59 ± 1.51 |

[a]H. E. Johns, in *Radiation Dosimetry* (ed. by G. J. Hine), p 593, Academic Press, New York, 1956.
[b]For 250 kvp, hvl 0.4 mm of Cu.
[c]C. W. Edington and M. L. Randolph, submitted for publication, 1958.

loss was found to be higher than that for 30-day lethality (Table 19), as noted by Carter *et al.*[19] It was not as high as anticipated, however, since the ratio of doses (neutrons to gamma rays) required to cause intestinal death (death within six days after irradiation) is approximately 1 to 4 (Sproul, Upton, Randolph, unpublished data). This discrepancy indicates that the weight loss of the small intestine 48 hr after irradiation does not accurately reflect the amount of injury present. This is further suggested by saturation of the weight loss response at about 800 r of gamma rays, a dose level far below that required for the production of intestinal death.

[19]R. E. Carter, V. P. Bond, and P. H. Seymour, *Radiation Research* 4, 413 (1956).

**Table 19. RBE of Radiations for Intestinal Weight Loss 48 hr After Irradiation**

| Radiations Compared | RBE – 1 SE for Intestinal Weight Loss | RBE for $LD_{50}/30$ Days* |
|---|---|---|
| X rays to gamma rays | 1.50 ± 0.43 | 1.39 |
| Neutrons to gamma rays | 2.02 ± 0.51 | 1.47 |
| Neutrons to x rays | 1.35 ± 0.28 | 1.05 |

*J. A. Sproul, Jr., and M. L. Randolph, *Radiation Research* 7, 452 (1957).

### Strain Differences in Susceptibility of Mice to Radiation-Induced Leukemia

G. E. Cosgrove, Jr. E. S. Ledford
F. F. Wolff T. Mack

**Introduction.** – The high susceptibility of mice of the RF strain to radiation-induced myeloid leukemia has not been observed to date in mice of other strains. These experiments were performed to study the effects of hybridization of the RF mouse on the susceptibility to this disease.

**Results.** – Susceptibility of (BALB/c × RF)$F_1$ mice to both myeloid and lymphoid leukemias is intermediate between that of the parental strains (Table 20). However, sex differences in the incidence of these diseases is similar in all three strains.

**Discussion.** – These results demonstrate that the high susceptibility of the RF mouse to induction of myeloid and lymphoid leukemias is transmitted in part to $F_1$ hybrid offspring of matings between RF males and BALB/c females.

**Table 20. Leukemia Incidence in Mice of $F_1$ Hybrid and Parental Strains**

| Strain | Sex | X-Ray Dose* (r) | Leukemia Incidence (%) | | |
|---|---|---|---|---|---|
| | | | Myeloid | Lymphoid, Thymic | Other |
| BALB/c | F | 0 | 1 | 0 | 10 |
| BALB/c | M | 0 | 0 | 0 | 12 |
| RF | F | 0 | 3 | 15 | 35 |
| RF | M | 0 | 7 | 1 | 40 |
| BALB/c × RF | F | 0 | 3 | 11 | 27 |
| BALB/c × RF | M | 0 | 1 | 2 | 14 |
| BALB/c | F | 300 | 5 | 1 | 5 |
| BALB/c | M | 300 | 11 | 2 | 15 |
| RF | F | 300 | 20 | 24 | 16 |
| RF | M | 300 | 55 | 8 | 18 |
| BALB/c × RF | F | 300 | 18 | 12 | 24 |
| BALB/c × RF | M | 300 | 25 | 7 | 12 |

*250-kvp x rays administered in a single dose to mice ten weeks of age (70 to 105 mice per treatment group).

The mode of transmission, although presumably genetic, may involve other mechanisms and requires further investigation.

### Life Span of Rat Erythrocytes

T. T. Odell, Jr. B. C. Caldwell

**Introduction.** – Various radioisotopes have been used in several studies of the life span of RBC in rats. The life span has been estimated to be 24 to 68 days. The study reported here was undertaken in order to make an estimate by a method not previously used for rats, namely, that of differential agglutination of introduced cells.

**Results.** – The animals employed in these experiments were male rats of the CC and DD strains raised in this laboratory. After removing approximately one-third the total blood volume of a DD recipient from a jugular vein, an equal volume of heparinized whole blood from a CC donor was injected by the same route. Five DD recipients were thus treated. On the first day after injection and at various intervals thereafter, the percentage of donor-type red cells was determined by a differential agglutination technique previously described (Odell *et al.*[20]). On day 1, the transfused cells were found to comprise 20 to 40% of the circulating population of red cells. This range includes the error (5 to 15%) to which all red-cell counting procedures are subject. Subsequent typing showed a gradual decrease of the donor-type cells. A few donor-type cells were found in all five rats at 61 days, while on day 67 a very few (less than 1%) donor cells were present in two rats.

**Discussion.** – The method used here placed the red-cell life span of rats at about 60 days, which is in good agreement with the majority of studies previously carried out with radioisotopes.

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[20] T. T. Odell, Jr., F. G. Tausche, and D. L. Lindsley, *Ann. N. Y. Acad. Sci.* **64**, 811 (1957).

# CELL PHYSIOLOGY

N. G. Anderson
J. F. Albright[1]
R. H. Shellhamer[2]
E. C. Horn[2]
W. Friedberg[1]
M. L. Anderson[2]
R. E. Canning
M. T. Guess

## Structure Formation by Liver Protein Solutions

N. G. Anderson M. L. Anderson

**Introduction.** – The production of artifacts during the course of tissue fixation and subsequent manipulation is well known. Less well appreciated is the fact that clear solutions of soluble tissue proteins can form a variety of structures not unlike many seen in living cells. This is an important consideration in the electron microscopy of isolated cell components, since some of the structures observed may have been produced in vitro. It is also of interest because the same mechanisms which produced the in vitro structures may be operative in structure formation in the living cell.

**Results.** – Clear extracts of rat liver were prepared as follows. Livers were perfused *in situ* with cold Locke's solution and homogenized in two volumes of cold 0.25 *M* sucrose. The homogenate or brei was centrifuged for 1 hr at 140,000 *g* to remove all particulate material. The top lipid layer was removed by suction, which resulted in a clear yellow solution containing no microscopically visible particles.

A slight turbidity appears after the solution has been standing for several hours in the cold, or after dialysis against isotonic sucrose, dilute pH 7.5 phosphate buffers, or against pH 5 acetate buffers. The structures seen after the first three treatments were concentrated by low-speed centrifugation and include (1) fine solid granules ranging from the limits of resolution of the phase contrast microscope to about 2 m$\mu$, and resembling mitochondria, (2) vesicles resembling superficially isolated nuclei but approximately 3 to 6 m$\mu$ in diameter, (3) a gel-like matrix material which often contained embedded particles, and (4) fibrils which tended to precipitate against the cover slip. At slightly lowered pH, more pronounced fibrils were formed, as shown in Fig. 16. These extend to the limits of resolution of the light microscope.

**Discussion.** – The importance of these results in the examination of fixed or fractionated tissues can only be assessed by extended studies with the electron microscope. The relation between these structures and intracellular ones can be approached by comparing the molecular compositions of the two. The authors propose to do this by means of serological and enzymatic studies. The fibrils produced by lowering the pH are of interest because spindle fibrils in living cells are rendered more easily visible by treating the cells with very dilute acid.

## Amino Acids and Peptides of Rat Liver Microsomes

N. G. Anderson J. F. Albright

**Introduction.** – There has been no conclusive demonstration that polypeptide intermediates are important in protein synthesis. To attack this problem and the allied question of similarity of chain segments in related proteins, methods for isolating peptides from the intracellular sites of protein synthesis (microsomes) and for separating them on ion exchange columns have been investigated.

**Results.** – In the initial series of experiments, microsomes were rapidly isolated from perfused rat liver and were extracted briefly with 1 *M* NaCl. The clear amber supernatant fluid resulting from centrifugation at 140,000 *g* for 2 to 3 hr contained an average of 17.2% of the microsomal nitrogen in seven experiments. Ultrafiltration through cellophane tubing, under nitrogen gas pressure, yielded a clear filtrate containing an average of 1.9% of the microsomal nitrogen. The free amino acids, as identified by paper chromatography after electrolytic desalting, were aspartic and glutamic acids, glycine, alanine, valine, and leucine (and/or isoleucine). A number of unidentified spots were also seen.

To avoid electrolytic desalting, ammonium formate was substituted for sodium chloride in the extraction procedure. The ammonium formate is removed by sublimation. With present facilities,

[1]USPHS Fellow.

[2]Consultant.

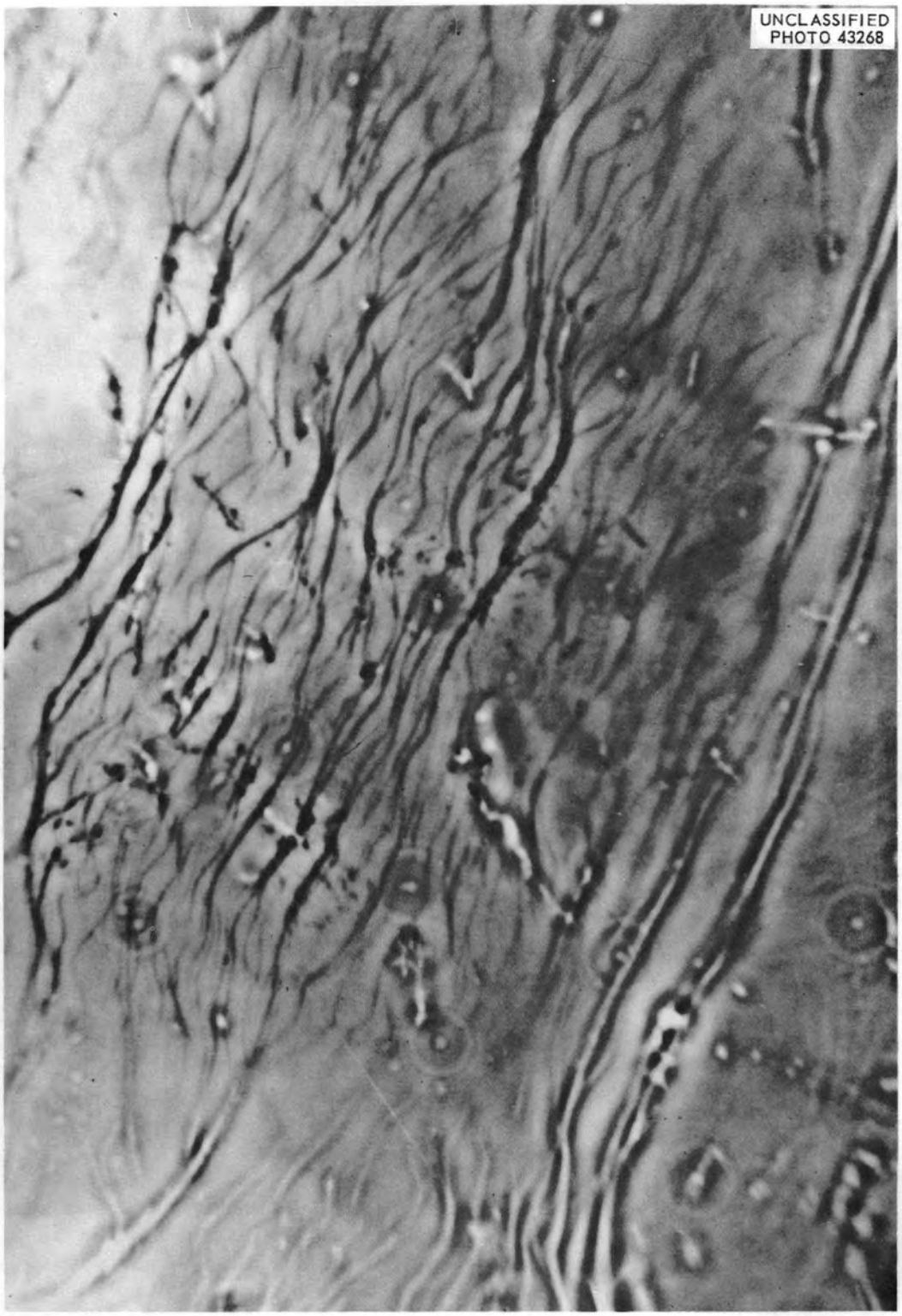


**Fig. 16. Fibrils Produced in Rat Liver Soluble-Phase Proteins by Dialysis Against 0.1 Ionic Strength pH 5 Buffer.**

24 rat livers may be processed in one day. In the ultrafiltrates obtained from these extracts, glutamic and aspartic acids, proline, alanine, valine, threonine, glycine, serine, cysteine, phenylalanine, and leucine (and/or isoleucine) have been identified. Again several ninhydrin-positive components were observed which were not known amino acids.

The filtrates from the ammonium formate extracts were fractionated by means of column chromatography on XE-64 cation exchange resin (Fig. 17). Elution with glass-distilled water resulted in the development of ten distinct peaks which were defined by passing the element through a micro-absorption cell and recording the changes in the absorption of light of 225-m$\mu$ wavelength.

Each of the fractions was collected separately and was further analyzed by quantitative ninhydrin estimations before and after acid hydrolysis. The optical density at 570 m$\mu$ may increase after acid hydrolysis by a factor of only 2 (peak P) or by as much as 12 times (peaks L and K); all the fractions react more intensely with ninhydrin after acid hydrolysis.

The absorption spectrum of each of the fractions was determined. Most of the fractions have an absorption maximum near 260 m$\mu$ as well as the broad maximum which extends from about 225 m$\mu$ down into the shorter ultraviolet region, which is characteristic of, but not specific for, peptide bonds.

The results of paper chromatography of some of the fractions before and after acid hydrolysis are presented in Table 21. The results show conclusively that these fractions do contain peptides. However, they do not reveal the precise composition of the peptides.

**Conclusions.** – The data presented do not permit a decision to be made regarding the source of the peptides under study. These peptides may be true intermediates in the path of protein synthesis, or they may be degradative products arising during the isolation and extraction procedure. This question must remain unanswered until results from isotope labeling experiments and analyses of other extraction procedures are at hand.

The absorption spectra of the fractions indicate, but do not prove, that some of the amino acids and peptides may be bound to purine and pyrimidine compounds. This possibility must be thoroughly investigated in view of the evidence implicating nucleic acids in protein synthesis and the general belief that nucleic acids serve as templates for protein reproduction.

## Studies on the Metabolism of Radioiodine-Labeled Tobacco Mosaic Virus in the Mouse and Rat

W. Friedberg

**Introduction.** – The resistance to metabolic breakdown of radioiodine-labeled TMV[3] (see Glossary) has been studied in the mouse and the rat.

**Results.** – The virus was labeled in vitro, and 0.5 mg per mouse was administered by tail-vein injection. Mice were killed at approximately weekly intervals, and the radioactivity of liver protein and intact spleen was measured in a well-type scintillation counter. To prevent re-utilization of released radioiodine, iodide was administered by intraperitoneal injection or in the drinking water. Thirty-five days after administration of the labeled virus, 0.34 and 0.052% of the injection dose persisted in the liver protein and spleen, respectively. In both the organs studied, the virus showed an increased biological half life ($T_{1/2}$) with increase in time. During the last two weeks of the experiment the $T_{1/2}$ of the virus was 30 days in the liver and 10 days in the spleen. Apparently the ability of the virus to persist is not related to the antibody-producing potentiality of the organ.

These results suggested the next experiment, that is, the determination of the role of mitotic activity in the turnover of the labeled virus. A comparison was made between normal livers of mature rats, where mitotic activity is low, and the regenerating liver of partially hepatectomized rats. Radioiodine-labeled TMV was administered via the saphenous vein by remote-control injection, and after ten days the median and left lobes of the liver were removed from half the rats. After another seven days all the animals were killed. (All the animals had been administered iodide in their drinking water as in the previous experiment.) Only preliminary results have been obtained so far; however, radioactivity comparisons between the entire regenerated liver and the right lobes of the normal livers indicate no significant difference

[3]The tobacco mosaic virus was a gift of I. Rappaport, Department of Botany, University of California, Los Angeles.

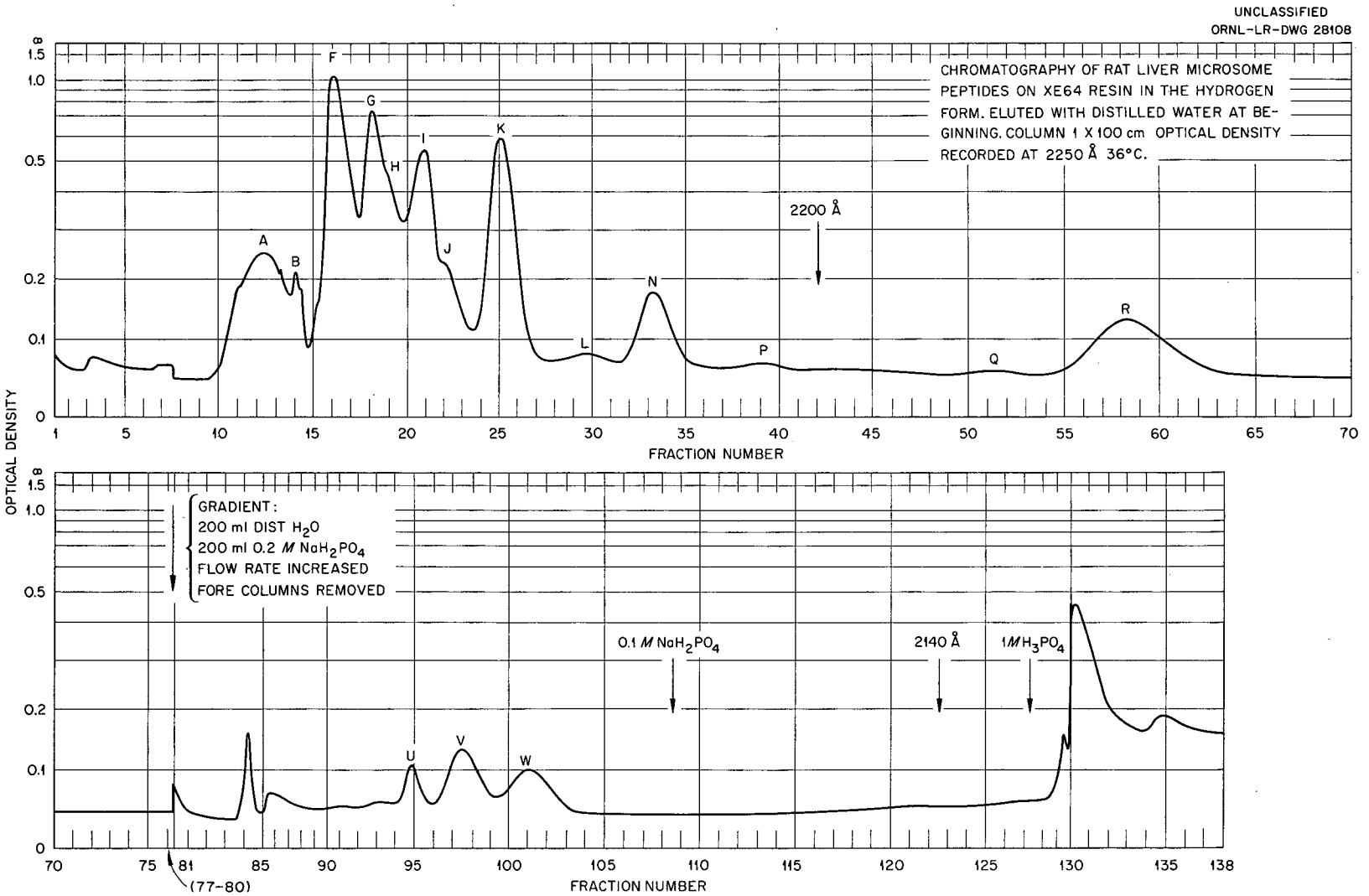


**Fig. 17. Chromatography of Rat Liver Microsome Peptides on XE-64 Ion Exchange Resin. Column dimensions: 1 × 100 cm; 36°C; water elution at start; 2250-Å absorption plotted against tube number.**

Table 21. Amino Acids in Fractions Eluted from Ion Exchange Column Before and After Acid Hydrolysis

| Column Fraction | Amino Acids Present Before Hydrolysis | Amino Acids After Hydrolysis | Amino Acids Liberated by Hydrolysis |
|---|---|---|---|
| F | Glu, Asp | Glu, Asp, Pro, Lys | Pro, Lys |
| G | Ala, Ser, Thr, Gly, Glu, Cys | Ala, Ser, Thr, Gly, Glu, Asp, Cys | Asp |
| I | Pro, Ala, Val, Glu | Pro, Val, Ala, Glu, Arg, Asp, Ser, Leu, and/or Iso | Arg, Asp, Leu and/or Iso |
| N | Phe, $\beta$-Ala(?), Ala | Phe, Ala, Val, Tyr, Thr, Glu, Gly, Ser, Asp, Cys, Leu/Iso | Val, Tyr, Thr, Glu, Gly, Ser, Asp, Cys, Leu/Iso |
| P | Phe | Phe, Val, Ala, Thr, Glu, Gly, Ser, Asp, Lys, Leu/Iso | Val, Ala, Thr, Glu, Gly, Ser, Asp, Lys, Leu/Iso |

in labeled virus turnover due to increased mitotic activity. Therefore, to generalize, it appears that the ability of the virus to persist better in one tissue than in another is not due to differences in mitotic activity.

The possibility that some of the radioiodine measured was not attached to virus protein is under study. Radioactivity was found in the hair of the rats, but the specific activity was considerably lower than in the liver and spleen. The thyroids also had some activity, but here again the specific activity was low. In the case of the thyroid, with its great ability to concentrate iodine, the iodide in the drinking water appeared to be effective in blocking re-utilization of released radioiodine. Butanol extracts of the livers contained no significant amount of radioactivity, indicating that the radioiodine in this tissue is not bound as an iodinated thyronine or as diiodotyrosine. Protein powders of the liver had very high specific activity, but additional measurements need to be made. Blood levels of radioactivity were only slightly above background and could not account for the radioiodine activity in any of the tissues studied.

**Conclusion.** – The ability of the TMV to persist is not related to the antibody-producing ability of the organ and is not greatly influenced by mitotic rate. The possibility that some of the radioiodine label may have been lost from the virus and attached to another molecule in the cell is under investigation.

# BIOCHEMISTRY

## NUCLEIC ACIDS, ENZYMES, AND RADIATION-PROTECTIVE COMPOUNDS

W. E. Cohn
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R. Shapira
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### Minor Constituents of Ribonucleic Acids

W. E. Cohn A. J. Bandy

The identity of the new pyrimidine nucleotide isolated from RNA (see Glossary) (the "fifth" nucleotide of RNA of Davis and Allen) has not yet been established, nor has it been proved conclusively that the sugar is ribose or that it is attached in the usual position to what is almost certainly a pyrimidine ring.

Hydrazinolysis has been used to destroy the pyrimidine ring (method of Baron and Brown), the reaction being followed by the loss in ultraviolet absorption. The residue, with and without acid treatment, has been chromatographed in the double solvent system used by Tolbert and has been examined directly for sugars. No amounts commensurate with the amounts of nucleotide degraded have been found, although smaller areas reacting with nonspecific carbohydrate sprays (e.g., aniline phthalate) have been found.

Hydrogenation of the nucleoside gives a product that, although retaining its spectrum (and hence not hydrogenated in the 5,6 position of uracil), migrates differently in both paper and column chromatography. It is proposed to explore this product further.

Treatment with anhydrous HF, a procedure devised and performed by D. Lipkin at Washington University, splits normal pyrimidine nucleotides to the free base, sugar (and some degradation products), and phosphate; our compound does yield an altered material which chromatographs very much like 5-hydroxymethyl uracil and has a spectrum almost identical with it. (It should be recalled that the spectrum of the undegraded nucleotide is quite similar to that of 5-hydroxymethyl uracil, also.) Here is another reaction of promise that, as with the hydrogenation reaction, awaits the production of more material for its further development.

In the meantime, at least one of the other minor constituents found in the digests of RNA used to prepare "X-uridylic" acid has been identified as a 6-substituted adenylic acid. The base derived from this nucleotide migrates on columns and on paper with 6-methylaminopurine and not with adenine, 2-methyladenine, or 6-dimethylaminopurine. Its spectral characteristics are nearly identical with those of 6-methylaminopurine.

The true role of both the above constituents (and of at least one other new nucleotide being investigated) in RNA structure is being investigated by isolation of polynucleotide material from RNase digests and of 5′ nucleotides (or nucleosides) from snake venom digests. The results of these tests will indicate whether the new minor constituents are indeed linked in the usual manner.

### RNA-Phosphorus Metabolism in *Escherichia coli* B

E. Volkin J. L. Countryman
M. H. Jones

Previous studies with bacteriophage T2-infected *Escherichia coli* B, where net synthesis of RNA is prevented, have shown an active turnover of one or more species of RNA, apparently representing only a small fraction of the total RNA.

The activity of this RNA may in some way be specifically related to the synthesis of materials necessary for phage production, but it is also possible that similar RNA activity is of importance whenever active synthesis of cellular material occurs.

Uninfected cultures of *E. coli* B were grown for varying periods of time in broth containing $P^{32}$-labeled inorganic phosphate, after which the nucleic acids were extracted and analyzed. Cultures in the lag, late lag, and logarithmic phases of growth were used. Synthesis of RNA begins early in the lag period and continues more rapidly than synthesis of DNA until an RNA/DNA ratio of about 7:1 is reached, toward the end of the lag period. This ratio remains constant during the logarithmic phase while vigorous synthesis of both DNA and RNA takes place.

When the cells in the logarithmic phase of growth assimilate $P^{32}$ orthophosphate continuously, an RNA/DNA specific activity ratio of at

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least 2 is observed initially. This value declines rapidly and then remains constant at about 1.3 instead of becoming unity. However, if only a limited isotope incorporation period is permitted, the initially high ratio drops to 1 within 8 min after removal of the isotope.

Subcellular fractions of logarithmic *E. coli* B were obtained by alumina grinding and subsequent differential centrifugation of cells which had incorporated $P^{32}$ for short periods of time. (In the material below, P1 = pellet at 20,000*g*, 20 min; P2 = pellet at 140,000*g*, 90 min; S = soluble fraction.) Cells grown in the presence of the isotope for 5 min have relative specific activities of the RNA's of P1, P2, and S of about 3.0, 1.0, and 1.5, respectively, but after another 30 min of isotope incorporation these values converge toward unity. When the isotope is removed after a short incorporation period, there is an immediate and persistent drop in the specific activity of P1-RNA, while the specific activities of P2- and S-RNA continue to rise for at least another 5 min before declining. Fifteen minutes after removal of the isotope, the specific activity of P2-RNA becomes the same as that of S-RNA, and the specific activity of P1-RNA declines to a point substantially below this value; P1-RNA, which is most sensitive to changes in isotope content of the culture, constitutes less than 5% of the total bacterial RNA. It is this fraction that exhibits the highest RNA turnover rate in the T2-infected system.

Thus, some heterogeneity of RNA metabolic activity has been demonstrated in uninfected bacteria, although turnover of RNA cannot yet be determined.

### RNA Turnover and DNA Synthesis in Bacteriophage-Infected Bacteria

L. Astrachan E. Volkin

A relation between RNA turnover and DNA synthesis has been indicated previously by experiments with *Escherichia coli* infected with bacteriophage T2. It has been demonstrated that the presence of chloramphenicol, added shortly after infection, not only inhibits DNA synthesis but also prevents RNA turnover, in that $P^{32}O_4$, which can be incorporated into RNA, is not subsequently released. Upon removal of the chloramphenicol, $P^{32}$ is released from RNA and incorporated into DNA. It has now been shown that if the labeled, infected cells are washed to reduce the acid-soluble radioactivity, $P^{32}$ subsequently incorporated into DNA comes, however devious the path, almost exclusively from the isotope previously found in RNA. However, a direct correlation between DNA synthesis and RNA turnover is not possible, since the rate of RNA turnover decreases considerably with time after infection, while the rate of net DNA synthesis increases. The RNA turnover rate is greatest immediately after infection and then decreases until it reaches a steady value 16 min after infection. Since mononucleotides released from RNA by alkaline hydrolysis have unequal specific activities, even when the RNA is synthesized immediately after infection, it is probable that the early burst of RNA metabolism is caused by the rapid formation of an RNA specific to the phage-infected host.

### Structure and Activity in Radiation-Protective Sulfhydryl Compounds

D. G. Doherty R. Shapira
T. W. McKinley, Jr.

The three most interesting compounds, AET, APT, and APMT,[2] previously shown to provide effective radiation protection when administered orally or intraperitoneally were investigated when given by the intravenous route. These isothiourea salts were dissolved in water and injected slowly into the tail vein of 101 × C3H mice. The chemical toxicities were the following: for AET, $LD_0$ = 110 mg/kg, $LD_{50}$ = 140 mg/kg; for APT, $LD_0$ = 135 mg/kg, $LD_{50}$ = 200 mg/kg; for APMT, $LD_0$ = 250 mg/kg, $LD_{50}$ = 300 mg/kg. It is interesting to note that, contrary to the findings for other routes of administration, AET is more toxic intravenously than APT. The slower conversion of APT to mercaptopropylguanidine at neutral pH may, in part, account for the decreased toxicity relative to AET. The relative effectiveness remains about the same, since 40 mg of AET per kilogram is required for 100% survival at 30 days, whereas 20 mg of APT per kilogram is required when given 20 min prior to 900 r of total-body

[2] D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 77–78; *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 63–64.

x irradiation. The radiation $LD_{50}$ for both compounds, however, is lower than that for other routes of administration, that is, 1200 r for AET and 1300 r for APT, intravenously, vs 1500 r for both compounds, orally. This decreased activity at high radiation dose levels is probably due to a lower concentration of protective agent in the gut when the chemicals are given intravenously than when they are given orally or intraperitoneally. In contrast with AET and APT, APMT does not lose its effectiveness upon intravenous administration, the minimum protective dose against 900 r being 50 mg/kg, while 200 mg/kg permitted a radiation $LD_{50}$ of 1100 r, comparable to that for the oral or intraperitoneal route. Studies made with labeled AET and APT showed that they were rapidly eliminated after intravenous administration. In accord with this finding, the radiation $LD_{50}$ 1 hr after administration was 900 r for AET and 1000 r for APT.

### Hydrolysis of Carboxyalkylisothioureas by Transamidinase and Arginase

D. G. Doherty R. Shapira

The transfer of the amidine group of isothioureas to amines is a well-known chemical preparative method for guanidines. The similarity between this reaction and the enzymic transfer of the amidine group in transamidination and in the formation of urea from arginine by arginase, as well as the structural similarities between the alkylguanidines and alkylisothioureas, prompted an investigation of the susceptibility of the alkylisothioureas to enzymic attack. The second ammonium sulfate fraction prepared from hog kidney cortex by the procedure of Ratner and Rochovansky[3] was used as the source of the transamidinase; bovine liver arginase was obtained commercially. The substrates were carboxyalkylisothioureas with chain lengths of two to six carbon atoms, and the extent of reaction was determined by a colorimetric sulfhydryl group estimation and by a manometric urea determination using urease. The optimum chain length for amidine transfer by transamidinase at pH 7.2 was four, represented by $\gamma$-carboxypropylisothiourea, the relative activities being 4C = 100, 5C = 63, 3C = 10, 6C = 7, and 2C = 2. In the absence of an acceptor such as glycine, urea was formed; with glycine present no urea was found, the product being presumably guanidoacetic acid. The arginase activity of this preparation was negligible at pH 7.2. In contrast, the arginase preparation did not appreciably hydrolyze these substrates below pH 8.5. In addition, the order of activity with respect to chain length was inverted, the five-carbon $\gamma$-carboxybutylisothiourea being the most susceptible. Thus it seems that transamidinase may also have arginase-like activity and, in the absence of an amino acid acceptor, can transfer the amidine group through an enzyme-amidine complex to water to form urea. A more highly purified enzyme preparation is essential if the details of this transfer process are to be verified.

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[3] S. Ratner and O. Rochovansky, *Arch. Biochem. Biophys.* 63, 277–95 (1956).

# ENZYMOLOGY AND PHOTOSYNTHESIS

G. D. Novelli M. J. Cormier P. Rogers, Jr.[1] A. N. Best
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## Purification of the Leucine-Activating Enzyme from *Escherichia coli*

G. D. Novelli A. N. Best

**Introduction.** – In the previous report,[3] it was stated that the tryptophan-activating enzyme from beef pancreas, although specific for tryptophan in the PP-ATP (see Glossary) exchange reaction, was highly nonspecific in the synthesis of ATP from a large number of aminoacyl adenylates. Since it had been previously found[4] that the leucine-activating enzyme from *Escherichia coli* exhibits specificity for leucyl-AMP in ATP synthesis when only a limited number of synthetic aminoacyl adenylates were available, it was felt that the specificity of this enzyme should be re-examined with a greater number of substrates. Therefore an investigation was started in order to improve the isolation and purification of the leucine-activating enzyme from extracts of *E. coli.*

**Results.** – *Escherichia coli* B was grown and harvested, and the crude extracts were prepared as previously described.[5] In attempting to improve the purification procedure, treatment of the extract with streptomycin sulfate was substituted for treatment with RNase for removal of the nucleic acid. The streptomycin sulfate treatment led to large losses in activity, and subsequent ammonium sulfate fractionation became highly erratic. Likewise, isoelectric precipitation did not allow for clean fractionation. It was eventually found that a combination of steps involving RNase treatment, ammonium sulfate fractionation, and treatment with calcium phosphate gel led to about a four- to tenfold purification. During the course of this study it was noted that the radioactive inorganic PP contained some insoluble material. After several trials the insoluble material was shown to be metaphosphate. When the PP solutions were purified by column chromatography over Dowex 1, and the fractions of *E. coli* were retested with the purified PP, the specific activities were found to be greater than those originally observed. Thus the over-all purification of the leucine-activating enzyme may be nearer tenfold than fourfold.

**Discussion.** – Now that it is known that the presence of metaphosphate in the PP preparations may suppress the expression of full enzymatic activity in the assay, the purification of the leucine-activating enzyme may be expected to proceed much more rapidly.

## Glycine Activation by Extracts of *Photobacterium fischeri*

M. J. Cormier G. D. Novelli

**Introduction.** – During comparative studies of the activation of amino acids, as measured by the exchange of inorganic PP with ATP or by the ATP-dependent formation of aminoacyl hydroxamates in extracts of *Ph. fischeri*, glycyl hydroxamate occurred without a corresponding glycine-stimulated PP-ATP exchange. This observation suggested that the mechanism of activation of glycine by these extracts might not involve the intermediate formation of glycyl adenylate. In order to resolve this point, an enzyme that catalyzes the ATP-dependent formation of glycyl hydroxamate has been purified seventeenfold from extracts of *Ph. fischeri.*

**Results.** – The chemical requirements for the formation of glycyl hydroxamate by the purified enzyme are glycine, ATP, $MgCl_2$, and hydroxylamine. The requirement for $Mg^{++}$ is not specific, since $Mn^{++}$ is equally effective. The hydroxamic acid was identified as glycyl hydroxamate by cochromatography with authentic glycyl hydroxamate on paper. All attempts to accumulate an intermediate in glycine activation have failed, thus indicating an enzyme-bound intermediate or an equilibrium favoring the backward reaction. The pH optimum for the enzyme lies at about 7.6. The enzyme appears to contain sulfhydryl groups, since $5 \times 10^{-5}$ *M* PCMB produces a 50% inhibition

[1] Fellow of the American Cancer Society.

[2] Research associate.

[3] R. A. McRorie and G. D. Novelli, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 82.

[4] J. A. DeMoss, S. M. Genuth, and G. D. Novelli, *Proc. Natl. Acad. Sci. U.S.* **42**, 325–32 (1956).

[5] J. A. DeMoss and G. D. Novelli, *Biochim. et Biophys. Acta* **22**, 49–61 (1956).

of the activity, and since cysteine partially reverses this inhibition. Specificity studies have indicated that the enzyme is specific for glycine. The purified enzyme does not support a glycine-dependent PP-ATP exchange, which suggests that the mechanism of this glycine-activating system is different from those amino acid activations which proceed through the intermediate formation of aminoacyl adenylates. From phosphate balance studies it has been shown that 1 mole of Pi is liberated from ATP for each mole of hydroxamate formed. Furthermore, an ATP-generating system can serve as an energy source for the activation of glycine. These observations strongly suggest that glycine is being activated in this system via a kinase type of reaction. The purified enzyme catalyzes a glycine-independent exchange of Pi with ATP, which is not affected by the addition of a mixture of 20 naturally occurring L-amino acids or hydroxylamine. It has also been observed that the Pi-ATP exchange reaction is purified along with the glycine-activating enzyme.

**Discussion.** – The failure to observe a glycine-dependent PP-ATP exchange eliminates the participation of glycyl adenylate as an intermediate in glycine activation. Likewise, the failure to observe a glycine-dependent Pi-ATP exchange eliminates the activation of glycine in a *primary step* which involves the elimination of Pi from ATP. Two possible reaction mechanisms that are not excluded by these experiments are illustrated below:

Scheme I

$$\text{E} + \text{ATP} \rightleftharpoons \text{E-ADP} + \text{Pi}$$

$$\text{E-ADP} + \text{glycine} \rightleftharpoons \text{E-glycine} + \text{ADP}$$

Scheme II

$$\text{E} + \text{ATP} + \text{glycine} \rightleftharpoons \text{E-glycylphosphate} + \text{ADP}$$

Scheme I represents the activation of the enzyme through interaction with ATP and the elimination of Pi. The ADP moiety on the enzyme is thus replaced by glycine, which replacement leads to the activation of glycine. The Pi-ATP exchange observed to occur in the absence of added glycine would be consistent with this scheme. Scheme II is a representation that is similar to what is generally believed to be the mechanism of glutamate activation in the glutamate-synthetase reaction. There is no information concerning the metabolic role of this glycine-activating system. Further work will be required to elucidate the exact mechanism of glycine activation and its metabolic function.

### Studies on the Synthesis of Ornithine Transcarbamylase by *Escherichia coli*

P. Rogers, Jr. G. D. Novelli

**Introduction.** – In a previous report,[6] the synthesis of OTC in suppressed cells of *Escherichia coli* was discussed as being a suitable system for the study of the *de novo* synthesis of a specific protein. The present study was designed to obtain information concerning the requirements for the synthesis of this enzyme in suppressed cells and protoplasts as a background for attempts to develop a cell-free system.

**Results.** – Suppressed cells are obtained by growing *E. coli* in a medium supplemented with 200 $\mu$g of arginine per milliliter. When such cells are harvested, washed, and then resuspended in a medium in the absence of arginine, a rapid synthesis of OTC begins immediately (without a lag, in contrast to the kinetics of induced enzyme synthesis) and reaches a maximum in about 60 min. Protoplasts of arginine-suppressed cells were prepared by the penicillin method of Lederberg[7] and by a modification of the lysozyme-Versene technique of Fraser and Mahler.[8] The synthesis of OTC by protoplasts exhibits the same kinetics as do the whole cells, except that the final level of enzyme activity is usually one-half that obtained with whole cells, and the synthesis proceeds at about one-third the rate observed with whole cells. Both cells and protoplasts require phosphate, $Mg^{++}$, and an energy source such as glucose. The addition of ATP or HDP did not improve the synthesis. While whole cells can effect a good synthesis with $(NH_4)_2SO_4$ as the nitrogen source, protoplasts require a complete mixture of amino acids for a complete expression of their synthetic capacity. Enzyme synthesis is stimulated by the addition of purines and pyrimidines, but not by 5′-mono- and triphosphonucleotides. The OTC formation occurs equally

[6] J. A. DeMoss and G. D. Novelli, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 83.

[7] J. Lederberg, *Proc. Natl. Acad. Sci. U.S.* **42**, 575 (1955).

[8] D. Fraser and H. R. Mahler, *Arch. Biochem. and Biophys.* **69**, 166 (1957).

well under aerobic or anaerobic conditions, but in either case the synthesis is markedly stimulated by shaking the suspension. Treatment of protoplasts with 6-mercaptopurine, ribonuclease, or chloramphenicol results in a strong inhibition of enzyme synthesis. Whole cells are completely prevented from synthesizing OTC by arginine concentrations as low as 20 μg/ml. However, with lysozyme protoplasts the enzyme synthesis cannot be suppressed below 20% of maximum by arginine concentrations as high as 1 mg/ml. Ornithine at 100 μg/ml has little or no effect on enzyme synthesis in either cells or protoplasts. Carbamylphosphate (4 μmoles/ml) does not suppress enzyme synthesis, although it causes a marked inhibition of growth. Citrulline, on the other hand, does suppress enzyme synthesis, presumably owing to its conversion to arginine.

**Discussion.** – The present studies indicate that the appearance of ornithine transcarbamylase activity when suppressed cells or protoplasts are incubated in the absence of arginine represents the synthesis of new enzyme molecules. The requirement for a nitrogen source and an energy source, together with inhibition by chloramphenicol, supports this view. Experiments with lysozyme protoplasts seem to magnify in general the requirements noted in cells. The stimulation by amino acids, by purines and pyrimidines, and by $Mg^{++}$ indicates that protoplasts act like leaky cells but can still synthesize considerable protein when properly supplemented. The kinetics of the synthesis of OTC (which starts immediately without a lag) suggests that if an RNA template is involved in the synthesis, then it (the template) might have been made during the growth phase and prevented from acting because of the presence of arginine. This suggestion implies that growth under conditions of enzyme suppression may be a way to separate, in time, the synthesis of specific RNA from the synthesis of the protein that is regulated by the RNA. Experiments to test this hypothesis are in progress.

### Studies on the Relation Between Ribonucleic Acid and Protein Synthesis

G. D. Duda G. D. Novelli

**Introduction.** – Several groups have observed that amino acid auxotrophs of *Escherichia coli*[9–11] can synthesize RNA, when protein synthesis is largely inhibited by the presence of chloramphenicol, only when the required amino acid is supplied. This finding has led to the idea that the activated amino acids (aminoacyl adenylates) may be a common intermediate in the synthesis of both RNA and protein. These compounds can be regarded on the one hand as activated amino acids that will become protein, or as activated nucleotides that will become nucleic acid. The investigation of this phenomenon in various cell preparations may provide information on the nature of RNA synthesis, particularly as it occurs during enzyme induction.

**Results and Discussion.** – The synthesis of the inducible enzyme β-galactosidase has been investigated in whole cells, protoplasts, and lysates of *E. coli* w. Contrary to the usual reports,[12] no requirement for $Mg^{++}$ could be found in any system. The lysates differ from the other two preparations in that amino acids are the only suitable nitrogen source. The RNase had no striking effect on protein synthesis in the preparations. In confirmation of the reports of Gros, protein synthesis in a leucine auxotroph of *E. coli* was found to be inhibited by chloramphenicol, while RNA synthesis was stimulated. Investigations are now in progress on the nature of the RNA synthesized in the presence of chloramphenicol, particularly with reference to the role of activated amino acids or nucleotides in the synthesis.

### Catalysis of the Formation of Amino Acid Hydroxamates by Extracts of Beef Tissue

M. P. Stulberg G. D. Novelli

**Introduction.** – An examination of data previously accumulated by this laboratory[13] has repeatedly demonstrated that certain amino acids do not exhibit the amino-acid-dependent PP-ATP exchange reaction, which is one of the manifestations of the

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[9] F. E. Hahn *et al.*; *Biochim. et Biophys. Acta* **26**, 469 (1958).

[10] F. Gros and F. Gros, *Biochim. et Biophys. Acta* **22**, 200 (1956).

[11] F. C. Neidhardt and F. Gros, *Biochim. et Biophys. Acta* **25**, 515 (1957).

[12] S. Spiegelman in *A Symposium on the Chemical Basis of Heredity* (ed. by W. D. McElroy and B. H. Glass), p 232–75, Johns Hopkins Press, Baltimore, 1957.

[13] G. D. Novelli, *Proc. Natl. Acad. Sci. U.S.* (in press).

postulated mechanisms for amino acid activation. With few exceptions, alanine, glycine, arginine, proline, and threonine react in amino acid activation systems from various sources to produce their corresponding hydroxamates but do not exhibit a PP-ATP exchange reaction. In addition, the amino acids which display an active PP-ATP exchange reaction make up only a small part of the total amino acid content of known proteins. Therefore it seemed possible that a significant number of amino acids may be activated by a mechanism other than that postulated for nine or ten amino acids.[14] An effort was made to isolate and purify the enzymes responsible for hydroxamate formation without the accompanying PP-ATP exchange in order to further study the mechanism of their activation.

**Results.** – Assays of beef liver, heart, and kidney exhibited amino-acid-dependent hydroxamate formation for alanine, glycine, proline, arginine, and threonine. These amino acids had previously been shown to be almost completely inactive in the PP-ATP exchange reaction in *Escherichia coli* and guinea pig liver.[13] Further fractionation of these activities by $(NH_4)_2SO_4$-acid precipitation techniques produced somewhat purer systems, but in all cases activities, though significant, were quite low.

**Discussion.** – These studies have demonstrated that the activation of glycine, alanine, proline, threonine, and arginine, as measured by hydroxamate formation, occurs in various beef tissues. The isolation of enzymes catalyzing this activation would require large quantities of starting material as well as an elaborate procedure because of the small activities found.

In order to pursue these studies further, it is necessary to have available a better source of starting material. The authors propose to examine a number of other tissues by means of the hydroxamic acid technique in the hope of finding a source of starting material that is sufficiently active to make an enzyme isolation attempt worth while.

[14] G. D. Novelli and A. N. Best, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 81.

### Photosynthesis

W. A. Arnold H. K. Sherwood

**Introduction.** – Experiments made in A. Szent-Györgyi's laboratory, last summer, show that muscle fibers have a magnetic asymmetry as large as that observed for many crystals. A report has been published in the *Proc. Natl. Acad. Sci. U.S.*

**Results and Discussion.** – The glow curves made with dried chloroplasts have been analyzed in order to determine the activation energies. The results were presented at the National Academy of Sciences–National Research Council *Symposium on the Photochemical Storage of Energy*, last September.

At the present time, measurements are being made on the photoconductivity of chlorophyll and carotene.

# MICROBIOLOGY

## TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson E. F. Phares
M. I. Dolin M. V. Long

### A Bacterial "Carbon Dioxide Enzyme"

E. F. Phares S. F. Carson
M. V. Long

**Introduction.** – Freshly prepared cell-free extracts of either *Propionibacterium pentosaceum* or *Veillonella gazogenes* can decarboxylate succinate, yielding $CO_2$ and propionate in equimolar amounts, whereas aged extracts do not. However, when aged extracts of both organisms are combined, the reaction goes to completion.[1,2] The *P. pentosaceum* extract contains the decarboxylase; the *V. gazogenes* extract contains an enzyme system (the "$CO_2$ enzyme") that converts the $C_1$ fragment, resulting from the decarboxylation reaction, to $CO_2$. A substantial part of the latter was found in the particulate fraction. We are studying an organism closely related to *V. gazogenes*, *Micrococcus lactilyticus* 221, and find the "$CO_2$ enzyme" from this organism to be exclusively particulate. The other components of the complete system can be demonstrated in the soluble fraction of any of the three organisms: the succinate-activating system, the CoA (see Glossary) transphorase, and the decarboxylase.

**Results and Discussion.** – The particulate fraction, containing the "$CO_2$ enzyme," is obtained by (1) centrifuging a cell-free extract of *M. lactilyticus* for 1 hr at 20,000*g*, and by (2) centrifuging the supernatant from the latter for 5 hr at 140,000*g*. The resulting pellet consists of three main components with Svedberg units of 11, 20, and 36; all these are of approximately equal specific activity with regard to the "$CO_2$ enzyme." The "activity" of the particulate fraction could not be obtained in a soluble form.

The soluble components of the system have been purified from cell-free extracts of *M. lactilyticus*. The CoA transphorase and the succinate-activating enzyme were partially purified by gel adsorption and elution and by $(NH_4)_2SO_4$ precipitation, followed by zone electrophoresis on starch. Each of these enzymes has been resolved from the other enzyme activities of the system. The succinic decarboxylase is less stable and does not stand salt and gel treatment so well as the other two enzymes; it has, however, been possible to partially resolve it from the transphorase and activating enzymes by zone electrophoresis.

### Properties of a Four-Electron Transfer DPNH Oxidase from *Clostridium perfringens*

M. I. Dolin

**Introduction.** – A four-electron DPNH oxidase (see Glossary) from the anaerobe *Clostridium perfringens* is being studied: (1) in order to obtain information on the role of electron transport to oxygen in the metabolism of obligate anaerobes, and (2) in order to learn something of the mechanism by which molecular oxygen is cleaved in a four-electron transfer reaction. Soluble four-electron-transfer oxidases[3] are known for ascorbate and catechol but have not been described for DPNH previous to the work with *Cl. perfringens*.[4] The "typical" DPNH oxidase catalyzes a two-electron transfer via flavin, with peroxide as the product. Four-electron transfer during DPNH oxidation (with water as the product) is regarded as characteristic of particle-bound oxidases, in which cytochrome oxidase is the terminal, oxygen-activating enzyme.

**Results.** – Initial enzyme extracts of *Cl. perfringens* have as high a specific activity for DPNH oxidation as do extracts of the strongly aerobic organism, *Azotobacter vinelandii*. On storage in the frozen state, however, the oxidase activity of the clostridial enzyme decays rapidly over the course of several weeks, while the cytochrome c reductase activity remains constant. Electrophoretic studies show that two species of cytochrome reductase can be distinguished in initial extracts: a slower moving component, devoid of oxidase activity, and a faster one which appears

[1] E. A. Delwiche, E. F. Phares, and S. F. Carson, *J. Bacteriol.* 71, 598–603 (1956).

[2] E. F. Phares, E. A. Delwiche, and S. F. Carson, *J. Bacteriol.* 71, 604–10 (1956).

[3] H. S. Mason, *Advances in Enzymol.* 19, 79 (1958).

[4] M. I. Dolin, *Bacteriol. Proc.* (*Soc. Am. Bacteriologists*), p 110 (1957).

to be identical with the oxidase. As the extract ages and oxidase activity decays, a greater proportion of the cytochrome reductase activity appears in the slower moving peak. Precipitation of the extract at pH 4.9 removes the slow moving component, leaving an oxidase that is electrophoretically indistinguishable from the remaining cytochrome reductase. The conversion of the oxidase to a cytochrome reductase devoid of oxidase activity can be brought about more rapidly (in minutes) by irradiation at 365 mμ. The cytochrome reductase formed has mobility and solubility characteristics similar to those of the cytochrome reductase formed during spontaneous decay of the oxidase activity.

Attempts to reverse the inactivation have not been successful, nor has any method of stabilizing the oxidase activity been found. Purification of the enzyme, therefore, is difficult. However, a rapid method for partial purification (based upon precipitation near the isoelectric point, followed by zone electrophoresis near the isoelectric point) yields preparations which have a turnover of 1000 moles of DPNH oxidized per minute per 100,000 g of protein, and a flavin content of about 0.3 mole per 100,000 g of protein. The oxidase activity is directly proportional to the content of bound flavin or FAD. Only one-half the total bound flavin, on a molar basis, is FAD, which suggests that there may be two different flavin prosthetic groups.

**Conclusion.** – The characteristics of the system described above suggest that the often-reiterated "characteristics" of flavoprotein oxidases (high $O_2$ requirement, formation of peroxide) are not in reality obligate properties of this class of enzymes. Water formed through the four-electron reduction of $O_2$ can be the product of a flavoprotein oxidase. It may be that such enzymes represent the first evolutionary attempts to adapt anaerobic organisms to a developing aerobic environment. The activated form of $O_2$ in this reaction is unknown. Simultaneous reduction of one molecule of $O_2$ by two bound flavin groups could result in the formation of $2H_2O$. Alternatively, some form of bound peroxide might be an intermediate. Whatever the mechanism, it appears that the very conditions that lead to loss of the four-electron transfer function of the oxidase lead also to its conversion to an enzyme that can still catalyze a one-electron transfer to cytochrome c, but which has physical properties different from those of the parent enzyme.

# PLANT BIOCHEMISTRY

N. E. Tolbert

A. H. Haber R. Rabson J. R. Loewenberg[1]

## Aging in Gamma-Irradiated Wheat Seedlings

N. E. Tolbert

**Introduction.** – In a previous report it was shown that photosynthetic processes in green plants were relatively insensitive to ionizing and ultraviolet radiation.[2] Equally insensitive to radiation was the photosynthetic process (greening) in etiolated plants when they were exposed to light.[3] In those two experiments, dosages of 150 kr of gamma radiation were necessary to produce a temporary inhibition of photosynthesis. After 24 hr, green plants returned to a normal rate of photosynthesis, and etiolated plants had greened normally. In this report some analyses are given of these irradiated plants for the subsequent survival period.

**Results.** – Etiolated plants, after exposure to an acute dose of 150 kr of gamma radiation, were kept in pots of soil in a constant-environment plant-growth chamber. For the first two to three weeks the plants appeared normal, but there was no new growth except for some general enlargement of the plant, probably from cell elongation. By the fourth week the plants were chlorotic, and fungal growth appeared on the dying leaves. At intervals, the respiration was measured, photosynthesis was examined by fixation of $C^{14}O_2$, and the products of the photosynthesis experiments were examined chromatographically (Table 22). The respiration rate increased at the end of the second week, part

[1]Research associate.

[2]L. P. Zill *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 101.

[3]F. B. Gailey and N. E. Tolbert, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 103.

**Table 22. Changes in Wheat Leaves After 150 kr of Gamma Radiation**

| Days After Irradiation | $Q_{O_2}$ ($\mu$l of $O_2$/hr/mg) | Total Soluble $C^{14}$ Fixed (c/m/mg tissue)* | Distribution (%) of $C^{14}$ in Solubles* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Sucrose | Glycine | Serine | Malic Acid | Glutamic Acid |
| 1 | 0.42 | 420 | 71 | Trace | 4 | 2 | 3 |
| 2 | 0.35 | 6610 | 71 | 1 | 9 | 1 | 2 |
| 5 | 0.29 | 9330 | 72 | 2 | 14 | 1 | 1 |
| 7 | 0.35 | 7600 | 62 | ** | 24 | 2 | 1 |
| 9 | 0.43 | 7110 | 51 | 6 | 30 | 3 | 1 |
| 12 | 0.59 | 4880 | 42 | 7 | 39 | 2 | 0.4 |
| 14 | 0.63 | 1870 | 22 | 9 | 49 | 5 | Trace |
| 16 | 0.71 | 2000 | 31 | 7 | 38 | 9 | Trace |
| 21 | 0.60 | 251 | 24 | 14 | 48 | 4 | 0 |
| 23 | 0.70 | 442 | 17 | 16 | 48 | 8 | 0 |
| 27 | 0.59 | 393 | 29 | 16 | 39 | 10 | 0 |

*All photosynthetic experiments were run under identical conditions for 1 hr in 1000 ft-c of light, in air enriched with tracer amounts of $C^{14}O_2$. The $C^{14}$ incorporated into the insoluble constituents was in each case approximately 20% of that incorporated into the soluble components.

**Included in the serine percentage.

of which may have been due to bacterial contamination on the dying leaves. The trend, even after the second day, was away from the normal pattern of sucrose synthesis and toward the incorporation of $C^{14}$ into glycine and serine. This same trend had previously been noted to a lesser degree in green plants immediately after irradiation.[2] During the last two weeks, several substances, such as glutamic acid, were not labeled during 1 hr of exposure to $C^{14}O_2$ in the light. Although the wheat plants had received a lethal acute dose of radiation, there was no inhibition of respiration or of photosynthesis, but growth was prevented. During the first two weeks after irradiation, there appeared on the epidermis of the leaves numerous droplets of a gummy exudate, which dried as beads and was collected and analyzed. Had the plants been watered by rain or spray, this phenomenon would not have been detected, since the exudate would have been washed off. The excreta contained much glucose, glutamine, and serine, as well as substantial amounts of fructose and many other amino acids. The exudation ceased between the second and third week when the photosynthetic rate dropped. During the first two weeks after irradiation, the leaves probably accumulated large amounts of the products of photosynthesis, since that process was not inhibited, although growth, which would have utilized the products, had ceased. This accumulation of photosynthesis products may also have been partially responsible for the drop in rate of photosynthesis and the change in the products of photosynthesis.

Between the second and fourth weeks, the plants showed a slow decline. No definite period of death occurred; rather, fungal growth slowly took over the leaves. This pattern of death in plants is in marked contrast to that found in animals exposed to massive dosages of acute radiation.

### Serine Biosynthesis in Plants During Photosynthesis

R. Rabson N. E. Tolbert

**Introduction.** – Experiments on the incorporation of the $C^{14}$ of $C^{14}O_2$ into glycine and serine during photosynthesis were described in an earlier report.[4] Additional experiments are herein reported which support the hypothesis that these two amino acids may arise from a two-carbon precursor derived from a sugar phosphate of the photosynthetic carbon cycle.

**Results.** – Coleus, soybean, and corn leaves were allowed to photosynthetically fix $C^{14}O_2$ for periods of 15 or 60 sec. Serine and alanine were isolated by a combination of paper and ion exchange chromatography and were then chemically degraded to determine the distribution of radioactivity in the carbon chain. In serine the radioactivity was nearly uniformly distributed among the three carbons, regardless of the period of photosynthesis. In contrast, the carboxyl carbon of alanine contained more radioactivity than either the $\alpha$ or $\beta$ carbon. In corn leaves after 15 sec of exposure to $C^{14}O_2$, the radioactivity was five times greater in the carboxyl carbon than in either of the other two carbons.

**Discussion.** – These results suggest that the three-carbon amino acids, serine and alanine, do not have a common precursor even though both are synthesized rapidly and in large amounts in the photosynthetic carbon cycle. Alanine was predominately carboxy-labeled in short-time experiments, as is phosphoglyceric acid. Thus alanine probably is derived from pyruvic acid, which in turn is formed from phosphoglyceric acid. The formation of serine, however, was via a pathway which will give a uniformly labeled compound. Thus the carbon chain of phosphoglyceric acid does not go intact into serine. One possible pathway of serine synthesis is as follows: pentose phosphate produces glycolaldehyde, which leads to glycolic acid, glyoxylic acid, glycine, and then serine.[5] The first two carbon atoms of ribulose diphosphate of the photosynthetic carbon cycle are uniformly labeled[6] in short-time photosynthesis and would thus give rise to uniformly labeled glycolic acid, glycine, and serine.

### Effects of Gibberellic Acid, Kinetin, and Light on the Germination of Lettuce Seed

A. H. Haber N. E. Tolbert

**Introduction.** – In a previous report it was mentioned that the stimulating effect of red light on

[4] N. E. Tolbert, R. Rabson, and P. C. Kerr, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 91.

[5] A. Weissbach and B. L. Horecker, in *A Symposium on Amino Acid Metabolism* (ed. by W. D. McElroy and H. B. Glass), p 741, Johns Hopkins Press, Baltimore, 1955.

[6] J. A. Bassham *et al.*, *J. Am. Chem. Soc.* **76**, 1760 (1954).

lettuce seed germination could be imitated by treatment with either gibberellins or kinins.[7-9] It has also been reported that far-red light, which inhibits germination, was ineffective on gibberellin-treated[7] or kinetin-treated[8] seeds. The authors previously demonstrated that the effects of gibberellic acid and kinetin on germination could be experimentally separated by temperature, gibberellic acid being ineffective at high temperatures, and kinetin at low temperatures.[7,10] In this project it was demonstrated that the red light effect can be experimentally separated from either the gibberellic acid effect or from the kinetin effect. The data also imply that the red–far-red photoreaction cannot be explained in terms of its regulating, within the seed, the production of endogenous gibberellin or kinin, substances which probably occur in higher plant tissues.[11,12]

**Results and Discussion.** – The temperature dependence of the activity of gibberellic acid and of kinetin was used to nullify their effects on germination after a 12-hr period at room temperature, during which time red and far-red light treatments were applied. During the 12-hr period neither gibberellic acid nor kinetin could reverse the far-red light inhibition of germination, although red light could. Thus the failure of other investigators to find a far-red effect upon seeds germinated in solutions of gibberellin or kinetin did not mean that gibberellin or kinetin uncoupled the germination mechanism from the red light requirement. Our experiments show that the action of red light can be experimentally separated from the action of either gibberellic acid or of kinetin. Considered together with the previous work,[7] it is now clear that the effects of the three germination-promoting agents (gibberellic acid, kinetin, and red light) can each be experimentally separated from the other two.[10] Consequently, the effect of the red–far-red photoreaction cannot be explained in terms of its having regulated endogenous levels of gibberellin-like or kinin-like substances.

[7]A. H. Haber and N. E. Tolbert, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 93–94.

[8]A. Kahn, J. A. Goss, and D. E. Smith, *Science* **125**, 645–46 (1957).

[9]C. O. Miller, *Plant Physiol.* **31**, 318–19 (1956).

[10]A. H. Haber and N. E. Tolbert, in *Proceedings of the International Conference on Photoperiodism in Plants and Animals* (to be published).

[11]B. O. Phinney *et al.*, *Proc. Natl. Acad. Sci. U.S.* **43**, 398–404 (1957).

[12]C. Danckwardt-Lilliesträm, *Physiol. Plantarum* **10**, 794–97 (1957).

## Distribution of Radioactivity Among Compounds Extracted from Lettuce Seeds Germinated in the Presence of $HC^{14}O_3^-$, $S^{35}O_4^{--}$, or $H_2P^{32}O_4^-$

A. H. Haber N. E. Tolbert

**Introduction.** – When dry lettuce seeds are given sufficient moisture, the time course of germination can be divided into the following phases: imbibition, activation, mitosis, protrusion of the radicle, and growth.[13] Although various enzyme activities have been found in extracts of seeds during the course of germination, very little is known of the metabolism of intact seeds during the phases before protrusion. However, it has been shown that lettuce seeds respire in the earliest phases of germination.[13] In attempts to explore the metabolic events occurring during germination, seeds were germinated in solutions containing $HC^{14}O_3^-$, $S^{35}O_4^{--}$, or $H_2P^{32}O_4^-$. At various times the seeds were extracted in boiling 85% alcohol and hot water, and the extracts were analyzed by chromatography. Under conditions of room temperature and lighting, the seeds used in these experiments entered the protrusion phase around 15 hr after the beginning of imbibition.

**Results and Discussion.** – Imbibition of water was complete after 4 hr and was unaffected by exposure to far-red light, either in the presence or absence of germination-promoting chemicals (gibberellic acid, kinetin, or thiourea).

The $C^{14}$ was fixed from $NaHC^{14}O_3$ into soluble compounds after only 1 hr of imbibition. Analysis of extracts after 3 hr of imbibition showed that about one-third the total fixed activity appeared in malic acid and that 12 to 15% appeared in glutamine, aspartic acid, citric acid, or glutamic acid. Other labeled compounds separated were asparagine, serine, glycine, succinic acid, fumaric acid, and glyceric acid (in decreasing order of activity). Alanine was not appreciably labeled. Although changes in patterns of distribution of $C^{14}$ among soluble compounds could be detected during the course of germination, the over-all qualitative patterns were similar during all phases

[13]M. Evenari, *Symposia Soc. Exptl. Biol.* **11**, 21–43 (1957).

of germination, even after protrusion of the radicle. The data suggest that the $C^{14}$ was fixed primarily by exchange carboxylations into organic acids and that the tricarboxylic acid cycle was operative in the earliest phases of germination. The total $C^{14}$ fixed into soluble compounds per seed was unaffected by continuous exposure during the first 12 hr to far-red light, which inhibits germination of these seeds (see "Effects of Gibberellic Acid, Kinetin, and Light on the Germination of Lettuce Seed," this report).

No $S^{35}$ activity was detected in any organic sulfur compounds through the first 18 hr, even though the radicles were visible after 15 hr. During the growth phase, $S^{35}$ was found in the sulfur-containing amino acids, primarily in methionine.

Seeds germinating in $KH_2P^{32}O_4$ that were extracted at any time prior to protrusion yielded only one labeled compound besides the orthophosphate. When $P^{32}$-phosphate was added to extracts prepared by boiling seeds for 30 min in water, this unknown compound did not appear to be labeled. It was labeled from $P^{32}$-phosphate in extracts prepared by boiling for short periods of time, as routinely used in the germination experiments. Since this unknown radioactive compound can thus be formed by the extraction procedure, it is very likely that no detectable $P^{32}$-phosphate was esterified by the intact seeds before protrusion of the radicle. About the time that the radicle had ruptured the seed coat, activity could be found in several phosphorus compounds. Considerable activity appears in phosphorylcholine and phospholipids, with traces appearing in several other compounds.

These results might be explained by postulating that drastic metabolic changes occur at about the time of protrusion, a possibility for which there is some justification.[14] Alternatively, these results might be explained by postulating that the seeds esterify orthophosphate before protrusion, but that the seed coat is impermeable to phosphate, thus preventing the $P^{32}$ from entering the seed until after the radicle has ruptured the seed coats. This alternative is supported by experiments performed on this same variety of seed in Evenari's laboratory, where it was shown that dinitrophenol stimulated the oxygen uptake of intact seeds during the earliest phases of germination.[14] Consequently, it may be inferred that respiration is coupled to phosphorylation during the earliest phases. When we used seeds with punctured coats, $P^{32}$-phosphate was esterified within 3 hr after the beginning of imbibition. The distribution of $P^{32}$ from these pricked seeds was similar after 3 hr to the distribution found in unpricked seeds at 18 hr (several hours after radicle protrusion). However, radicle protrusion occurred no sooner in pricked seeds than in unpricked controls. Thus the results suggest an impermeability of the seed coat to orthophosphate.

Far-red light exposure in the presence or absence of gibberellic acid, kinetin, or thiourea did not appear to alter either the apparent impermeability of the seed coats to phosphate or the distribution of $P^{32}$ in extracts of pricked seeds.

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[14] M. Evenari *et al.*, *Bull. Research Council Israel* **D6**, 33–37 (1957).

# BIOPHYSICS

J. S. Kirby-Smith[1]
M. L. Randolph
J. Jagger
J. N. Dent[2]
S. Kondo[3]
D. L. Parrish

## Attempts to Photoreactivate Enzymes in Vitro

J. Jagger

**Introduction.** – Transforming factor, DNA (see Glossary), has been successfully photoreactivated in extracts of *Escherichia coli*.[4] That photoreactivation (PR) of enzymes in cell extracts might also be possible was suggested by C. W. Shuster and J. L. Larimer (personal communication), who found apparent PR of triosephosphate dehydrogenase in extracts of mouse brain. The present experiments were undertaken to determine whether enzymes can indeed be photoreactivated in vitro. Aside from the intrinsic interest of such a result, the study of PR with enzymatic assays would be far easier than the current studies with transformation assays. These experiments were carried out in cooperation with N. G. Anderson and W. D. Fisher of the Cell Physiology Group.

**Results.** – The "soluble phase" of rat liver cell extracts (ratlivsol)[5] was used. This contains considerable RNA, but no DNA or large particles; it is physically similar to the bacterial extracts used by Rupert *et al.*[4] Very thin samples were irradiated at 0°C under quartz cover slips with 2537-Å radiation. Immediately after the ultraviolet treatment, photoreactivating light (PR light) from fluorescent lamps was administered for periods from 2.5 to 40 min. The catalase (by perborate assay) and rhodanese (by KCN assay) activities of (1) dialyzed and frozen, (2) undialyzed and frozen, and (3) freshly extracted ratlivsol were studied. In all cases, exponential inactivation with ultraviolet rays was obtained with fairly high precision, $10^6$ ergs/mm$^2$ (60 min) reducing the catalase activity to about 10% and the rhodanese activity to about 40% that of controls. The PR was attempted (1) at 0 and 25°C, (2) with and without added magnesium ion, and (3) with and without addition of unirradiated ratlivsol. Neither inactivation nor reactivation of either enzyme by the PR light was found. In addition, purified beef liver catalase was inactivated, added to ratlivsol, and treated with PR light under various conditions. Again, no PR was found.

**Discussion.** – It is concluded that catalase and rhodanese cannot be photoreactivated in ratlivsol. Pending proof that ratlivsol is indeed a photoreactivating system, this is a strong indication that these particular enzymes cannot be photoreactivated even in the living cell.

## Responses of Salamander Thyroids to TSH Administration

W. G. Lynn[6] J. N. Dent

**Introduction.** – A study of the effects of goitrogens upon thyroid activity in the salamanders *Triturus viridescens* and *Desmognathus fuscus* has revealed significant differences between the two species.[7] The results indicate that either (1) the pituitary gland of *Triturus* fails to show any increased production of TSH (see Glossary) after goitrogen administration or (2) the thyroid of this animal is particularly insensitive to TSH. To test the latter possibility an investigation has been made of the effects of TSH administration upon the thyroid histology and the radioiodine uptake in these two salamanders.

**Results.** – The histological changes in the thyroid gland following TSH treatment were found to be essentially the same in *Triturus* and *Desmognathus*. Thyroids of animals killed 12 hr after a single injection of one Junker-Schoellmann unit of TSH showed no differences from those of controls. However, thyroids of animals which received daily injections of one J-S unit for 2, 4, 6, or 8 days exhibited marked hyperplasia, hypertrophy, and colloid depletion. The effect was relatively long-lasting; thyroids of animals injected

[1] On leave; Guggenheim Fellowship.

[2] Consultant.

[3] Visiting investigator from abroad.

[4] C. S. Rupert, S. H. Goodgal, and R. M. Herriott, *J. Gen. Physiol.* 41, 451 (1958).

[5] N. G. Anderson, *Exptl. Cell Research* 11, 186 (1956).

[6] Research participant, summer 1957.

[7] J. N. Dent and W. G. Lynn, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 81–82.

for four days and killed as long as eight days after the last injection still showed maximal response.

The uptake of $I^{131}$ by the thyroids of *Desmognathus* 18 hr after the last of five daily injections of TSH was significantly higher than that of control thyroids. The radioactivity in the thyroid region then declined in both, although the radioactivity in the experimental thyroids remained higher than that in the controls throughout the succeeding 72 hr during which periodic counts were made. In *Triturus* the $I^{131}$ level at 18 hr after the fifth TSH injection was much higher than that in control thyroids, and, while the controls showed a steady decline, the treated animals showed a further rise in $I^{131}$ uptake by the thyroid which reached a peak at about 40 hr. At 90 hr the radioactivity in the thyroids of the experimental animals was still approximately twice that in the control thyroids.

**Discussion.** – The results demonstrate that the thyroid gland of *Triturus* is not insensitive to TSH. The histological responses of the thyroid in *Triturus* were entirely comparable with those in *Desmognathus* in nature, extent, duration, and in the dosage required for maximal effects. And the influence of TSH administration upon radioiodine uptake was even greater in *Triturus* than in *Desmognathus*. It is clear therefore that the failure of the *Triturus* thyroid to show any hypertrophy after goitrogen administration must be attributed to some unusual condition in the pituitary of this animal such that the usual increase in TSH production following thyroid inhibition does not occur. If this is the case, then the very strong physiological response to injected TSH found in these experiments would be expected on the basis of findings for the goldfish, which shows a similar condition.[8]

## Free Radicals Induced in Amino Acids by Radiation

M. L. Randolph D. L. Parrish

**Introduction.** – The study of radiation-induced free radicals by this group has followed the lines of instrumentation study and the measurement of the average energy absorbed per long-lived free radical formed in several amino acids.

**Results.** – Measurements with single-crystal, microgram samples of diphenylpicrylhydrazyl (DPPH), some of which were loaned to us by R. Livingston of the Chemistry Division, indicated a minimum detectable signal (i.e., signal height equal to circuit noise height) of $5 \times 10^{-11}$ mole or less. The sensitivity is poorer for samples with wider resonance patterns and for wet samples. Free radicals produced by $Co^{60}$ gamma irradiation of glycine, glycylglycine, $\beta$-alanine, and leucine have been measured. A sample of the raw data, the chart record of the derivative of an absorption curve for irradiated glycine, and the derived absorption curve are given in Figs. 18 and 19. By comparing the areas of absorption curves for irradiated dry amino acid samples with the areas for DPPH standards, it is found that the average amounts of absorbed energy per long-lived (at least days) free radical formed are approximately as follows: glycine, 160 ev; glycylglycine, 100 ev; $\beta$-alanine, 1300 ev; and leucine, 1200 ev. The glycylglycine absorption curve shows two equal or nearly equal peaks, the leucine four unequal peaks, and the $\beta$-alanine one broad peak which may well be the envelope of unresolved components. By using the full width of the DPPH curve, about 1.7 oersteds (ref 9), as a calibration of the scan speed of the magnetic field, glycine satellites are found at 9 oersteds on either side of the central peak. The changes in glycine resonance curves with time following irradiation are being observed also. In cooperation with A. D. Conger of the Genetics Group preliminary measurements of radiation-induced free radicals in barley seed have been made, and fewer radicals are being found in wet than in dry seed.

**Discussion.** – Further instrumentation development is required to achieve sensitivity comparable to that achieved by Commoner *et al.*[10] Our values for glycine indicate that several times as much energy is absorbed per free radical formed as was found by Zimmer *et al.*[11] We also find the satellites to be only about half as far from the central peak as they report. Our data are in rough agreement with earlier data taken by J. S. Kirby-Smith in this laboratory.

[8] O. A. Berg and A. Gorbman, *Proc. Soc. Exptl. Biol. Med.* **86**, 156 (1954).

[9] R. Livingston and H. Zeldes, *J. Chem. Phys.* **24**, 170–71 (1956).

[10] B. Commoner, *et al.*, *Science* **126**, 57 (1957).

[11] K. G. Zimmer, L. Ehrenberg, and A. Ehrenberg, *Strahlentherapie* **103**, 3 (1957).

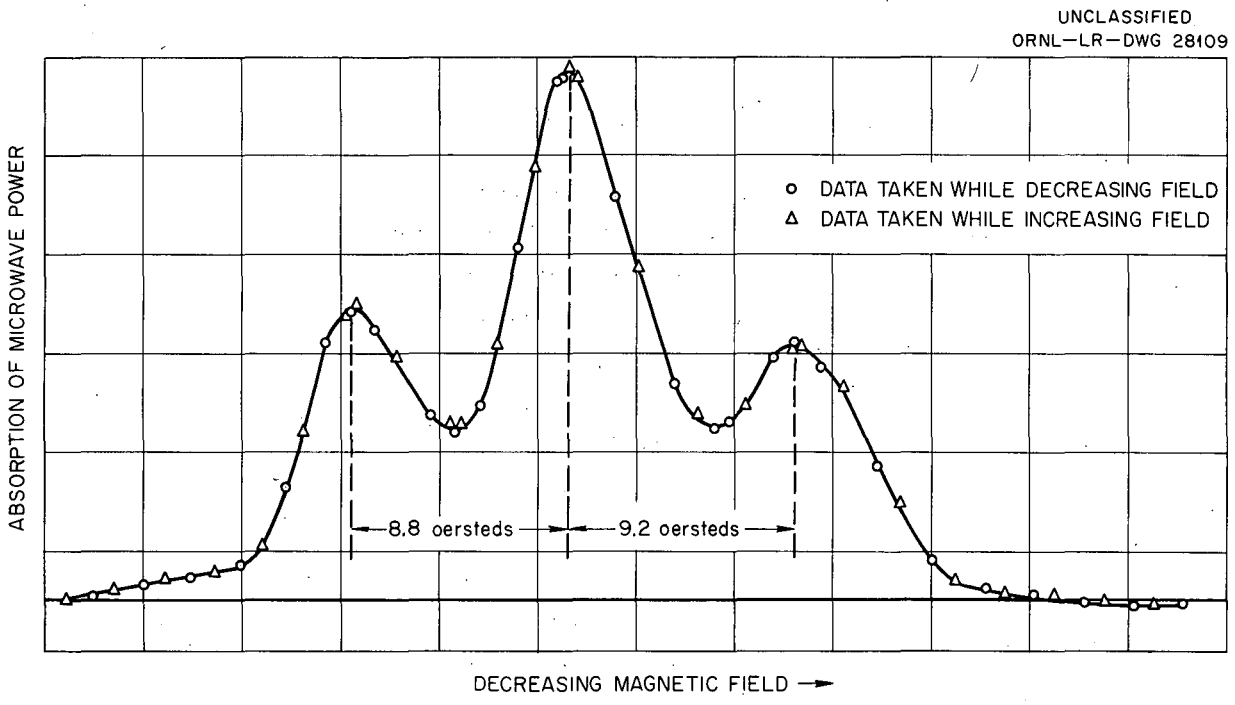


**Fig. 18. Absorption Curve for Irradiated Glycine.**

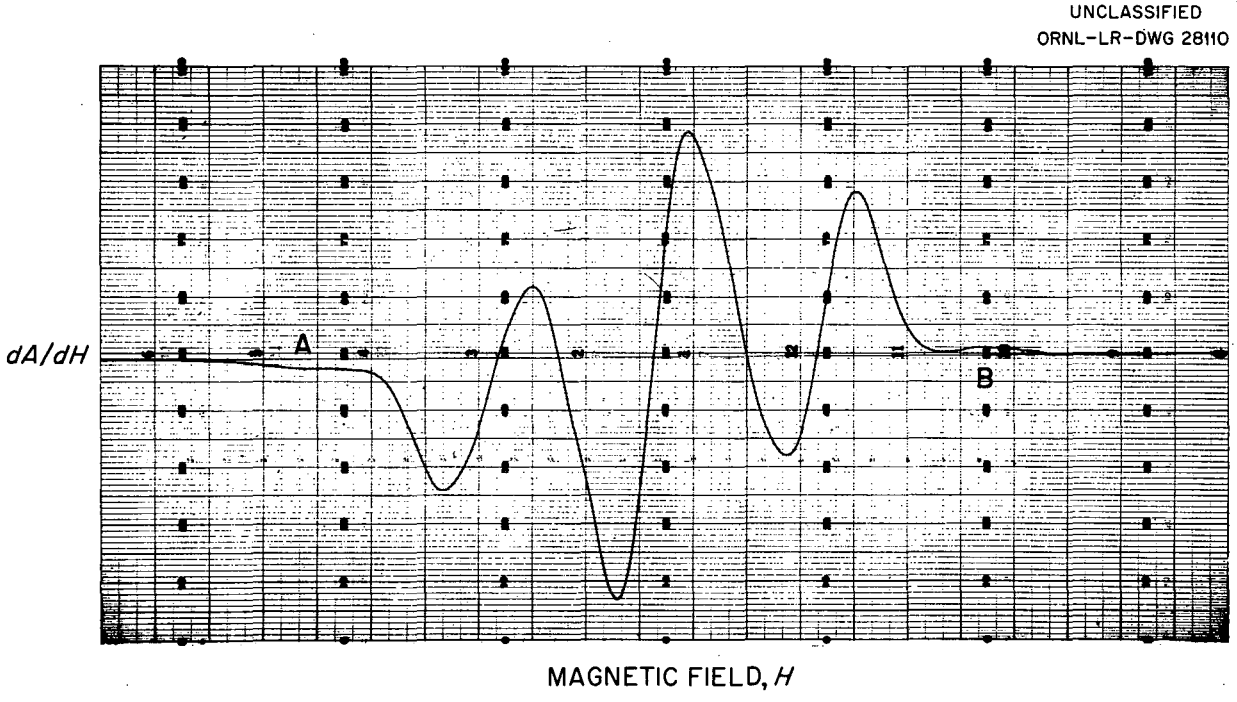


**Fig. 19. Derivative of Absorption Curve for Irradiated Glycine. Small peaks A and B at ends of pattern are real.**

## GLOSSARY

| | |
|---|---|
| ADP | adenosine diphosphate |
| AET | *S*,2-aminoethylisothiuronium·Br·HBr |
| AMP | adenosine monophosphate |
| APMT | 3-aminopropyl-*N*′-methylisothiuronium·Br·HBr |
| APT | *S*,3-aminopropylisothiuronium·Br·HBr |
| ATP | adenosine triphosphate |
| CoA | coenzyme A |
| DNA | deoxyribonucleic acid |
| DNase | deoxyribonuclease |
| DPNH | reduced diphosphopyridine nucleotide |
| FAD | flavinadenine dinucleotide |
| HBM | homologous bone marrow |
| HDP | hexose diphosphate |
| IBM | isologous bone marrow |
| LET | linear energy transfer |
| MEG | 2-mercaptoethylguanidine |
| OTC | ornithine transcarbamylase |
| PCMB | *p*-chloromercuribenzoate |
| Pi | inorganic phosphate |
| PP | pyrophosphate |
| RBC | red blood cells |
| RBE | relative biological effectiveness |
| RBM | rat bone marrow |
| RNA | ribonucleic acid |
| RNase | ribonuclease |
| TMV | tobacco mosaic virus |
| TSH | thyroid-stimulating hormone |

## PUBLICATIONS AND LECTURES

**Publications.** – The list of open-literature publications for the period is comprised of a book-length symposium, the second one published in 1957, 58 papers, and 36 abstracts. Forty-nine papers and 37 abstracts are in press.

| AUTHOR(S) | TITLE OF ARTICLE | PUBLICATION |
|---|---|---|
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| ——— | A Simple Method for Observing Refractive Index Gradients in Liquids | *Biochim. et Biophys. Acta* 25, 428 (1957) |
| Arnold, W. A. | Decay of the Delayed Light Emission in *Chlorella* | *Research in Photosynthesis* (ed. by H. Gaffron *et al.*), p 128–133, Interscience, New York, 1957 |
| Arnold, W., R. Steele, and H. Mueller | On the Magnetic Asymmetry of Muscle Fibers | *Proc. Natl. Acad. Sci. U.S.* 44, 1–4 (1958) |
| Cohn, W. E., and J. X. Khym | Separation of Nucleotides of Ribonucleic Acid | *Biochemical Preparations* (ed. by D. Shemin), vol V, p 40–48, Wiley, New York, 1957 |
| Cohn, W. E., E. Volkin, and J. X. Khym | Separation of 5′-Deoxyribonucleotides | *Biochemical Preparations* (ed. by D. Shemin), vol V, p 49–55, Wiley, New York, 1957 |
| Congdon, C. C. | Bone Marrow Transplantation in Animals Exposed to Whole-Body Radiation | *J. Cellular Comp. Physiol.* 50, supp 1, 103–108 (1957) |
| ——— | Experimental Treatment of Total-Body Irradiation Injury: A Brief Review | *Blood* 12, 746–754 (1957) |
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| Cormier, M. J., and J. R. Totter | Quantum Efficiency Determinations on Components of the Bacterial Luminescence System | *Biochim. et Biophys. Acta* 25, 229–237 (1957) |
| Dent, J. N., and C. W. Sheppard | The Distribution of Lithium in Amphibian Gastrulae Autoradiographically Demonstrated | *J. Exptl. Zool.* 135, 587–594 (1957) |
| DiLuzio, N. R., K. A. Simon, and A. C. Upton | Effects of X Rays and Trypan Blue on Reticuloendothelial Cells | *Arch. Pathol.* 64, 649–656 (1957) |
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| Doherty, D. G., R. Shapira, and W. T. Burnett, Jr. | Synthesis of Aminoalkylisothiuronium Salts and Their Conversion to Mercaptoalkylguanidines and Thiazolines | *J. Am. Chem. Soc.* 79, 5667–5671 (1957) |

| AUTHOR(S) | TITLE OF ARTICLE | PUBLICATION |
|---|---|---|
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| Schwartz, E. E., A. C. Upton, and C. C. Congdon | A Fatal Reaction Caused by Implantation of Adult Parental Spleen Tissue in Irradiated $F_1$ Mice | *Proc. Soc. Exptl. Biol. Med.* 96, 797–800 (1957) |
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| Wilbur, K. M., N. Wolfson, C. B. Kenaston, A. Ottolenghi, M. E. Gaulden, and F. Bernheim | Inhibition of Cell Division by Ultraviolet-Irradiated Unsaturated Fatty Acid | *Exptl. Cell Research* 13, 503–509 (1957) |
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**Scientific Society Lectures and Traveling Seminars.** – Twenty-three of the lectures given by Division personnel were before professional society groups. The remaining 26 were included in the ORINS-ORNL Traveling Lecture series.

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Adler, H. I. | An After-Effect of Ionizing Radiation on a Mutant of *Escherichia coli* | Soc. Am. Bacteriol. (Ky.-Tenn. Branch), Lexington |
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| Anderson, N. G. | Cell Physiology at the Molecular Level | Dept. of Zoology, University of Wisconsin, Madison |
| ——— | Glass in Biological Research | American Scientific Glass-blower's Society, Oak Ridge |
| ——— | Ultracentrifugation Through Packed Starch | Biophys. Soc., Cambridge |
| Anderson, B., and T. T. Odell, Jr. | Chemical Studies of Mucopolysaccharides from Rat Blood Platelets | Soc. Exptl. Biol. Med. (Southeastern Section), Richmond |
| von Borstel, R. C., and M. L. Pardue | Comparison of Radiation-Induced and Genetically Contrived Dominant Lethality in Habrobracon and Drosophila [*Genetics* 42, 401 (1957)] | Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Stanford |
| Cohn, W. E. | Minor Constituents of Nucleic Acids | Wellcome Research Laboratories, New York |
| ——— | As above | Dept. of Biochemistry, New York University |
| ——— | The New Nucleotides | Dept. of Chemistry, Washington University, St. Louis |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Congdon, C. C. | Bone Marrow Transfusions | East Tennessee Baptist Hospital, Quarterly Staff Meeting, Knoxville |
| ——— | Experimental Treatment of Irradiation Injury | Tennessee Chapter of the Society of Sigma Xi, University of Tennessee, Knoxville |
| ——— | Foreign Bone Marrow Reaction | Biology Operation, Hanford Laboratories, Richland |
| ——— | Role of Marrow Transplants in Recovery from Ionizing Radiation Injury | University of Washington Medical School (Conjoint Clinical Conf.), Seattle |
| Conger, A. D. | Biological Experiments at Atomic Bomb Explosions | Dept. of Biology, Longwood College, Farmville, Virginia |
| ——— | Radiation Protection in Mammals | Dept. of Science, Longwood College, Farmville, Virginia |
| ——— | As above | Dept. of Biology and National Science Foundation sponsored program, University of Virginia, Charlottesville |
| ——— | As above | Dept. of Biological Sciences, Florida State University, Tallahassee |
| ——— | RBE and LET of Gamma Rays, X Rays, and Fast Neutrons | Dept. of Biol., University of Virginia, Charlottesville |
| ——— | Relative Biological Efficiency of Gamma Rays, X Rays, and 14-, 2.5-, and 1-Mev Fast Neutrons | Depts. of Botany, Agronomy, and Biochemistry, University of Florida Medical School, Gainesville |
| Fabergé, A. C. | Relation Between Chromatid-Type and Chromosome-Type Breakage-Fusion-Bridge Cycles in Maize Endosperm [*Genetics* **42**, 368–369 (1957)] | Am. Inst. Biol. Sci. (Genet. Soc. Am.), Stanford |
| Haber, A. H. | Effects of Gibberellic Acid, Kinetin, and Light on Germination of Lettuce Seeds | Dept. of Botany, University of Tennessee, Knoxville |
| Haber, A. H., and N. E. Tolbert | Effects of Gibberellic Acid, Kinetin, and Light on the Germination of Lettuce Seed | International Symposium on Photoperiodism in Plants and Animals, Gatlinburg |
| ——— | Greening in Particles in Etiolated Wheat Leaves Exposed to Light [*Plant Physiol.* **32**, ix (1957)] | Am. Inst. Biol. Sci. (Plant Physiol. Soc.), Stanford |
| ——— | Interactions of Kinetin and Adenine in the Germination of Lettuce Seeds [*Plant Physiol.* **32**, xlix (1957)] | Am. Inst. Biol. Sci. (Plant Physiol. Soc.), Stanford |
| Hollaender, A. | Problems in Radiation Protection | Cancer Research Institute, New England Deaconess Hospital, Boston |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Krieg, D. R. | A Test for Interaction Between Pseudoalleles to Produce a Recombinant Phenotype | Cold Spring Harbor Phage Conf., Long Island |
| ——— | Comments on Ultraviolet-Induced Mutations in Bacteriophage T4 | Cold Spring Harbor Phage Conf., Long Island |
| Lindsley, D. L., and C. W. Edington | Sex-Linked Recessive Lethals Whose Expression is Determined by the Y Chromosome [*Genetics* 42, 384 (1957)] | Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Stanford |
| Makinodan, T. | Effect of Irradiation on Antigen-Host Relationship | Carnegie Institution of Washington, Dept. of Embryology, Baltimore |
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| Novelli, G. D. | The Activation of Amino Acids | Society of Sigma Xi, University of Georgia, Athens |
| ——— | Some Problems Concerning the Activation of Amino Acids | Natl. Acad. Sci. (Symp. on Activation of Amino Acids), Buffalo |
| Oakberg, E. F. | X-Ray Damage and Recovery in the Mouse Testis [*Anat. Record* 128, 597 (1957)] | Am. Inst. Biol. Sci. (Am. Soc. Zoologists), Stanford |
| Oakberg, E. F., and R. L. DiMinno | X-Ray Sensitivity of Different Stages of Primary Spermatocytes of the Mouse [*Genetics* 42, 386–387 (1957)] | Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Stanford |
| Perry, R. P., and M. E. Gaulden | Ultraviolet Microbeam in Studies of Nucleolar Function | Biophys. Soc., Cambridge |
| Rabson, R., and N. E. Tolbert | Biosynthesis of Serine in Plants [*Plant Physiol.* 32, xxxv (1957)] | Am. Inst. Biol. Sci. (Plant Physiol. Soc.), Stanford |
| ——— | Biosynthesis of Homoserine from Aspartic Acid in Alaska Peas [*Plant Physiol.* 32, xxxv (1957)] | Am. Inst. Biol. Sci. (Plant Physiol. Soc.), Stanford |
| Randolph, M. L. | The Detection, Dosimetry, and Radiobiological Action of Fast Neutrons | Dept. of Physics, University of Cincinnati, and the Robert A. Taft Sanitary Engineering Center, Cincinnati |
| Randolph, M. L., and A. D. Conger | Principles of Analyses of Biological Effects Versus Physical Parameters of Irradiation | Biophys. Soc., Cambridge |
| Rohrbaugh, L. M., and N. E. Tolbert | Effects of Indoleacetic Acid on Metabolism in Tomato and Oat Plants [*Plant Physiol.* 32, xviii (1957)] | Am. Inst. Biol. Sci. (Plant Physiol. Soc.), Stanford |
| Russell, L. B., and M. K. Freeman | Comparison of the Effects of Acute and Fractionated Irradiation on Fertility of the Female [*Anat. Record* 128, 615–616 (1957)] | Am. Inst. Biol. Sci. (Am. Soc. Zoologists), Stanford |

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|---|---|---|
| Russell, L. B., and L. Wickham | The Incidence of Disturbed Fertility Among Male Mice Conceived at Various Intervals After Irradiation of the Mother [*Genetics* 42, 392–393 (1957)] | Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Stanford |
| Russell, W. L. | Genetic Effects of Radiation [*Radiology* 69, 444 (1957)] | Radiological Soc. North Amer., Chicago |
| ——— | Genetic Hazards of Radiation as Interpreted from Animal Studies | Dept. of Agronomy, University of Florida, Gainesville |
| Schwartz, D. | Cytogenetic Studies with Maize | Dept. of Zoology, University of Chicago, Chicago |
| ——— | As above | Argonne National Laboratory, Chicago |
| de Serres, F. J. | A Genetic Analysis of an Insertional Translocation Involving the ad-3 Region in *Neurospora crassa* [*Genetics* 42, 366–367 (1957)] | Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Stanford |
| Shapira, R. | Transamidination | Dept. of Biochemistry, Vanderbilt University, Nashville |
| Stapleton, G. E., and N. A. Sicard | Radiation Studies on Synchronous Cultures of *Escherichia coli* ($15_T$–) | Soc. Am. Bacteriol. (Ky.-Tenn. Branch), Lexington |
| Upton, A. C. | Hazards to Man of Ionizing Radiations | Nuclear Science Seminar of the Office of Naval Research, ORINS Training Bldg., Oak Ridge |
| ——— | Hematological Effects of Radiation | ORINS Medical Division, Oak Ridge |
| ——— | Induction of Myeloid Leukemia by X Rays | Gordon Research Conf. on Cancer, New London, New Hampshire |
| Volkin, E. | RNA Metabolism in the T2 System | Cold Spring Harbor Phage Conf., Long Island |

**Visiting Lecturers.** – Investigators from other laboratories who have lectured on the Biology Seminar series are as follows:

| LECTURER | AFFILIATION | TOPIC |
|---|---|---|
| W. Bergmann | Department of Chemistry, Yale University, New Haven | Nucleotides of Sponges or Evolution on a Molecular Level |
| J. H. Taylor | Department of Botany, Columbia University, New York | Current Studies on the Replication of Chromosomes |
| D. Metcalf | Children's Cancer Research Foundation, Boston | Observations on a Lymphocytosis Stimulating Factor in Blood and Tissues |

| LECTURER | AFFILIATION | TOPIC |
|---|---|---|
| M. E. Case | Department of Botany, Yale University, New Haven | Gene Conversion at the *Pan-2* Locus of *Neurospora* |
| W. Mörikofer | Physikalisch Meterologisches Observvatorium, Davos-Platz, Switzerland | The Radiation Program for the International Geophysical Year |
| E. B. Lewis | Division of Biology, California Institute of Technology, Pasadena | Some Studies of Conversion and Pseudoallelism at the White "Locus" |
| ——— | | The Bithorax Pseudoalleles and Differentiation |
| M. J. D. White | Department of Zoology, University of Missouri, Columbia | Mechanisms of Adaptive Chromosomal Polymorphism and Their Coadaptation in Insect Populations |
| R. R. Becker | Department of Chemistry, Columbia University, New York | Synthesis and End Group Studies of Polypeptidyl Proteins |
| G. C. Webster | Department of Agricultural Biochemistry, Ohio State University, Columbus | Some Apparent Intermediate Steps in Amino Acid Incorporation into Protein |
| P. Koller | Chester Beatty Research Institute, London, England | Immunological Characteristics of Radiation Chimeras |
| Ö. Winge | Carlsberg Laboratorium, Copenhagen, Denmark | The Yeasts as a Tool of Genetic Research |
| D. Stanković | Faculty of Agriculture, Zemun, Yugoslavia | Science in Yugoslavia |
| P. W. Wilson | Department of Bacteriology, University of Wisconsin, Madison | Present Day Problems in Biological Nitrogen Fixation |
| J. J. Trentin | Department of Anatomy, Baylor University, College of Medicine, Houston | Consequences of Bone Marrow-Induced Radiation Recovery |
| H. A. Schneiderman | Department of Zoology, Cornell University, Ithaca | Perspectives in Insect Endocrinology |
| R. S. Caldecott | Department of Agronomy and Plant Genetics, Institute of Agriculture, University of Minnesota, St. Paul | Modification of X-Ray-Induced Injury in Barley by Posttreatment with Oxygen |
| H. Swift | Department of Zoology, The University of Chicago, Chicago | The Cytochemistry of Polytene Chromosomes |
| ——— | | RNA and Cell Fine Structure |
| J. C. Nichol | Department of Chemistry, University of Minnesota Duluth Branch, Duluth | Developments in the Theory of Moving Boundary Electrophoresis |
| R. Ceppellini | Institute for the Study of Human Variation, Columbia University, New York | Evidence for Multiple Factors (Antibodies?) Reacting with Nuclear Components in the Serum of LED Patients |
| ——— | | Gene Interaction at the ABO Locus in Man |
| A. Bendich | Sloan-Kettering Institute for Cancer Research, New York | Fractionation of DNA and Its Biological Implications |
| L. Hamilton | Sloan-Kettering Institute for Cancer Research, New York | Nucleic Acid Turnover in Leukocytes and the Function of Lymphocytes |

| LECTURER | AFFILIATION | TOPIC |
|---|---|---|
| T. Iwamura | Department of Zoology, The University of Texas, Austin | Synchronous Culture of *Chlorella* with Special Reference to Changes of Nucleic Acid Bases During Its Life Cycle |
| E. R. Dempster | Department of Genetics, University of California, Berkeley | The Correlation Between Mental Traits in Man |

**Speakers at Professional Meetings, Spring 1958.** – Thirty-seven papers on research in the Biology Division are scheduled to be given before the end of June. The societies and speakers are listed below:

*Association of Southeastern Biologists* – M. E. Gaulden (coauthor, R. P. Perry), W. G. Lynn (coauthor, J. N. Dent), and R. T. Brumfield

*Eleventh Annual Biology Research Conference* – K. C. Atwood, R. D. Owen, and G. D. Novelli

*Federation of American Societies for Experimental Biology* – N. G. Anderson (coauthor, J. F. Albright), L. Astrachan, W. E. Cohn, C. C. Congdon, M. J. Cormier, G. E. Cosgrove (coauthors, E. E. Schwartz, A. C. Upton, and C. C. Congdon), J. L. Countryman (coauthor, E. Volkin), D. G. Doherty (coauthor, R. Shapira), W. Friedberg, N. Gengozian (coauthor, T. Makinodan), J. W. Goodman, T. Makinodan (coauthor, N. Gengozian), G. D. Novelli (coauthor, Audrey N. Best), T. T. Odell (coauthor, B. Anderson), R. A. Popp (coauthor, L. H. Smith), P. Rogers, Jr., C. W. Sheppard (coauthor, E. B. Darden), L. H. Smith (coauthor, J. C. Tohá), N. E. Tolbert, and A. C. Upton (coauthor, Frances F. Wolff); in addition to these, A. C. Upton will be one of four speakers on a joint society symposium on "The Biology of the Cancer Cell"

*Society of American Bacteriologists* – H. I. Adler, S. F. Carson (coauthor E. F. Phares), M. I. Dolin, and G. E. Stapleton (coauthor, N. A. Sicard)

*Symposium on the Noxious Effects of Low-Level Radiation: Physical Elements and Biological Aspects* (Lausanne, Switzerland) – A. Hollaender

*Ciba Foundation Symposium:* "Carcinogenesis: Mechanism of Action" – A. C. Upton

*Tenth Annual Midwest Cancer Conference* (Wichita) – A. C. Upton (two talks)

*American Association for Cancer Research* – A. C. Upton (coauthor, F. F. Wolff) and E. E. Schwartz

**Eleventh Annual Biology Research Conference.** – As previously announced, the subject of this conference, to be held in Gatlinburg on April 2–6, will be "Genetic Approaches to Somatic Cell Variation." The program, which has been considerably revised, follows:

*WEDNESDAY, APRIL 2*

MORNING

Chairman: Curt Stern, University of California, Berkeley

*The Nucleus and Somatic Cell Variation:* Curt Stern, University of California

*The Cytoplasm and Somatic Cell Variation:* Boris Ephrussi, Centre National de la Recherche Scientifique, Gif sur Yvette (Seine et Oise), France

*Cellular and Possibly Nuclear Changes upon Immuzination:* Albert H. Coons, Harvard University Medical School

AFTERNOON

Chairman: George D. Snell, Roscoe B. Jackson Memorial Laboratory

*Erythrocyte Antigen Mosaicism:* Charles W. Cotterman, Wadley Research Institute and Blood Center

*Somatic Variation in Human Erythrocyte Antigens:* K. C. Atwood, Oak Ridge National Laboratory

EVENING

Smoker

Host: Union Carbide Nuclear Company

*THURSDAY, APRIL 3*

MORNING

Chairman: J. F. Danielli, University of London King's College

*Histocompatibility Changes in Tumors:* Georg Klein, Karolinska Institutet

*Genetic Variation in the Somatic Cells of Maize:* R. A. Brink, The University of Wisconsin

*Melanotic Tumors of Drosophila:* Claudio Barigozzi, University of Milan

AFTERNOON

*EXCURSION – GREAT SMOKY MOUNTAINS* (The group will leave from the Mountain View Hotel at 2:00 PM. In case of inclement weather, the program will continue, and Saturday morning will remain open for a tour of the Biology Division of Oak Ridge National Laboratory.)

EVENING

Reception and dinner

*FRIDAY, APRIL 4*

MORNING

Chairman: Hilary Koprowski, The Wistar Institute of Anatomy and Biology

*Correlation of Chromosomal with Physiological Changes in Tumors:* Theodore S. Hauschka, Roswell Park Memorial Institute

*Chromosome Rearrangement and the Formation and Alteration of Tumors:* Charles E. Ford, Medical Research Council, Radiobiological Research Unit, Harwell

AFTERNOON

Chairman: S. E. Luria, University of Illinois

*Genetic Changes in Cells in Tissue Culture:* Marguerite Vogt, California Institute of Technology

*Genetic Studies on Human Somatic Cells in Vitro:* T. T. Puck, University of Colorado Medical Center

EVENING

Chairman: E. L. Tatum, The Rockefeller Institute for Medical Research

Round-Table Discussion: *Molecular Basis of the Cause and Expression of Somatic Cell Variation.*
Discussion leaders:
Michael Heidelberger, Institute of Microbiology, Rutgers University
Rollin D. Hotchkiss, The Rockefeller Institute for Medical Research
W. Eugene Knox, Cancer Research Institute, New England Deaconess Hospital
Lloyd W. Law, National Cancer Institute
Daniel Mazia, University of California, Berkeley
G. D. Novelli, Oak Ridge National Laboratory
Sol Spiegelman, University of Illinois

***SATURDAY, APRIL 5***

MORNING

Chairman: Herschel Roman, University of Washington

*Cell Population Dynamics and Somatic Change:* J. Werner Braun, Institute of Microbiology, Rutgers University

*Summary:* Joshua Lederberg, University of Wisconsin

The following people have been invited to be special discussion leaders:

Elio Borghese, Generale dell'Universita di Cagliari
F. M. Burnet, The Walter and Eliza Hall Institute of Medical Research
L. L. Cavalli-Sforza, Instituto Sieroterapico Milanese "Serafino Belfanti"
Ruggero Ceppellini, The University of Turin Medical School
C. A. Clarke, David Lewis Northern Hospital
Dr. Clauss, Max-Planck-Institut für Meeresbiologie
J. Hämmerling, Max-Planck-Institut für Meeresbiologie
Gustavo Hoecker, Universidad de Chile
P. B. Medawar, University College, London
N. Avrion Mitchison, University of Edinburgh
Ray D. Owen, Oak Ridge National Laboratory

**ORNL Advisory Committee for Biology.** – The annual meeting of the Committee was held on November 4, 5, and 6, 1957, in the Biology Division. The first day of the meeting was devoted to reviews of the work in progress in the Division. On the other days, Committee members were conducted on tours of the Division facilities and were given opportunities for consultation with individual investigators. Members of the Committee were E. A. Doisy, St. Louis University School of Medicine; A. B. Hastings, Harvard Medical School; Karl Sax, The Arnold Arboretum, Harvard University; and Curt Stern, Department of Zoology, University of California, Berkeley.

**Bone Marrow and AET Conference.** – The latest in the series of conferences on the use of bone marrow and AET in the treatment of radiation injury and certain blood diseases was held on January 10 and 11 in the Biology Division Building. These conferences represent a concerted effort from a

number of laboratories in research on these problems. Representatives of the following laboratories and hospitals attended the two-day session: National Cancer Institute, Harvard Medical School, Children's Cancer Research Foundation (Boston), New England Medical Center, Peter Bent Brigham Hospital, Imogene Bassett Hospital (Cooperstown), Veterans' Adminstration Hospital (New Haven), St. Luke's Hospital (Newberry, N. Y.), George Washington University, Walter Reed Army Medical Center, Jefferson Medical Center, New Orleans Charity Hospital, Naval Medical Center (Bethesda), Parke, Davis, & Co., Agricultural and Technical College (Greensboro), Columbia University, ORINS Medical Division, UT-AEC Agricultural Project, USAEC (Oak Ridge and Washington), and ORNL Biology Division.

The next meeting of this group will take place in Philadelphia on Monday, April 14.

**Education.** – Members of the Division who have engaged in educational activities at other sites are as follows: A. D. Conger, who gave a month-long course in radiobiology at Florida State University, Tallahassee; and W. L. Russell, who presented a refresher course in genetic effects of radiation at the Radiological Society of North America, in Chicago.

Within the Division, two courses of instruction have been offered. A. C. Upton, C. C. Congdon, and G. E. Cosgrove planned and gave the instruction in the pathology course, which was held twice a week from the middle of October 1957 to the middle of January 1958. A course in biophysics, organized by M. L. Randolph, has just gotten under way. This group will meet twice a week until about April 1.

R. F. Kimball and J. S. Kirby-Smith continue their research, under Guggenheim Fellowships, at the Karolinska Institutet in Stockholm and at St. Bartholomew's Hospital in London, respectively.

During the fall classes at ORSORT, seven lectures were given by the following Division members: L. B. Russell, W. L. Russell, E. F. Oakberg, G. E. Stapleton, and A. C. Upton.

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