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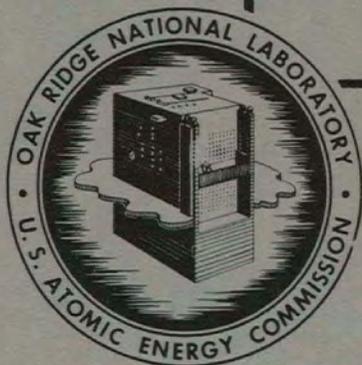
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BIOLOGY DIVISION QUARTERLY PROGRESS REPORT

FOR PERIOD ENDING NOVEMBER 10, 1951



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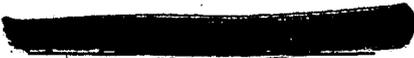
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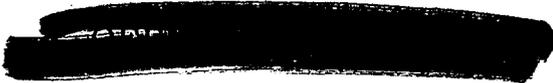
**BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT
for Period Ending November 10, 1951**

Alexander Hollaender, Director

Edited by E. J. Slaughter

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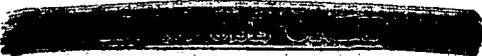
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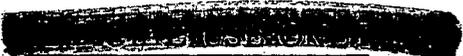
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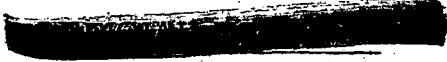
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REVIEW OF RESEARCH PROGRESS

Alexander Hollaender

CYTOGENETICS

Dr. Kimball C. Atwood, formerly of Columbia University, joined the Cytogenetics Section in the past quarter. Dr. Atwood has set up experiments for studying radiation effects on Neurospora conidia.

The studies on radiation protection have revealed that a number of bacterial strains kept at certain temperature levels after X irradiation will recover to a certain degree. For example, Escherichia coli B/r, which has an optimum temperature for growth of 37°C, has a fiftyfold recovery if kept at 18°C for at least 12 hours after exposure and then elevated to 37°C.

Development of the pattern of protection by chemicals on bacteriophage now includes experiments on the "indirect effect" as well as the "direct effect." In spite of the fact that phage shows no oxygen effect, it can be protected by chemicals.

Studies of the oxygen effect in maize have brought out that pollen irradiated in air shows 3.3 times as many breaks as that irradiated in a 100 per cent nitrogen atmosphere. The decrease in breakage by this factor agrees well with results reported for similar experiments with Tradescantia and Drosophila.

A triploid yeast has apparently been isolated. Clones of this or higher ploidy isolated by methods described should constitute a source of information about the genetic behavior of polyploids, as well as provide excellent material for radiation studies.

A study of the comparative effects of P³² β rays and X rays shows that, for comparable doses, mitosis is more adversely affected by X than by β rays.

Studies of the effects of X rays on mutation rate at specific loci of the autosomes in Drosophila have been initiated. The results from these experiments will give a better basis for the comparison of the rates of X-ray-induced mutation in mice and Drosophila.



In the study of radiation protection by chemicals, mecholyl bromide has been found to protect mice against radiation at about the same level as cysteine. X rays have been determined to have very little effect on an adaptive enzyme in E. coli. The radiation, however, interferes with the formation of fresh enzyme.

MAMMALIAN GENETICS AND DEVELOPMENT

Dr. Eugene F. Oakberg, formerly of the Department of Genetics, Iowa State College, joined the Mammalian Genetics and Development Section last quarter. Dr. Oakberg, a specialist in vertebrate histology, will assist in the supervision of the Section's large program on the measurement of radiation-induced mutation rates in mice.

Interesting information was obtained on the X-ray-induced translocation of semisterility in mice.

PATHOLOGY AND PHYSIOLOGY

Lymphocytes have not been found to exert any protective effect against radiation injury. It has been found that the incidence of leukemia in a special strain of mice with a high normal incidence of leukemia can be reduced by cortisone. X-ray-induced leukemias can be reduced by the same means. Similar and almost simultaneous findings were made by Kaplan at Stanford University Medical School. Information of increasing interest, especially in regard to hormone production, has been obtained from pituitary tumors induced by treatment of mice with large quantities of radioactive iodine.

MICROBIOLOGY

The work of the Microbiology group will be the subject under discussion at the 1952 Biology Research Conference which has been set for April 10, 11. A tentative program has been arranged but will be reserved until its final form can be given in the next Quarterly Report.

BIOCHEMISTRY

Information concerning the structure of nucleic acids continues to be made available on the basis of compounds isolated by the ion-exchange method. The isotope effect in the urease-catalyzed hydrolysis of urea- C^{14} , which has been investigated in cooperation with the Organic Chemistry group, gave the expected isotopic effect and failed to verify the reverse effect which has been reported by Meyerson and Daniels.

PLANT PHYSIOLOGY

Development of the application of ion-exchange methods for sugar separation is constantly opening up new fields of considerable importance in plant physiology. It has now been possible to remove the borates from the sugars by several methods. Interesting results may soon be forthcoming from the work on the use of radioautographs in callus cultures.

BIOPHYSICS

The Biophysics group has completed setting up the 300-curie γ -ray source, which is now available for general use. It is an especially handy tool for the irradiation of microorganisms, allowing very close proximity of the subject to the source. This is an important feature, since many microorganisms are very radioresistant.

**PRESENTATION OF RESEARCH RESULTS
TO THE SCIENTIFIC PUBLIC**

Publications. This period has seen the open publication of thirty-four articles written by members of the Biology Division. Following the plan announced some time ago, unclassified Biology ORNL reports are being issued in the reprint form. This series does not follow the old ORNL number sequence but is being designated as the ORNL-Reprint series, beginning with number 1. Papers published in open literature and in Laboratory reports are listed.

The volume of proceedings of the 1949 Biology Research Conference in Oak Ridge is now in press. The Journal of Cellular and Comparative Physiology will bring out this "Symposium on Radiation Microbiology and Biochemistry" as Supplement 1, volume 39, February 1952. This will be the third symposium so published, with preparation of the fourth, the 1951 proceedings, to get under way at once.

<u>AUTHOR(S)</u>	<u>TITLE OF PAPER</u>	<u>OPEN PUBLICATION</u>
Arnold, W. A., E. W. Burdette, and J. B. Davidson	A recording Warburg apparatus	Science, 114: 364-367, 1951 (Oct.)
Burnette, W. T., Jr., G. E. Stapleton, M. L. Morse, and A. Hollaender	Reduction of the X-ray sensitivity of <i>E. coli</i> B/r by sulfhydryl compounds, alcohols, glycols, and sodium hydro-sulfite	Proc. Soc. Exptl. Biol. Med., 77: 636-638, 1951
Carlson, J. Gordon and Rachel McMaster	Nucleolar changes induced in the grasshopper neuroblast by different wave lengths of ultraviolet radiation and their capacity for photorecovery	Exptl. Cell Research, II: 434-444, 1951 (Sept.)
Carson, S. F., J. W. Foster, W. E. Jefferson, E. F. Phares, and D. S. Anthony	Oxidative formation of lactic acid by a fungus	Arch. Biochem. Biophys., 33: 448-458, 1951 (Oct.)
Carson, S. F., E. H. Mosbach, and E. F. Phares	Biosynthesis of citric acid	J. Bact., 62: 235-238, 1951 (Aug.)
Cohn, W. E.	Some results of the applications of ion-exchange chromatography to nucleic acid chemistry	J. Cellular Comp. Physiol., vol. 38, Suppl. 1: 21-40, 1951

<u>AUTHOR(S)</u>	<u>TITLE OF PAPER</u>	<u>OPEN PUBLICATION</u>
Doherty, D. G.	The enzymatic hydrolysis of glyconyl peptide derivatives (Abstract)	Abstracts of Papers of 12th International Congress of Pure and Applied Chemistry, New York, Sept, 1951, p. 98
Doherty, D. G.	The synthesis of glyconyl peptides (Abstract)	Abst. of Papers 12th Inter. Congr. Pure and App. Chem., New York, Sept, 1951, p. 98 and (in part) Chem. Eng. News, 29: 3948, 1951
Furth, J.	Recent studies on the etiology and nature of leukemia	Blood, VI: 964-975, 1951 (Nov.)
Gaulden, Mary E. and J. Gordon Carlson	Cytological effects of colchicine on the grasshopper neuroblast <u>in vitro</u> with special reference to the origin of the spindle	Exptl. Cell Research, II: 416-433, 1951 (Sept.)
Geckler, R. P. and R. F. Kimball	A new method for determination of separation times in conjugating <u>Paramecium aurelia</u> (Abstract)	Microbial Genetics Bulletin, (Cold Spring Harbor)#5, Oct. 1951, pp. 14, 15.
Harrington, Nyra and R. W. Koza	Effect of X radiation on the desoxyribonucleic acid and on the size of grasshopper embryonic nuclei	Biol. Bull., 101: 138-150, 1951
Hollaender, A. (ed.)	Symposium on Biochemistry of Nucleic Acids	J. Cellular Comp. Physiol., vol. 38, Suppl. 1, 1951 (July)
Hollaender, A., G. E. Stapleton, and W. T. Burnett, Jr.	The modification of X-ray sensitivity by chemicals	Isotopes in Biochemistry, ed. by G.E. W. Wolstenholme, Churchill, Ltd., London, 1951, pp. 96-113
Hollaender, A., G. E. Stapleton, and W. T. Burnett, Jr.	Chemical aspects of radiation sensitivity on living cells (Abstract)	Abstracts of Papers of 120th Meet. Am. Chem. Soc., p. 14C
Mosbach, E. H., E. F. Phares, and S. F. Carson	Degradation of isotopically labeled citric α -ketoglutaric and glutamic acids	Arch. Biochem. Biophys., 33: 179-185, 1951(Sept.)

<u>AUTHOR (S)</u>	<u>TITLE OF PAPER</u>	<u>OPEN PUBLICATION</u>
Mosbach, E. H., E. F. Phares, and S. F. Carson	Wolff-Kishner reduction of pyruvic and 3-formylpropionic acids	J. Am. Chem. Soc., 73: 5477-5478, 1951(Nov.)
Phares, E. F.	Degradation of labeled propionic and acetic acids	Arch. Biochem. Biophys., 33: 173-178, 1951(Sept.)
Underwood, Newton and A. H. Doermann	Photography of bacteriophage plaques	Microbial Genetics Bulletin (Cold Sp. Hbr.) #5, Oct. 1951, p. 17.
Whittinghill, Maurice	Some effects of gamma rays on recombination and on crossing over in <u>Drosophila melanogaster</u>	Genetics, 36: 332-355, 1951 (July)
<u>AUTHOR (S)</u>	<u>TITLE OF PAPER</u>	<u>PROJECT PUBLICATION</u>
Arnold, W. A., E. W. Burdette, and J. B. Davidson	A recording Warburg apparatus	ORNL Reprint # 14
Bigelow, R. R., J. Furth, M. C. Woods, and R. H. Storey	Endothelial damage by X rays disclosed by lymph fistula studies	ORNL Reprint # 9
Bizzell, O. M., W. T. Burnett, Jr., P. C. Tompkins, and L. Wish	Phosphorus-bakelite beta-ray source	ORNL Reprint # 4
Burnette, W. T., Jr. G. E. Stapleton, M. L. Morse, and A. Hollaender	Reduction of the X-ray sensitivity of <u>E. coli</u> B/r by sulfhydryl compounds, alcohols, glycols, and sodium hydrosulfite	ORNL Reprint # 12
Carlson, J. Gordon and Rachel McMaster	Nucleolar changes induced in the grasshopper neuroblast by different wave lengths of ultraviolet radiation and their capacity for photorecovery	ORNL Reprint # 11
Christenberry, K. W. and J. Furth	Induction of cataracts in mice by slow neutrons and X rays	ORNL Reprint # 10
Darden, E. B., Jr. and C. W. Sheppard	A thimble type gamma-ray dosimeter and the measurement of the radiation from lumped and distributed type sources	ORNL-1002
Darden, E. B., Jr. C. W. Sheppard, and L. C. Emerson	Gamma-ray contamination in the thermal neutron exposure facility of the Oak Ridge reactor	ORNL-1003

<u>AUTHOR (S)</u>	<u>TITLE OF PAPER</u>	<u>PROJECT PUBLICATION</u>
Furth, J. and J. Moshman	On the specificity of hypervolemia and congestive changes in tumor-bearing mice	ORNL Reprint # 8
Hollaender, A. (Director) (E. J. Slaughter, Editor)	Biology Division Quarterly Progress Report for Period Ending May 10, 1951	ORNL-1026
Hollaender, A. (Director) (E. J. Slaughter, Editor)	Biology Division Quarterly Progress Report for Period Ending Aug. 10, 1951	ORNL-989
Mosbach, E. H., E. F. Phares, and S. F. Carson	Degradation of isotopically labeled citric α -ketoglutaric and glutamic acids	ORNL Reprint # 13
Oginsky, E. L., et al., H. C. Lichstein, and S. F. Carson	The influence of vitamin B ¹² on oxidation by a mutant strain of <u>E. coli</u>	ORNL Reprint # 6
Sheppard, C. W.	New developments in potassium and cell physiology: 1940-50	ORNL Reprint # 1
Sheppard, C. W. and Gertrude Beyl	Cation exchange in mammalian erythrocytes III. The polytic effect of X rays on human cells	ORNL Reprint # 5
Sodaro, R. M. and C. W. Sheppard	A technique for studying vasodilation and vasoconstriction using P ³² -labeled erythrocytes	ORNL Reprint # 3
Strehler, B. L. and W. A. Arnold	Light production by green plants	ORNL Reprint # 7
Teas, H. J.	Effect of canavanine on mutants of <u>Neurospora</u> and <u>Bacillus subtilis</u>	ORNL Reprint # 2

Scientific Society Lectures and Traveling Seminars. The traveling seminar season opened with Dr. S. F. Carson's lecture before the Bacteriology Department of the University of Illinois on "Comparative Biochemistry of Propionic Acid Fermentation." This was followed with a lecture by Dr. W. A. Arnold before the Biology Club of the Miller School of Biology, University of Virginia. Dr. Arnold's subject was "Light Production by Green Plants."

Twenty-two papers have been presented at scientific societies during this quarter. The Division was represented by twelve speakers at the American Institute of Biological Science meeting in Minneapolis, one of the most important meetings of the year for biologists. All papers presented are listed.

Billen, Daniel (H. C. Lichstein)	Ky.-Tenn. Branch, Soc. Am. Bact., Louisville	The effect of X radiation on the adaptive formation of formic hydrogenlyase in <u>E. coli</u>
Baker, W. K.	Tenn. Acad. Sci., Clarksville	Species and speciation from the standpoint of the geneticist
Khym, J. X.	Gordon Conference on Ion Exchange, New Hampton, N. H.	Some biological applications of ion exchange
Cohn, W. E.	Gordon Conference on Nucleic Acids, New Hampton	The application of ion exchange to the problems of nucleic acid structure and composition
Kimball, R. F.	<u>Paramecium</u> Genetics Symposium, Indiana Univ., Bloomington	1. Modification of the effects of X rays on stock 90 of variety 1 of <u>Paramecium aurelia</u> 2. Further investigations of the inheritance of reduced vigor
Sheppard, C. W.	Am. Heart Assoc. Symposium, Augusta	Isotope experiments on capillary permeability and interrelated circulatory mixing
Schwartz, Drew	Gen. Soc. Am. (AIBS), Minneapolis	A case of male sterility in maize involving gene-cytoplasm interaction
Kirby-Smith, J. S. (given by A. D. Conger)	As above	Effects of infrared radiation on the X- and γ -ray-induced chromosomal aberrations in <u>Tradescantia</u> pollen tubes
Conger, A. D. (L. M. Fairchild)	Gen. Soc. Am. (AIBS), Minneapolis	The induction of chromosomal aberrations by oxygen
Woods, M. C. (R. H. Storey, J. Furth)	Am. Physiol. Soc., Salt Lake City	Albumin exchange between blood and lymph compartments and estimation of lymph volume
J. C. Kile (W. L. Russell and L. B. Russell)	Gen. Soc. Am. (AIBS), Minneapolis	Failure of hypoxia to protect against the radiation induction of dominant lethals in mice
Noggle, G. R.	Bot. Soc. Am. (AIBS),	Phosphatase activity of lily anthers
Taylor, J. Herbert*	Genetics Soc. Am. (AIBS)	Autoradiographic measurements of desoxypentose nucleic acid (DNA) synthesis during the mitotic and meiotic cycles

* Consultant

Riley, H. P.* (N. H. Giles, Jr. and A. V. Beatty)	Gen. Soc. Am. (AIBS) Minneapolis	The oxygen effect on X-ray-induced chromatid aberrations in <u>Tradescantia</u> microspores
Giles, N. H., Jr.* (A. V. Beatty and H. P. Riley)	As Above	The relation between the effects of temperature and of oxygen on the frequency of X-ray-induced chromosome aberrations in <u>Tradescantia</u> microspores
Kimball, R. F. (Nenita Gaither)	As Above	Modification of the action of X rays upon <u>Paramecium aurelia</u>
Hollaender, A. (G. E. Stapleton W. T. Burnett, Jr.)	Am. Chem. Soc., Div. Biol. Chem., New York	Chemical aspects of radiation sensitivity of living cells
Doherty, D. G.	12th Internat. Congr. Pure and Appl. Chem., New York	The enzymatic hydrolysis of glyconyl peptide derivatives
Doherty, D. G.	As Above	The synthesis of glyconyl peptides
Billen, Daniel (G. E. Stapleton and A. Hollaender)	Bot. Soc. Am. (AIBS) Minneapolis	Probable mechanisms of protection of some chemicals against radiation
Noggle, G. R. (L. P. Zill)	Am. Soc. Plant Physiol. (AIBS), Minneapolis	Applications of ion-exchange methods to the quantitative analysis of sugars in plant extracts
Khym, J. X. (L. P. Zill)	As Above	The separation of sugars by ion exchange

Four papers are scheduled to be given at the annual meeting of the AAAS in Philadelphia, December 26-31, 1951. The following people will present work of the Biology Division at the American Society of Zoologists: Dr. Liane B. Russell (coauthors - W. L. Russell and Mary H. Major), Dr. R. P. Geckler (coauthor - R. F. Kimball), Dr. J. S. Dent (coauthor - E. L. Hunt), and Dr. G. R. Noggle. Mr. A. W. Burke, Jr. and Miss Frances Gamble will report on some work done prior to joining the Biology Division. Instead of attending the AAAS, Dr. R. F. Kimball will journey to the West Coast to attend a symposium on "Some Biological Effects of Radiation" at a meeting of the Western Society of Naturalists. His talk at this meeting will be "Some Factors Influencing the Sensitivity of Microorganisms to X rays."

* Consultant

Visiting Lectures. A series of seminars by summer Research Participants was given at the close of the season. The only other visiting speaker during the quarter was Dr. Joseph Weiss, of the Department of Chemistry, University of Durham, Kings College (England), who spoke twice before the Division assembly. His subjects were "The Action of Ionizing Radiations on Amino Acids in Aqueous Systems" and "The Action of X rays on Nucleic Acids in Aqueous Systems."

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball (Leader)

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

Division Delay By X Rays In Paramecium

(Kimball, Gaither, King)

For a clear understanding of division delay by radiation, it is necessary to have a detailed picture of the distribution of the delay to various intervals after irradiation. Such a picture was obtained for ultraviolet-induced delay and reported in Quarterly Report ORNL-727. An experiment has now been performed for the same purpose with X rays.

The animals were placed in small drops under mineral oil and exposed to the unfiltered beam of the Maxitron machine according to the techniques given in Quarterly Report ORNL-989. Intensity was calculated to be approximately 68 kr per minute. Exposures of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 minutes were made and controls were kept, after which thirty animals were isolated from each exposure group. Observations of these 270 animals and their progeny were made every hour until at least six divisions were completed. A single animal from each line of descent was transferred to fresh culture medium twice daily to keep the numbers to be counted small. The time between successive divisions was calculated from the records of numbers of animals present at each observation. The arithmetic means of the reciprocals of these times are plotted in Fig. 1 for three dose groups and the control. For comparison, a similar plot for the lowest dose group (500 ergs/sq mm) from the ultraviolet experiment reported in ORNL-727 is given.

Two features are immediately apparent. The general pattern of distribution to the various intervals after irradiation is the same for X rays and ultraviolet. There is a major difference between the two in the relatively greater effect of X rays upon the first interval. A greater "immediate" effect is also reflected in the fact that the 4-minute exposure to X

* Research Participant

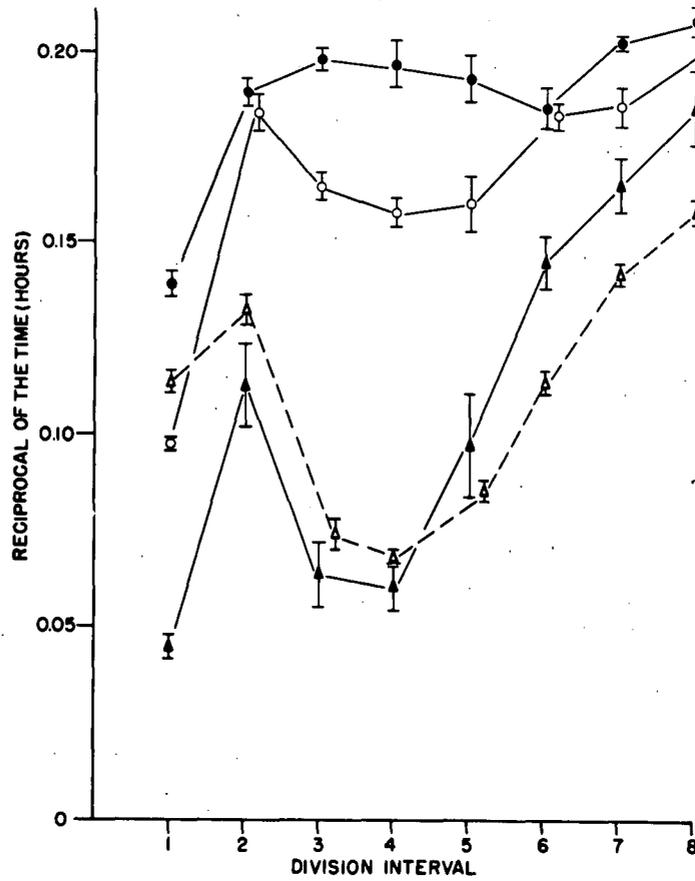


FIG. 1

The reciprocals of the times for successive division intervals after irradiation. The first interval is the period between irradiation and the first division; the second, the period between the first and the second divisions; etc. The data are for three different doses of X rays and, for comparison, a single dose of ultraviolet.

X rays

- - 0.5 min
- - 2.0 min
- ▲ - 4.0 min

Ultraviolet

- △ - 500 ergs/sq mm

rays resulted in the death without division of 21 out of the 30 animals isolated whereas, in the ultraviolet experiment, a dose of 2500 ergs/sq mm, five times the one plotted, produced no death before the first division.

A characteristic feature of the delay by higher doses of ultraviolet is a very long cessation of division usually after two or three divisions. Such

a cessation period may last for as long as three weeks before recovery of the normal division rate takes place. No long cessation of this sort has been found for X rays, but the reason is now clear. The maximum dose which does not kill all animals before the first division is equal in its effect upon later intervals to a relatively low dose of ultraviolet; and so long cessation periods are not to be expected. The basic pattern of delay by the two types of radiation is the same, only the relative importance of particular parts of it being different.

The Structure of the Macronucleus and the Effect of Radiation upon It

(Kimball)

Kimball (Anat. Record, 105:543, 1949) and Kimball and Gaither (J. Cellular Comp. Physiol., 37:211-233, 1951) have reported upon a change in the structure of the macronucleus following exposure to ultraviolet. The change was observed in living material under the Zeiss phase microscope. Two types of material in the macronucleus can be seen with this microscope: (1) small, round, dark bodies; and (2) a lighter background material which appears, when the macronucleus is broken by compression, to consist of many tightly packed bodies near the limits of visibility of the microscope. After ultraviolet, the dark bodies fused with one another into large vacuolated masses. Since this behavior resembled rather closely similar changes reported in nucleolar material in other organisms, the hypothesis was advanced that the dark bodies were nucleoli and the very small bodies, chromosomes.

The detailed visible structure of the macronucleus of ciliates has never been clearly interpreted, so it appeared worth while to check this by specific staining techniques. Accordingly, animals were exposed to a heavy dose of ultraviolet, fixed several hours later in 2 per cent osmic acid, imbedded in paraffin, and sectioned at 3 μ . Sections were stained either with the Feulgen and light green combination or with pyronin and methyl green. Unirradiated controls were treated in the same way. Large vacuolated masses were clearly visible in the irradiated material stained, as the hypothesis predicted, with light green or with pyronin. The background material stained uniformly with Feulgen or with methyl green as would be the case if chromosomal material were involved. Thus, the hypothesis that the dark bodies under the Zeiss phase are nucleoli appears to be firmly established.

So far, similar changes in macronuclear structure have not been found even after 250 kr of X rays. This is rather surprising in view of Duryee's

(J. Natl. Cancer Inst. 10:735-796, 1949) finding of very similar changes in the nucleoli of amphibian oocytes following doses of the order of 50 kr of X rays. The reason for this apparent difference is not clear.

Modification of the Effects of X Rays upon Paramecium

(Kimball, Gaither, King)

Culture fluid. - In the previous quarterly report (ORNL-989), it was shown that paramecia exposed to X rays in phosphate buffer with 3 per cent culture fluid added (D) were distinctly more affected than animals exposed in undiluted culture fluid (C). It seemed possible that the protective effect of C as compared to D might be due either to protection by the complex ingredients of C against injurious substances formed in the medium or by some alteration of the condition of the animals by D to make them more radiosensitive. To test this matter, animals were exposed for three quarters of an hour either to C or to D, then placed in C or D and irradiated as soon thereafter as possible. In all cases, they were removed to C immediately after irradiation and isolated, each into a separate container.

The results are shown in Table 1. Surprisingly enough all treatment groups were nearly alike save the group which was kept in C until just before irradiation but irradiated in D. This group was more affected by the X rays. It appears that the nature of the medium at the time of irradiation

TABLE 1

THE EFFECT OF CULTURE MEDIUM (C) AND CULTURE MEDIUM DILUTED TO 3 PERCENT WITH PHOSPHATE BUFFER (D) UPON THE ACTION OF X RAYS ON PARAMECIUM AURELIA

Pretreatment for 3/4 hour with	Medium during irradiation	Dose of X rays (min)	Number of treated animals	Division rate as per cent of control	Per cent survival
C	C	1	60	93	100
C	D	1	55	79	72
D	C	1	60	97	100
D	D	1	54	93	100
C	C	2	60	78	100
C	D	2	54	--	0
D	C	2	60	72	72
D	D	2	54	70	24

is important, suggesting that injurious substances produced in the medium play a role. However, the condition of the animals is important also, since those which were kept for some time in D no longer show a marked effect of the medium at the time of irradiation, though the somewhat higher death after 2 minutes exposure in D suggests a slight effect.

The tentative hypothesis can be advanced that C animals, but not D, respond to something produced in the D medium by X rays. It can be assumed that this material is not produced to the same extent in the C medium, perhaps because of a protective effect of the complex ingredients (lettuce infusion, living bacteria, and bacterial products) of this medium.

Oxygen tension. - Several experiments have been performed to check the effect of low oxygen tension on X-ray-induced division delay. In general, they show that division delay is unaltered or increased when the irradiation takes place under hypoxial conditions. This is, on the whole, in line with the findings reported in ORNL-989 but more cases were found in which there was no effect of hypoxia, suggesting a variable response. In any case, there is no evidence of protection. This contrasts with the protection repeatedly found against the mutational effect (ORNL-989). This protection has now been checked for the postautogamous mutational changes produced by high doses (ca. 100 kr) of X rays. After recovery from the immediate effects, the animals were sent through autogamy and the per cent which failed to survive 4 days was checked. In the group of autogamous clones exposed in air 66 per cent failed to survive, while in the group exposed in 2 per cent oxygen, 47 per cent failed to survive.

Variability In Tradescantia Pollen

(Conger, Fairchild)

The dry pollen grains of Tradescantia have shown, in the past, a remarkable variability from week to week in response to radiation. This unaccountable variability between experiments may be by as much as a factor of two. Examples of this are shown in the Figs. 2 and 3, wherein two X-ray experiments and two α -particle experiments are compared.

The two X-ray experiments (Fig. 2), made about a month apart, were supposedly identical treatments, yet the amount of chromosomal breakage induced was about two and a half times greater in one experiment than in the other. The same is true of the two α -ray experiments (2 weeks apart).

It is important to point out that the results within any 1 day's experiment are consistent among themselves, the variation being between days. It is not due to errors in radiation dosimetry.

The same sort of day-to-day variation is appearing in our experiments on inducing chromosomal breakage in these pollen grains with oxygen. This is demonstrated in Fig. 4. We have more data showing the daily variation between single treatment points, but it does not illustrate the consistency of results within any 1 day that is shown by these dose curves.

The variability between the oxygen experiments could easily be due to any of a number of treatment factors such as excessive desiccation of the pollen by the dry gas or changes in permeability, but it is difficult to see how the results in the radiation experiments can be explained by differences in environmental conditions at the time of treatment. As a matter of fact, such differences in response to radiation can be obtained only with considerable effort, for example, by extremes of temperature (0° and 35°C), at least when the microspores in the flower buds are used. No such extremes were encountered in these experiments.

The cause of the variation in these experiments is difficult to guess. Most of the ordinary conditions, such as temperature and humidity, do not vary enough to account for the magnitude of the differences, on the basis of experience with microspores in the flower buds. However, the pollen grains, dry and exposed to the air, are a very different material from the microspores, a wet tissue enclosed in the buds. We suspect that it is more likely due to some biological variation in the conditions of the cells themselves, arising from changes in the vigor and growth of the plants.

Experiments seeking the factors which may be causing this have been started but results are not yet available for discussion.

The Effect of Oxygen Tension on X-Ray-Induced Chromosome Breakage in Maize

(Schwartz, Clark)

Results of experiments described in the last Quarterly Report, ORNL-989, indicate a definite protection of reduced oxygen tension against the induction of chromosome breakage by X rays. Pollen irradiated in air showed 3.3 times as many breaks as pollen irradiated in a 100 per cent nitrogen atmosphere. The nitrogen-treated pollen was preflushed with the gas for 3 minutes. A steady flow of nitrogen was passed through the treatment chamber during the 4 1/2 minutes of radiation. The dosage given was 1000 r.

Chromosome breakage was determined genetically rather than cytologically. Genes a (aleurone color) and sh (shrunken endosperm) are situated on the long arm of chromosome 3 and show very close linkage (0.25

FIG. 2

Two X-Ray Experiments

- - cd - isocd, Experiment 18
- ▲ - normal cells, " "
- - cd - isocd, Experiment 19
- △ - normal cells, " "

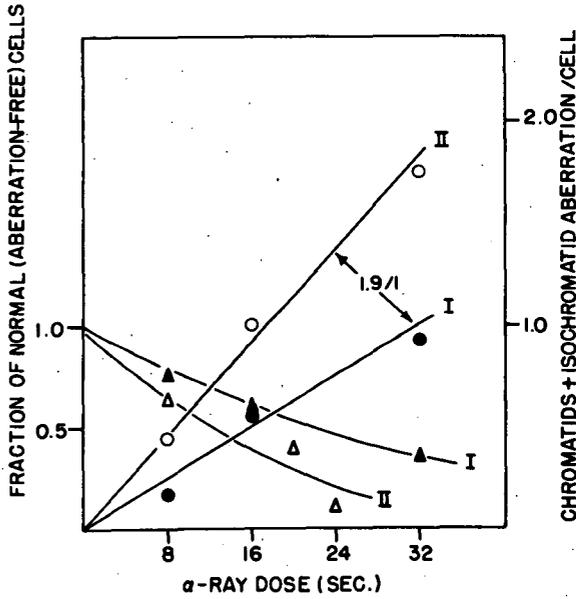
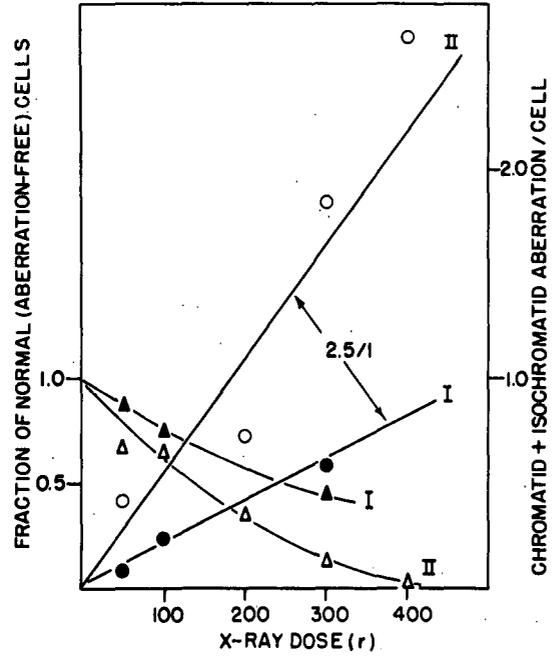


FIG. 3

Two X-Ray Experiments

- - cd - isocd, Experiment 5
- ▲ - normal cells, " "
- - cd - isocd, Experiment 7
- △ - normal cells, " "

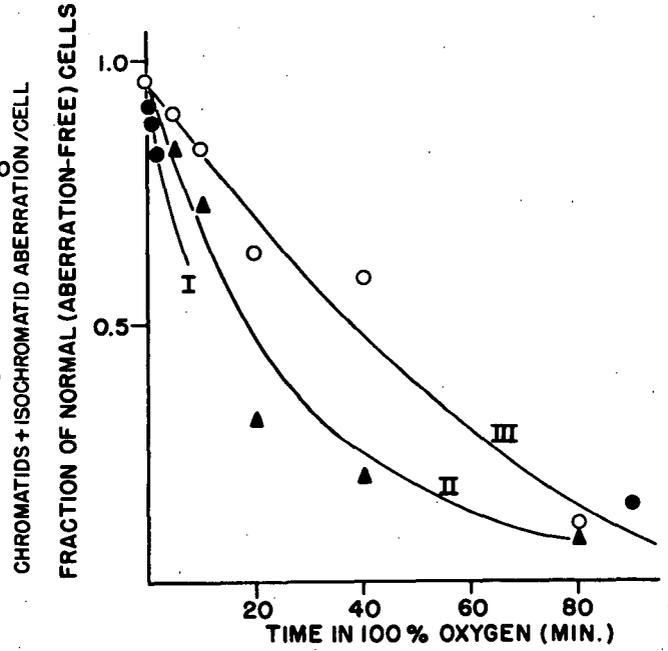


FIG. 4

Three Oxygen Experiments

- - Experiment, Aug. 27
- - " , Oct. 11
- ▲ - " , Oct. 15

per cent crossing over). Pollen carrying both dominant genes, A Sh, was X-rayed and used to pollinate tester plants homozygous recessive for both genes, a sh. The resultant seeds were examined and chromosome breakage was detected by the occurrence of colorless and/or shrunken seeds. Three classes of effects were observed and scored: (1) whole seed losses of A and Sh (due to deletion of the portion of the chromosome carrying both genes), (2) whole losses of either A or Sh (due to point mutations), and (3) mosaics-seeds having areas of both A-Sh and a-sh tissue (due to a single break distal to the A-Sh loci resulting in dicentric formation and subsequent bridge-breakage-fusion cycle in the endosperm). Results are given in Table 2. The decrease of chromosome breakage by a factor of 3.2 agrees well with the results reported for similar experiments with Tradescantia and Drosophila.

The experiment was set up to determine the relative protection of reduced oxygen tension against gross chromosomal deletions as compared to point mutations. However, the frequency of point mutations in both the air and nitrogen treatments was so low that a valid comparison is not possible.

TABLE 2
FREQUENCY OF SEEDS SHOWING CHROMOSOME BREAKAGE FOLLOWING
IRRADIATION IN AIR AND NITROGEN

Gas	No. of seeds scored	Whole losses of <u>A</u> and <u>Sh</u> (%)	Whole losses of <u>A</u> or <u>Sh</u> (%)	Mosaics (%)	Total (%)
Air	6158	2.517	0.065	0.650	3.232
N ₂	4505	0.933	0.022	0.066	1.021
Air/N ₂		2.7	2.9	9.9	3.2

The Behavior of an X-Ray-Induced Ring Chromosome in Maize

(Schwartz, Clark)

A mechanism has been described (ORNL-989) whereby a ring chromosome involving the nucleolar organizer was converted into a stable rod chromosome. Plants carrying the converted rod appeared as green offspring in the progeny of self-pollinated striped plants. Further studies were conducted in an attempt to complete the cycle by changing the rod

back to a ring. Some of the converted rod chromosomes are duplicated for a large segment of chromosome 6. If during meiosis, the duplicated sectors of the same chromosome pair and crossing over occurs between them, a ring chromosome will be formed. The formation of these rings should result in the appearance of striped plants in the progeny of self-pollinated F_1 green plants, due to the instability of ring chromosomes. This is precisely what was found. Fifteen F_1 green plants were self-pollinated. Of these, 14 segregated only green and white offspring in good 3:1 ratios. However, one plant segregated stripes in addition to the green and white progeny, thus completing the ring \rightarrow rod \rightarrow ring cycle.

Plants heterozygous for the ring and a normal chromosome 6 show a very high frequency of bridges in the meiotic anaphase (ORNL-989). Since each bridge contains the nucleolar organizer region, the distribution of nucleoli in the quartet stage following meiosis was investigated. Thirty-four per cent of the quartets showed abnormal nucleolar distribution, eight abnormal types were encountered (Fig. 5). Types a and b occurred with the highest frequency. All these abnormal quartet types can be explained by (1) the absence of the nucleolar organizer, (2) the presence of two organizers in one cell, (3) the presence of a large deficiency in chromosome 6 inactivating the nucleolar organizer, and (4) the presence of a large duplication on chromosome 6 inactivating the nucleolar organizer.

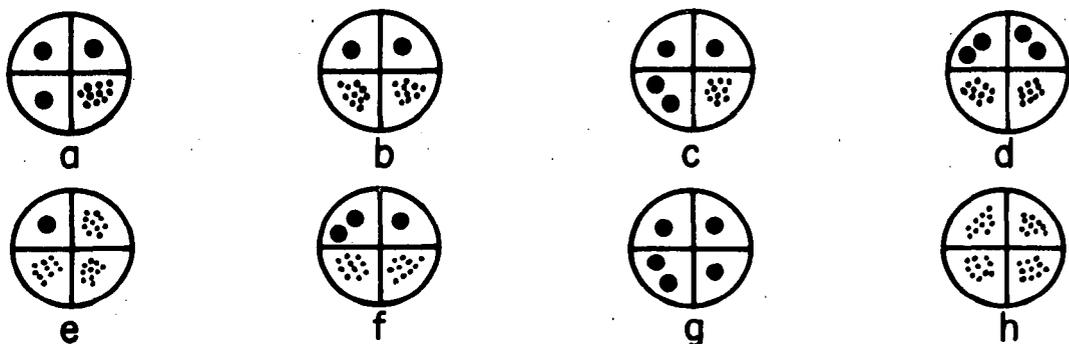


FIG. 5

The Distribution of the Nucleoli in the Abnormal Quarter Types
 (☒ - diffuse condition of nucleolar material)

Radiation Effects on Neurospora Conidia

(Atwood, Mukai)

It is possible to measure, in some cases directly and in some indirectly, the following aspects of the effect of radiation (or other agents) on the conidia of Neurospora.

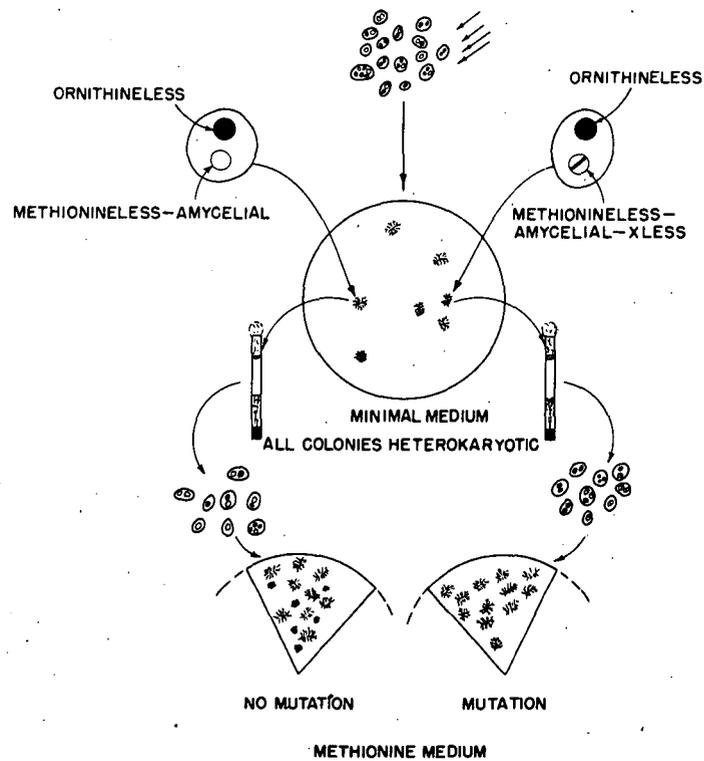
1. Surviving fraction of cells
2. Surviving fraction of nuclei
3. Fraction of nuclei containing at least one recessive lethal mutation
4. Frequency with which separately induced recessive lethal mutations are homologous
5. The degree to which all of the above effects are independent of each other

Such determinations, particularly where they can be made simultaneously on a single treated suspension, permit definite conclusions regarding the relative response to radiation of the genetic apparatus, the nucleus as a whole, and the extranuclear constituents of the cell.

It will be useful to review the principles underlying the estimation of the quantities 2, 3, and 4 above, since these are, for the most part, unpublished. The surviving fraction of nuclei may be estimated indirectly from a comparison of the surviving fraction of (initially) heterokaryotic conidia on minimal medium with the surviving fraction of the same suspension plated on medium containing the growth factor required by the separate components. To make this clear let us consider a conidium with n nuclei, some of which are A-less and some B-less. This cell can initiate growth on minimal medium. Now assume that radiation has destroyed all but one of the nuclei. Evidently this last surviving nucleus must be either A-less or B-less, and the cell can initiate growth on medium containing factors A and B, but not on minimal medium. If we have a suspension of such cells, the surviving fraction on minimal will be smaller than on A+B medium, and this difference is a function of the number and distribution of nuclei and the surviving fraction of nuclei, as distinct from other cell constituents. The number and distribution of nuclei in the conidia can be found by direct counts.

The fraction of nuclei containing at least one recessive lethal mutation may be estimated directly by a somewhat more complicated procedure. The conidia of a heterokaryon between ornithineless and methionineless-amycolial are plated in minimal medium. Although many homokaryotic cells are present in the suspension, only those cells which are heterokaryotic can grow. The resulting colonies are isolated to tubes

and allowed to form conidia. These conidia are brought into aqueous suspension and streaked on the surface of agar medium with added methionine, a separate streak being made for the conidia of each isolate. Where conidia homokaryotic for methionineless-amycolial are viable they will produce colonies having the distinctive amycolial morphology. Thus, two morphologically different types of colony will ordinarily appear after streaking; the normal type which originates from heterokaryotic conidia, and the amycolial from one of the homokaryotic classes. However, if a recessive lethal mutation is present in the amycolial-labeled nucleus of the conidium which gives rise to the initial heterokaryotic isolate, then all conidia from this isolate which are homokaryotic for amycolial will be inviable because they are also homokaryotic for the lethal mutation. The presence of the lethal mutation is then revealed by the absence of amycolial colonies in the streak, and the mutation can be maintained in heterokaryotic form for further study. Figure 6 represents this procedure diagrammatically.



The frequency with which separately induced lethal mutations are homologous may be found directly by the following procedure:

Suspensions are prepared of the conidia of heterokaryons known to carry lethal mutations in their amycelial components. These suspensions are intimately mixed two-by-two, and cultures are established using each mixture as an inoculum. A few of the conidia from each culture contain at least one amycelial nucleus from each of the two contributing heterokaryons, but no other nuclei. Cells of this constitution will produce (amycelial) colonies only if the lethal mutations carried by the contributing heterokaryons are not homologous. Consequently the presence of amycelial colonies in streaks made with the conidia of such cultures reveals that the mutations are not homologous.

We are beginning to apply these methods to the study of the effect of ionizing radiation, and some preliminary results will be reported. Figure 7 shows survival curves for an aqueous suspension of conidia of the heterokaryon (ornithineless)-(methionineless-amycelial) taken simultaneously on minimal and supplemented media using 250 kv X rays filtered

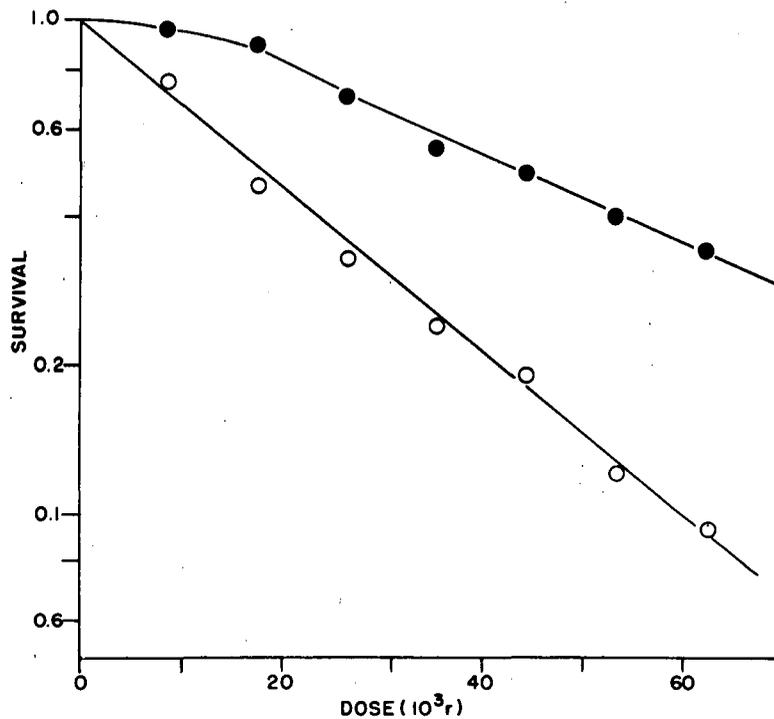


FIG. 7

Survival of Conidia of Heterokaryon (29997)-(4894 - 422) A in Aqueous Suspension. 250 kv of X rays, 3 mm of Al.

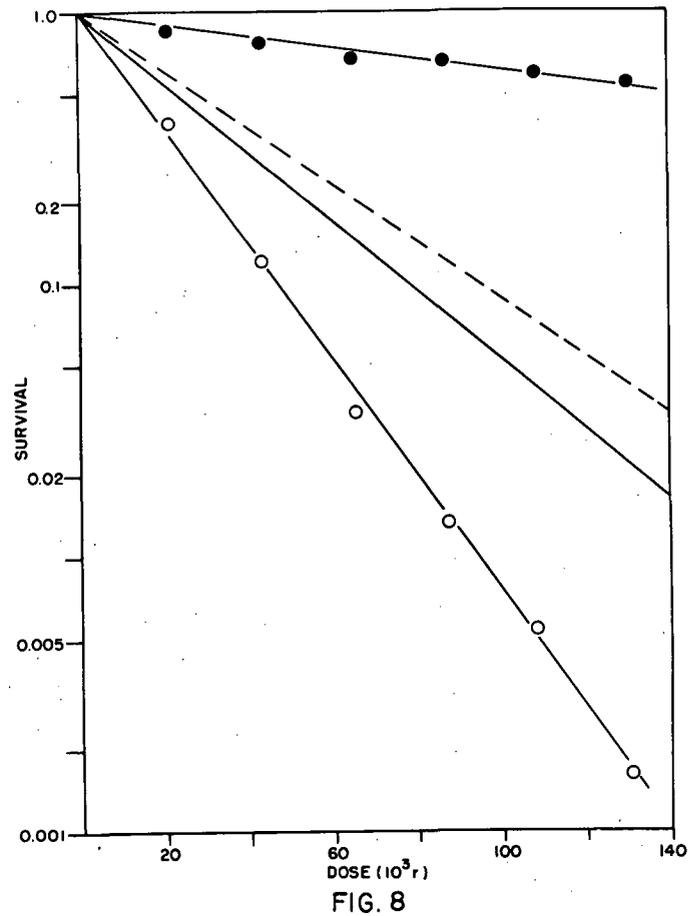
- - medium supplemented with arginine and methionine
- - minimal medium

with 3 mm of Al. The average number of nuclei per conidium in this suspension is close to two, with about 50 per cent of the cells binucleate. On minimal medium a simple exponential, or single target curve is obtained. This result can be readily explained, since it is known that at least two viable nuclei must be present to insure cell viability. Thus it will be sufficient to inactivate just one nucleus in a cell to transform the cell from a heterokaryon to a homokaryon and render it inviable on minimal medium. On supplemented medium, however, all the nuclei must be inactivated in order to kill the cell (provided, of course, that it survives extranuclear effects). We see in Fig. 7 that a multitarget curve on supplemented medium is in fact obtained. These findings are consistent with the conclusion that the inactivation of the cells within the dose range studied is largely, if not entirely, a nuclear phenomenon.

The multitarget curve rapidly becomes linear (on a semilogarithmic plot) as the dose increases, and its ultimate slope is a measure of the sensitivity of single (last surviving) nuclei in the cells. Comparison of the slope of the curve on minimal medium with the ultimate slope of the curve on supplemented medium reveals a difference of a factor of 1.7. One might expect them to differ by a factor of approximately 2.0, since the probability of inactivating either one or two nuclei is twice that of inactivating a single nucleus. This discrepancy becomes understandable when the part played by recessive lethal mutation is taken into account.

Recessive lethal mutations have no effect on the survival of multinucleate cells except where the same mutation is present in each nucleus, a rare eventuality if we assume a reasonable number of possible different mutations. In a cell which is effectively uninucleate, however, any one of the possible recessive lethal mutations will lead to inactivation. Thus, in the experiment shown in Fig. 7 the survival on minimal medium will not be influenced by recessive lethal mutations, but on supplemented medium these mutations will depress the survival in the region of ultimate slope, in so far as they occur. The appropriateness of this line of reasoning can be tested, since we have a direct method for the estimation of recessive lethal mutations.

Fig. 8 shows the results of an experiment in which survival on minimal medium and the frequency of nuclei carrying at least one recessive lethal mutation were determined simultaneously, using the method outlined in Fig. 6 for the mutation data. To facilitate comparison of mutation with survival, the frequency of unmutated nuclei has been plotted. Now, if we assume that the inactivation of heterokaryotic cells results from the inactivation of either one of two nuclei (and this is a reasonable approximation), we can obtain a hypothetical curve for the survival of a single nuclei with respect to processes other than recessive lethal mutation. The probability that a nucleus simultaneously survives these processes and the recessive lethal mutation process is the product of the



Conidia of Heterokaryon (29997)-(4894 - 422)A on Aerial Hyphae.
250 kv of X rays, 3 mm of Al.

- - unmutated nuclei
- - survival on minimal medium
- - theoretical survival of single nuclei
- - theoretical survival of uninucleate conidia

separate survivals. Thus by reference to the curve for unmutated nuclei in Fig. 8, we can compute the expected survival curve for uninucleate, or effectively uninucleate cells. When the slopes of this theoretical curve and the survival curve of the heterokaryon on minimal medium in Fig. 8 are compared, they are found to differ by a factor of 1.7 as is expected from the results of the experiment in Fig. 7.

Having at hand a structure of hypothesis which predicts the survival of uninucleate cells, we have begun experiments with a genetic strain of *Neurospora* which produces solely uninucleate conidia. The results will be presented in a subsequent report.

The Effect of Oxygen on Lethal Mutation in Neurospora

(Atwood, Mukai)

The production of chromosome breakage by treatment of Tradescantia pollen with oxygen has recently been reported by Conger (ORNL-989:23-31, 1951). In Neurospora, chromosome breakage followed by the loss of acentric fragments would be revealed as lethal mutation by the heterokaryon method shown in Fig. 6. Dry conidia of the heterokaryon (ornithineless)-(methionineless-amycelial) attached to aerial hyphae were treated with 100 per cent oxygen at 100 lb/sq in. for periods up to 100 minutes, then brought into suspension and plated. No significant effect on survival was noted. The data for recessive lethal mutation are given in Table 3. The mutation frequencies in treated material are not significantly greater than in the control, at the 5 per cent level. Conger has reported that in Tradescantia a 100-minute exposure gives an effect approximately the equivalent of 1200 r of X ray. Since 1200 r would have a negligible effect on Neurospora, experiments involving much longer treatments with oxygen are in progress.

TABLE 3

TREATMENT OF DRY CONIDIA WITH 100 PER CENT OXYGEN AT 100 LB/SQ IN.
HETEROKARYON METHOD

Duration, minutes	Control	10	30	100
Total isolates	100	119	119	239
Number of isolates carrying lethals	2	0	0	6

X-Ray Sensitivity of Bacteriophage T2H in the Presence of Various Selected Chemicals

(Doermann, Hill)

One of our previous quarterly reports concerned itself with the X-ray sensitivity of bacteriophage T2H in the presence of several protective chemicals which had been shown to be protective to bacteria by the Radiation Protection Group of this Laboratory (see Quarterly Report ORNL-1026:20). More particularly these experiments were concerned with protection of phage against the so-called "direct effects" of radiation, and it was noted that two groups of chemicals gave some measure of protection, namely the sulfhydryl compounds, cysteine and BAL, and the alcohols.

We have now made some studies of the protective effects of certain chemicals against the "indirect effect" also. When phage is irradiated in the absence of protective substances, the single-hit type of exponential survival curve is not observed. Rather, plotting logarithm of survival against dose, it bends downward in the manner of a multiple-hit curve. The indirect effect as defined by Watson (Dissertation, Indiana University, 1950) is obtained by subtracting the direct effect from this curve. The experimental technique is to irradiate two series of control tubes at different doses, the one series a broth suspension of phage and the other a suspension of phage in phosphate buffer (15 M, pH 6.9). The substances to be tested are added to buffer suspensions. The broth curve simply serves to measure the direct effect of the radiation. Of the substances tested, it may be qualitatively stated that all afforded some protection against the indirect effect. These substances are listed in two groups: (1) Those which gave complete protection against the indirect effect, that is, they made the buffer curve superimposable on the broth curve, are cysteine, methionine, glutamate, and tryptophane. All were tested at 0.005 M concentration. It should be noted that the cysteine curves were brought up higher than the broth curve, due to protection against the direct effect. (2) Those which gave partial protection against the indirect effect are glycine, α -alanine, β -alanine, serine, norleucine, and lysine which were tested at 0.005 M ethanol and 0.01 M formate. The shapes of the survival curves in the second class are of some interest. In all cases in the latter group, with the exception of serine, they were of the multiple-hit type and were located intermediate between the broth curve and the buffer curve. The serine curves, on the other hand, were also intermediate between the two control curves, but were of the single-hit type.

Watson's results indicated that the phage particles inactivated in buffer were largely prevented from reproducing, due to impairment of their ability to adsorb onto the host cells. Those inactivated in broth, although capable of adsorbing, were unable to propagate for some other reason. Our results, as well as those of Watson could still be explained on the moderately simple hypothesis that the inactivation of the adsorptive capacity of the phage is a multiple-hit phenomenon which is largely mediated through the suspending medium. The single-hit phenomenon could be simply a lethal action on the reproductive structure of the phage particle. This might be the result of direct absorption of energy or of an indirect effect. One would then interpret the broth curve as resulting from the total direct effect of the radiation plus part of the indirect inactivation of the reproducing structure. The serine curve might possibly give the total inactivation on this structure. The cysteine curves giving maximum protection may measure exclusively the total direct effect. The other curves would then measure combinations of direct and indirect effects on both structures.

To test whether this hypothesis can be seriously considered, more experiments must be made. More specifically, a more complete test is necessary to see whether one can measure separately the four types of killing, namely, the direct and indirect single-hittypes and the direct and indirect multiple-hittypes. Since Watson has already worked out the techniques for separating the effects on the phage particle, it seems possible that such an approach could succeed.

Isolation of Triploid Yeast

(Pomper, Daniels)

Polyploid yeast have been reported in the literature (DeLong and Lindgren, *Bact. Proc.*, p. 63, 1951; Tobias, Univ. of Cal., Report UCRL-960: 43-69, 1950). Data of their isolation, stability, and genetic characteristics are lacking.

An attempt has been made in this laboratory to isolate a triploid clone from *Saccharomyces cerevisiae*. This yeast normally exists as a diploid; its ascospore isolates may be kept as haploids (since our strain is heterothallic). The approach employed was as follows: It is known that mating type is controlled by a single major gene pair (a/a); hence it might be possible by irradiation to cause a mutation at this locus. If mutation occurred in a diploid normally heterozygous for mating type to render it homozygous, conjugation might ensue between such a diploid and an appropriate haploid to produce a triploid.

The experiment was designed in two parts, viz., tests with and without irradiation of the diploid. Conditions were such that a prototroph recovery (Pomper and Burkholder, *Proc. Natl. Acad. Sci.* 35: 456-464, 1949) would be possible if a triploid were produced. This was done as follows: diploid ($Tr^+ / Tr^- Me^+ / Me^- Ad^- / Ad^- Ur^- / Ur^-$) x haploid ($Tr^- Me^- Ad^+ Ur^+$), permitting recovery on an agar lacking all four factors (Tr = tryptophan, Me = methionine, Ad = adenine, Ur = uracil). Both irradiated and unirradiated diploid cells were mixed with haploids of both mating types (four separate "crosses") in the usual procedure for yeast conjugation, and plated (after incubation) on agar lacking all four factors. No colonies appeared on the plates in which the irradiated diploid was employed; colonies did appear in both tests with the unirradiated diploid. We have no explanation for the failure of the irradiated diploid test to give colonies.

Single colony isolations were made from the plates. Microscopic examination showed cells that were large and rather more oval than our usual diploid or haploid clones. We decided to continue on the tentative assumption that these isolates were triploids, and both genetic and irradiation tests were made in an effort to evaluate the assumption.

The genetic tests were made by inducing sporulation. It should be noted that haploids have never (in our hands) given abundant sporulation, and further, that the particular diploid employed in the original cross did not sporulate when tested under usual conditions. The fact that the triploid isolates sporulated appeared to support the assumption that they were different from both the diploid and the haploid. Analysis of the ascospore isolates seemed to both support and contradict the assumption. As shown in Table 4, segregation was abnormal for three genes, normal for two.

TABLE 4
ANALYSIS OF SEGREGATIONS OF ASCOSPORES

Ascospore	Mating Type	Segregations			
		Tr	Me	Ad	Ur
Cross 1: Haploid (<u>a</u> Tr ⁻ Me ⁻ Ad ⁺ Ur ⁺) x Diploid (<u>a/a</u> Tr ⁺ /Tr ⁻ Me ⁺ /Me ⁻ Ad ⁻ /Ad ⁻ Ur ⁻ /Ur ⁻)					
541.1	-	+	-	+*	+*
.2	a	-	+	+*	-
.3	a	-	+	-	+
.4	-	+	-	+*	+*
544.1	a?	-	-	+	+
.2	a	+	+	-	+
.3	a	-	-	-	+
.4	-	+	+	+	+
555.1	a?	+*	+	+*	+
.2	a	+	-	+	+*
.3	-	-	+	+	-
.4	-	-	-	+	+
Cross 2: Haploid (<u>a</u> Tr ⁻ Me ⁻ Ad ⁺ Ur ⁺) x Diploid (<u>a/a</u> Tr ⁺ /Tr ⁻ Me ⁺ /Me ⁻ Ad ⁻ /Ad ⁻ Ur ⁻ /Ur ⁻)					
547.1	-	+	+	+	+*
.2	-	+	-	+	+
.3	a	-	+	+	+
.4	a	-	-	+*	-
552.1	a?	+	+	+*	+
.2	a	-	-	+	+
.3	a?	+	+*	+	+*
.4	a	-	-	-	+
554.1	-	-	+*	+	-
.2	-	+	-	+	+
.3	a?	-	-	-	+*
.4	a	+	+*	+*	+*

* = Those cultures that grew more slowly in the absence of a given factor than they did in the control tube (containing all factors).

Support for the theory comes from the abnormal mating behavior of the isolates; contradiction from the segregations of the biochemical requirements. The fact that the segregations for Tr and Me were apparently those for single gene pairs, while those for Ad and Ur showed an excess of independent ("+" types, made us suspect that perhaps the original ("triploid") isolates were, after all, merely double mutants of an unstable sort (selected in such a way that the Tr and Me segregations would be normal, but those for Ad and Ur abnormally high for independence.

At this point, irradiation studies were initiated on the four ascospores of ascus 541 (A541.1, A541.2, A541.3, and A541.4). Our earlier studies had shown a marked difference in survival after ultraviolet irradiation between haploid and diploid strains, permitting differentiation. It was thought that, if the test clones were not triploid (and instead were diploid double revertants), the ascospores should give survival curves characteristic of haploids. This was not the case. As shown by the survival curves in Fig. 9, there is considerably variation among the four clones. While the data are not good enough to determine state of ploidy by extrapolation (Norman, Exptl. Cell. Research, 2:454, 1951), they are strongly suggestive at least of a difference. Further, tests were made on the two triploid clones that had been used in the genetic study, and both showed a high order of resistance.

Thus, it is now felt that most of the data support the assumption that a triploid or its equivalent was formed and isolated, although caution is still required. Further tests are in progress (1) to clarify the mating behavior of the ascospore isolates, (2) to attempt sporulation of possible diploid ascospore isolates as an aid in understanding the peculiar segregational behavior of the biochemical markers, and (3) to refine the irradiation techniques adequately to permit a ploidy estimate. It is not at all clear at this time that our original basic hypothesis, viz., that mating type is mutable, has been fulfilled. Experiments now in progress may elucidate this point.

If triploids (or clones of higher ploidy) can be isolated by the methods employed, they will be a source of information about the genetic behavior of polyploids, as well as providing excellent material for studies of irradiation effects as influenced by gene number.

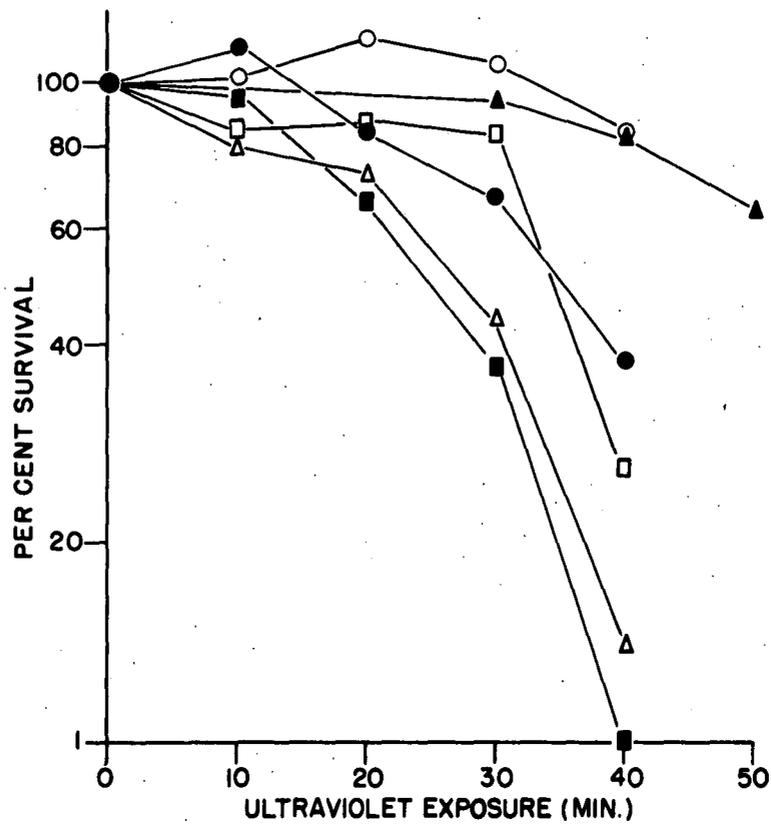


FIG. 9

○ - A541.1
● - A541.2
■ - A541.3

□ - A541.4
△ - A205.3 (known haploid)
▲ - P4(A92.2 x A93.2) (known diploid)

EFFECTS OF RADIATION ON RATE OF MITOSIS

Mary Esther Gaulden (Leader)

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Comparison of Effects of Beta Rays and X Rays on Mitosis

(Gaulden; Darden, Biophysics; Nix)

Phosphorus-bakelite plaques served as sources of pure, high intensity β rays (Nucleonics 8: 17, 1951). Accurate calibration of the surface dose rates of the plaques was made with an instrument designed by Sheppard and Abele (AECU-655). X rays of 125 kvp were used and were measured in air with a Victoreen thimble chamber.

For β irradiation of neuroblasts embryos were dissected from eggs and embryonic membranes, yolk, and appendages removed. The head and abdomen were cut away. The exposure dish consisted of a rubber hydrochloride membrane (0.001 in. thick) stretched between two close fitting aluminum rings (i. d. - 3 cm). The dish was filled to a depth of 8 mm (maximum penetration of β rays - 7 mm) with artificial culture medium and the embryos placed, ventral surface down, on the bottom (neuroblasts lie at ventral surface). The freshly cut ends adhere to the membrane holding the neuroblasts firmly against it during exposure. A plaque of desired intensity was then brought into close contact with the bottom of the dish. Thus the cells were separated from the source only by the rubber hydrochloride membrane. The culture medium provides a uniform material around and above the cells, a condition which eliminates discontinuities in the system and thereby allows for accurate calculations of the intensity of the radiation at the level of the cells. For exposure to X rays embryos were prepared as above but irradiated in a polystyrene dish.

The amount of energy dissipated by 1 r of X rays was considered to be 91 ergs/g of tissue and that dissipated by 1 rep of β rays 83 ergs/g. Dose rates of β rays were accordingly adjusted to equal those of X rays in terms of energy dissipated by a conversion factor of 1.1. The final dose of β rays was calculated as the dose delivered at the center of the

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neuroblast (rubber hydrochloride membrane absorbs 5 per cent of surface dose and the thin layer of epidermis plus the top half of the neuroblast absorbs about 2 per cent). Two doses of radiation were used, namely, 8 and 64 r of X rays and 8.8 rep and 70.4 rep of β rays. The lower dose does not depress mitosis to zero and therefore permits analysis for differences in depth to which mitosis is depressed by the two types of radiation. The higher dose depresses mitosis to zero, resulting in periods of inhibition and recovery that are of considerable duration.

Following irradiation the embryos were made into hanging-drop preparations and the neuroblasts observed with a microscope enclosed in an incubator maintained at 38°C. The number of cells in mid-mitosis was recorded every 22 minutes for 5-6 hours after irradiation. Since 22 minutes is the average duration of mid-mitosis at 38°C, and since the treatments used do not affect this duration, the number of cells completing mitosis in a given period of time can thus be determined. Data were obtained from 12 embryos treated with each dose of X rays and 12 with each dose of β rays as well as 12 embryos that received no treatment.

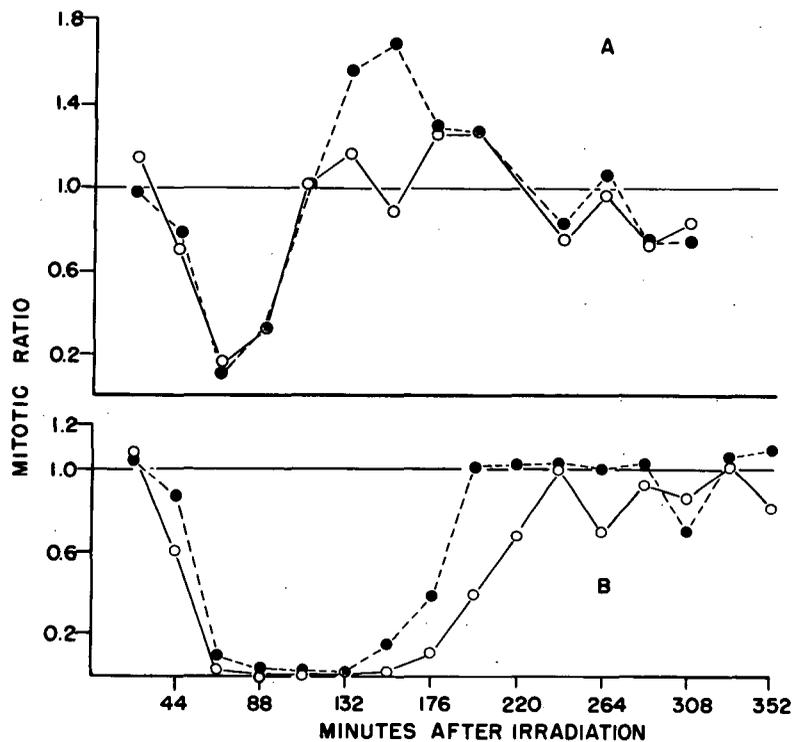


FIG. 10 A

FIG. 10 B

● - 8.8 rep β rays
○ - 8 r of X rays

● - 70.4 β rays
○ - 64 r of X rays

Figure 10 shows that at both doses X rays and β rays affect mitotic activity similarly with respect to the extent to which it is depressed but differently with respect to recovery. At the lower dose a statistically significant greater number of cells recover after treatment with β rays than with X rays (132 and 154 minutes after treatment). At the higher dose β irradiated cells begin to recover from inhibition sooner and more cells recover than was the case with cells which were X-rayed. Those differences are statistically significant. It appears therefore that for comparable doses of radiation X rays affect mitosis more adversely than do β rays.

Immediate Effects of X Radiation on Mitotic Stages

(Carlson, Harrington)

Studies have been completed in which the immediate effects of irradiation on each stage of mitosis from very late prophase through anaphase were observed. Grasshopper embryos were treated with 64, 256, 512, 1024, or 4096 r of X rays in a period of time no greater than 1.5 minutes. Studies of aceto-carmine smears supplemented observations made with living culture preparations.

In the untreated, normal neuroblast the very late prophase stage is initiated with spheration of the cell just a few minutes before the breakdown of the nuclear membrane which initiates prometaphase. During metaphase the proximal portion of the chromosomes are situated approximately in the equatorial plane of the cell. Anaphase extends from the initial separation of sister chromatids at their extreme proximal ends to the time when the chromosomes lose their sharp outlines and the cleavage furrow is almost complete, which marks the beginning of telophase. Neuroblasts are classed as early telophases until the nucleoli become visible. Average times for these stages are shown in Table 5.

After 64 r, neuroblasts which were in metaphase or anaphase during irradiation proceed normally. The chromosomes are not sticky or highly refractive, no bridges or fragments are noted, and mitotic rate is normal (Table 6).

Treatment of metaphases and anaphases with 256 r produced no detectable effects. Neuroblasts irradiated in late prophase or prometaphase show about two to five bridges per cell at late anaphase. The chromatin is sticky (Table 7). The late prophase stage is sometimes prolonged by this dose, and the cleavage furrow may be delayed by about 15 minutes. Although cleavage does not occur normally, the chromatin proceeds through its normal mitotic cycle and assumes the appearance of early telophase

TABLE 5

COMPARISON OF THE MID-MITOTIC TIMES IN MINUTES (32°C) BETWEEN CONTROL AND IRRADIATED CELLS. EACH VALUE IS AN AVERAGE OF 25 TO 50 CELLS.

Treatment	Mid-mitotic Stages						Prometaphase to Early Telophase
	Prometa-phase	Meta-phase	Early Anaphase	Middle Anaphase	Late Anaphase	Early Telophase	
Control	3.7	11.0	3.8	3.4	4.4	8.5	25.9
512 r	4.6	15.3	5.5	3.9	5.4	18.9	33.8

TABLE 6

Dose of X rays (r)	Mitotic Stage When Irradiated			
	P _{8c}	P ₁₀	M ₂	A ₂
64	0	0	0	0
256	+	+	0	0
512	+	+	0	0
1024	+	+	+	0

0 = normal (no appreciable delay)

+ = mitotic delay

TABLE 7

PERCENTAGE OF CELLS SHOWING STICKINESS AFTER IRRADIATION

Dose of X rays (r)	Stage at which Irradiation Occurred				
	P _{8c}	P ₁₀	M ₂	A ₂	A ₄
64	14.2	16.6	0	0	-
256	46.4	31.5	10.5	0	0
512	92.8	61.9	40.0	0	0
1024	88.8	94.1	71.4	10.0	0
4096	100.0	100.0	100.0	66.6	0

threads. Timings show that early telophase stages are slowed up considerably and are nearly twice that of the normal cells. The cleavage furrow is apparently completed soon after the nucleoli reappear. The very late prophase stage is more sensitive to X rays since the above effects appear in a greater degree in nuclei irradiated in this stage than in nuclei irradiated in prometaphase.

After 512 r, cells in very late prophase at irradiation exhibit bridges and extreme stickiness of the chromosomes at anaphase. Cleavage is greatly delayed. The bridges do not lose their sharp outline as soon as the other chromatin material, i. e., they retain an anaphase appearance much longer than the other chromosomes. The cleavage delay of a cell is correlated with the number of bridges present. Eventually the cleavage furrow cuts through the chromosomal bridges, and then the nucleoli reappear. Cells in which very late prophase is prolonged by radiation also have slightly longer metaphase and anaphase periods as compared to the controls. The interval from prometaphase to cleavage is longer in the treated than the control cells, cleavage coinciding with the reappearance of the nucleoli. Neuroblasts irradiated during prometaphase and metaphase show about one to three bridges per cell, and some cleavage delay. Nuclei receiving 512 r in the anaphase stages proceed normally through the mid-mitotic division. Neuroblasts in early telophase are delayed by 512 r (Table 5).

All cells irradiated in very late prophase, prometaphase, metaphase, and early anaphase with doses of 1024 or 4096 r show extreme stickiness, numerous bridges, and greatly retarded division (Tables 6 and 7). The cleavage time may be delayed as much as 2 hours. The cells are so greatly affected that it is extremely difficult to make observations on them in the living culture preparations.

Effect of Radiation on Metabolism of Grasshopper Egg and Embryo.

(Tipton, St. Amand)

Observation on the effects of X-ray exposure of Chortophaga eggs, 14 days old (26°C), has been extended.

In the last Quarterly Report (ORNL-989:47) we stated that no effect was apparent at X-ray doses as low as 4700 r. At that time conclusions were tentative, based on the total respiration during 4 hours after treatment.

From a re-examination of these data, plus that of additional experiments, it appears that, on the basis of respiration during the first 2 hours

postirradiation, Chortophaga eggs are adversely affected by X rays at a dosage of 4700 r if the respiratory measurements are made in an oxygen atmosphere and at 38°C. The X-ray exposure was made at room temperature (22-29°C), and in room air.

As is indicated in Table 8, if the Warburg flask is flushed with room air instead of oxygen, the respiration of the eggs is not affected by an X-ray dose of 6640 r; and the deleterious effect of 40,000 r is significantly less than that in oxygen.

TABLE 8
RESPIRATION OF 14-DAY EGGS
(TEMPERATURE - 38°C)

Oxygen Atmosphere				Room Atmosphere			
X-ray dosage (r)	No. of flasks	No. of eggs	Cu mm O ₂ /100 eggs/hr on basis of first 2 hours postirradiation	X-ray dosage (r)	No. of flasks	No. of eggs	Cu mm O ₂ /100 eggs/hr on basis of first 2 hours postirradiation
0	19	570	80.09	0	6	180	72.26
64-68	1	30	90.76	4,427	1	30	70.40
2,244	1	30	95.00	6,640	1	30	82.30
4,680	1	30	60.50	29,000	2	60	48.75
7,020	1	30	58.15	40,000	2	60	61.77
10,000	2	64	71.25				
20,000	2	60	70.16				
30,000	3	95	59.08				
40,000	3	95	27.43				
Total	33	1029			12	360	

Drosophila Genetics: Variegation Caused by X-Ray-Induced
Rearrangements at the Peach Locus in Drosophila virilis

(Baker)

In the previous Quarterly Report (ORNL-989) mention was made of the position effects caused by the induction of translocations in wild type males of Drosophila virilis with breakage points presumably near the peach locus. A more detailed account can now be given. Four different rearrangements have been detected which produce the peach-mottled eyes.

T(Y;5)pe^{m1} - Genetic evidence indicates the presence of a translocation between the Y and the fifth chromosome. No abnormality is observed in salivary gland chromosomes, but genetic evidence indicates that chromosome 5 is broken distal to the peach locus. Brain smears of hyperploid males show no size differences of the chromosomes. On this cytological evidence it is presumed that both the fifth and the Y chromosomes are broken close to the centromere. Direct evidence for the presence of the translocation is seen in the offspring of the pe^{m1} females. One-half the male offspring have an extra chromosome (Y) present in the brain cells. In this case the heterochromatin of the Y chromosome is brought close to the peach locus.

T(3;5)pe^{m2} - Both genetic and cytological evidence indicate the presence of a translocation between chromosomes 3 and 5. Chromosome 3 is broken in the 3D1 region of the salivary gland chromosomes (Griffen's map) while chromosome 5 has one break at or near the centromere and another in the A7 region. The intervening region between the breaks (almost the whole length of the fifth chromosome) is inverted. The break in the third chromosome and the distal break in the fifth chromosome are in the euchromatic regions.

T(4;5)pe^{m3} - Genetic evidence indicates a translocation between the fourth and fifth chromosomes although no rearrangement is observable in salivary gland chromosomes. Genetic evidence indicates that the break in the fifth chromosome is distal to the peach locus. It is assumed that both the fourth and fifth chromosomes are broken close to the centromere.

T(3;5)pe^{m4} - Both genetic and cytological evidence indicate the presence of a translocation. The break in the third chromosome is close to 3B2c, a euchromatic region, while the break in chromosome 5 is proximal to the basal heterochromatin in this chromosome. Indirect evidence which can be verified by further tests indicates that the break in the fifth chromosome is still distal to the peach locus.

The importance of these findings is twofold. The type of position effect causing variegation has usually been attributed to the fact that heterochromatin has been brought close to a gene normally located in a euchromatic region. (In a very few cases the reverse situation has been found.) The peach locus in Drosophila virilis is presumably located in the basal heterochromatin. However, it appears from the data that bringing either foreign euchromatin (the 3:5 translocation) or foreign heterochromatin close to the locus produces variegation.

The last three of these cases of position effects were originally detected among about 400 male F_1 offspring whose fathers had received 2000 r units of X radiation. With this relatively high rate of breakage of the fifth chromosome near the peach locus, the possibility of studying the effects of different agents on the X-ray sensitivity to breakage of a short chromosome region presents itself. This is feasible because of the ease with which the variegated eyes may be detected.

RADIATION PROTECTION

Alexander Hollaender (Leader)

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E. H. Anderson	Ruth W. Whittle
Daniel Billen	A. W. Burke, Jr.
W. T. Burnett, Jr.	

Effect of Suboptimal Incubation Temperatures on Survival of Irradiated Escherichia coli

(Stapleton, Grayson, Hollaender)

Several types of pretreatments (prior to irradiation) have been shown to modify the X-ray sensitivity of Escherichia coli B/r. Reduction of the oxygen concentrations of irradiated suspensions, as well as the addition of various organic or inorganic chemical agents to the suspensions prior to irradiation has resulted in an over-all reduction of the X-ray sensitivity of these cells. The sensitivity of E. coli has also been shown to be dependent on the conditions under which the cells were cultured before irradiation. To date, there have been no indications that removal of oxygen, or addition of chemical agents as posttreatments can change the sensitivity of the bacteria. The effect of cultural conditions after irradiation on survival of bacteria has only recently been investigated.

Reduction of the temperature of living cells after irradiation has been shown by some investigators to aid in the recovery of some types of living cells from the effects of ionizing radiation. In some cases the apparent recovery was merely a postponement of the radiation damage, since the radiation effects occurred quite normally when the cells were returned to room temperature and metabolic activity was resumed.

Recovery of bacterial cells from the effects of ionizing radiations has not been demonstrated by the usual techniques, for example, by comparison of the efficiency of various intensities of the radiation or by a comparison of efficiency of single versus fractionated doses of the radiation. Likewise, holding of irradiated cells at low temperatures after irradiation has not been shown to aid in recovery (Latarjet-C. R. Acad. Sci. 217:186, 1943). We have investigated the effect of holding irradiated bacterial cells on nutrient agar plates at suboptimal incubation temperatures. The irradiated washed-cell suspensions were diluted and surface plated at various temperatures from 6°C to 42°C. The plates were allowed to come to equilibrium at the various temperatures prior to seeding with the irradiated cells, then the suspensions were rapidly plated and the plates

returned to the incubators at the various temperatures for 24 hours. This period was longer than the time required for optimal recovery at any of the temperatures used. After this holding period the plates were removed to a 37°C incubator and, after 24 hours incubation at this temperature, the colonies were counted to determine the survival. The plating technique was considered to be the most advantageous for these studies, since by this method one could be sure that colonies developing would represent the actual number of surviving cells, not confused by growth or cell division.

It was found that, although no recovery took place when the cells were held at the low temperatures (0 - 6°C) for as long as 96 hours, holding for much shorter periods of time at intermediate temperatures (12 - 30°C) resulted in greater survival than was obtained at 37°C. As can be seen in Fig. 11, the survival of bacterial cells is maximal for any dose, when the plates are incubated at ~18°C. It seems evident that recovery does not involve a simple decay of a toxic substance produced by the radiation, otherwise the recovery at the lower temperatures should be similar to that obtained at 18°C. One interpretation of such data might be that the curves relating survival as a function of the holding temperature are a measure of two processes which occur in the irradiated cells, these processes having different temperature coefficients. At the lower temperatures a process akin to synthesis is the predominant one, while at the temperatures above 18°C, a destructive process is more important. The survival at any temperature, then, depends on the relative rates of these two processes.

It is also apparent from Fig. 11, that the magnitude of the recovery depends on the number of cells affected by the radiation. If one plots the survival fraction of cells as a function of X-ray dose, at the temperatures for minimal and maximal recovery, (18 and 37°C) as in Fig. 12, it is clear that a constant fraction of the affected cells are recoverable by holding at the lower temperature.

It seemed appropriate to study the rates of recovery at the various temperatures. In order to investigate this portion of the problem, it was necessary to prepare large numbers of identical sets of seeded plates. After various intervals of time sets of plates were removed from the incubator at the lower temperature, quickly warmed to 37°C, and then incubated at this temperature for 24 hours. This technique was considered satisfactory to stop the recovery process at the desired time, since no recovery occurs at 37°C. The results of this investigation are shown in Table 9. The surviving fraction at various times for four of the temperatures are studied.

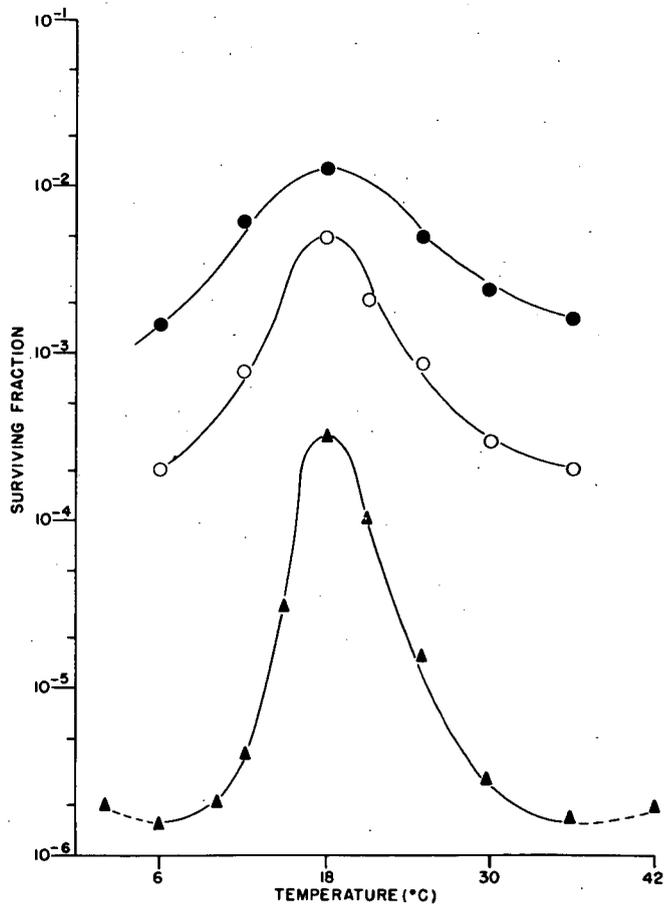


FIG. 11

Survival at Several X-Ray Doses as a Function of Incubation Temperature

- - 40,000 r
- - 60,000 r
- ▲ - 80,000 r

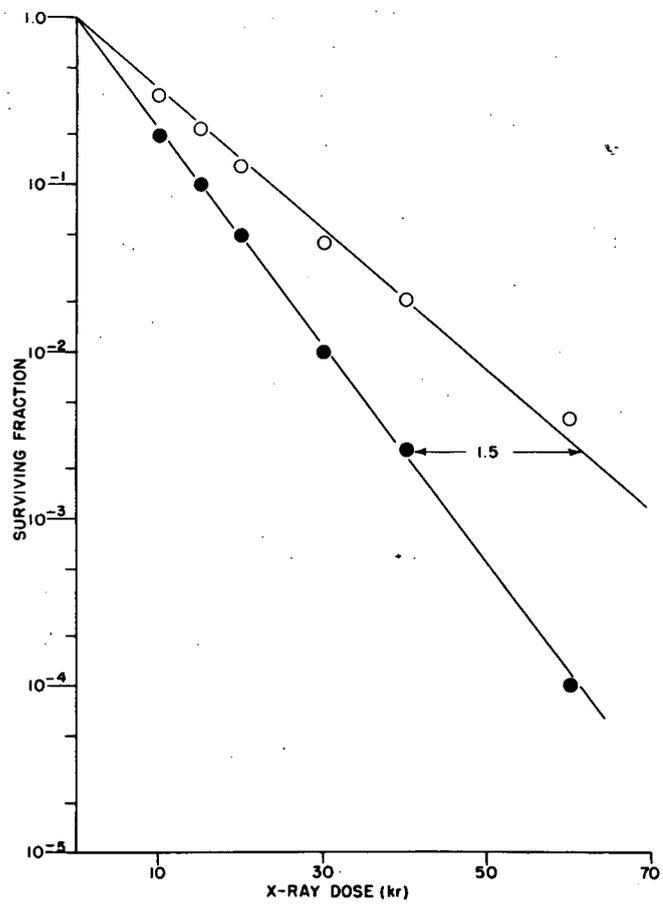


FIG. 12

Recovery at Various X-Ray Doses (Aerobic B/r 20-hour culture cells irradiated)

- - holding at 18°C
- - holding at 37°C

TABLE 9
 RATE OF RECOVERY OF *E. COLI* B/r AT VARIOUS TEMPERATURES
 X-RAY DOSE = 66,000 r

Holding Time Hours	Survival Fraction x 10 ⁻⁵			
	12°C	15°C	18°C	25°C
0	1.03	1.03	1.03	1.03
2	1.56	2.05	2.26	2.60
4	2.16	3.65	4.52	5.60
6	2.65	6.00	9.05	6.20
10	4.13	9.20	50.20	7.30
12	7.00	11.0	100.0	7.50
22	10.0	12.5	120.0	7.50

Platings were made at the temperatures indicated and at the various times sets of plates were removed to 37°C.

It is apparent that the initial rate of recovery increases with increase in temperature over the range studied. It should be noted that, although the initial rates of recovery increase with temperature, the final recovery is greatest at about 18°C. This is consistent with the hypothesis that the relative rates of the two processes, one synthetic and the other destructive, determine the survival at any temperature. On this basis, it would seem that temperatures near 18°C present the most advantageous balance between the rates of the processes.

Work is continuing on this problem from the standpoint of establishing whether or not recovery can occur in the absence of growth, and also whether bacterial division is intimately concerned with cessation of recovery at various temperatures, as has been suggested for other living cells. The importance of this phenomenon in correlating mutation and lethal effects is quite obvious.

Effect on Adaptive Enzyme Formation

(Billen, Lichstein)

The data presented (Fig. 13) show that X radiation of resting cell suspensions of *E. coli*, in doses of 60,000 r or greater, prevents completely the ability to synthesize formic hydrogenlyase, while having no effect on preformed hydrogenlyase activity. (Summary of paper in press, J. Bact.)

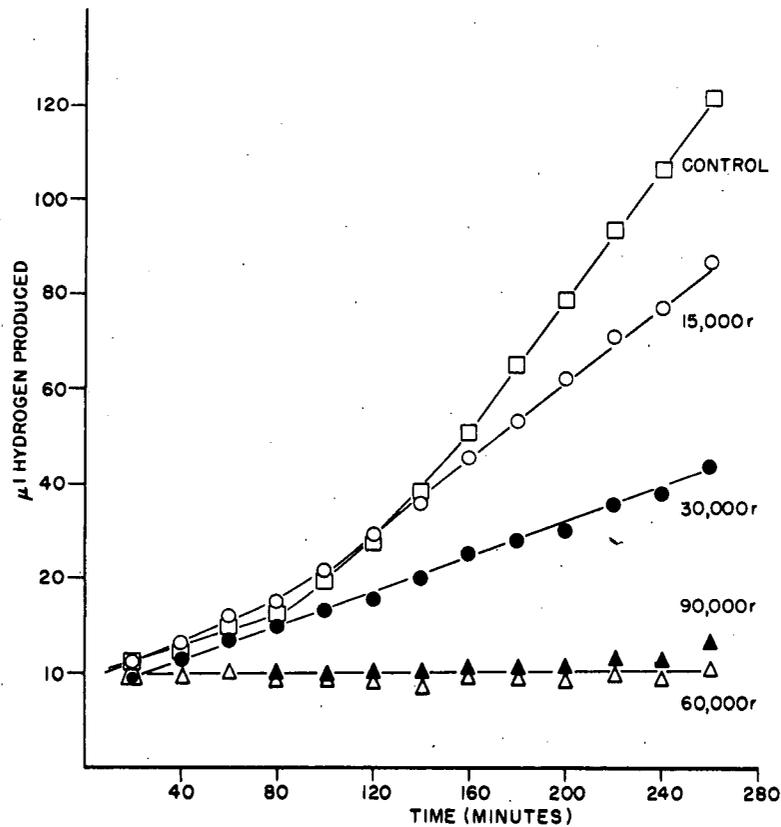


FIG.13

The effect of X radiation on the adaptive formation of formic hydrogenlyase by resting cell suspensions of Escherichia coli.

The Effect of Mecholyl Bromide on the Mortality of X-Irradiated Mice

(Burnett, Burke)

A few chemicals have recently been screened in this laboratory as possible protective agents for X-irradiated mice. The most effective one tested is mecholyl bromide, a drug which produces parasympathetic stimulation. In therapeutic doses, mecholyl slows the heart rate, lowers blood pressure, constricts the bronchioles, dilates the peripheral blood vessels, constricts the pupils, increases intestinal tone and peristalsis, causes salivation and flushing, and stimulates the detrusor muscle of the bladder (Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, Macmillan, 1941). The indications are that the 28-day LD₅₀ for mice is increased about 50 per cent when mecholyl bromide is given by intraperitoneal injection 1 to 5 minutes before X irradiation.

In a screening test 17 C57 black female mice, 12 - 16 weeks of age and averaging 23 g in weight, were irradiated with 700 r of X rays following the intraperitoneal injection (1 - 5 min preirradiation) of 0.2 ml of a solution containing 0.2 mg of mecholyl bromide (9 mg/kg) of body weight. Fourteen were alive 28 days later. Only six of a group of 15 mice given 0.2 ml of saline intraperitoneally survived a 700 r dose of X rays. All of a group of five mice given 19 mg/kg of mecholyl bromide survived 700 r. In another experiment 8-months-old female mice of the 101 x C₃H strain averaging 26 g in weight were given 8 mg/kg of mecholyl. Although the colony was infested with a Pseudomonas, a condition which seems to have increased the death rate of the control mice, it would appear that mecholyl reduced the general effects of both the radiation and the bacteremia which was evidently induced or aggravated by the radiation.

In later experiments, we have used exclusively the C57 black strain of mice supplied by the Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine. The mice are exceptionally disease-free, usually well-tempered, and easy to handle.

The results indicate that, while the 28-day LD₅₀ for untreated males is approximately 600 r, the 28-day LD₅₀ for males injected intraperitoneally with 19 - 150 mg/kg of mecholyl (3 - 5 min preirradiation) is approximately 900 r. At the 150 mg/kg dose level, mecholyl bromide is toxic, giving about 20 per cent mortality within a few hours in both irradiated and controls. A dose of 300 mg/kg results in 100 per cent mortality within a few minutes after injection.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF
RADIATION IN MICE

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Semisterility in Mice Caused by X-Ray-Induced Translocation

(Wickham)

Data have been presented by W. L. Russell, Gower, Jones, and Henderson (ORNL-644, ORNL-727) on the incidence of partial sterility in male and female offspring of irradiated male mice. Twenty-two males and 15 females were tested. Four males and six females were semisterile. Some of the progeny of these semisterile animals have been tested to maintain the various lines and to determine whether or not the semisterility is inherited in a manner that would be in accordance with the segregation of reciprocal translocations. A supply of semisterile animals greater than the amount needed to maintain the lines was kept on hand to use in an attempt to obtain cytological evidence of reciprocal translocations. Thirty-eight male offspring of the semisterile animals were tested for semisterility. With the exception of one line that had only two males available, four males from each line were tested. Four females* were placed in the pen with each male, and when the females became pregnant they were moved to single pens. They were kept there until 3 days after the litters were born; then they were returned to the same male. The use of four females to bear the young of each male allowed the test to be made in a shorter time; and keeping the females from the males until 3 days after the delivery of the young eliminated the litters that would have been conceived during postpartum estrus. These litters would be disturbing in a fertility test since a fertile female will sometimes have a smaller litter if the litter is conceived during postpartum estrus.

Of the 38 males tested, 20 were semisterile, 15 were fertile, and 3 were sterile. There was at least one semisterile animal in each of the ten groups representing the ten semisterile lines. The distribution of the number of litters and the mean litter sizes of the 38 males is shown in Table 10.

* Throughout the tests described in this report, matings were made to F₁ hybrids of two inbred strains (101x C₃H). These animals were selected because of their high fertility.

TABLE 10

DISTRIBUTION OF MALES WITH REGARD TO NUMBER OF LITTERS
AND MEAN LITTER SIZE

No. of litters:	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
No. of males:	3	-	-	-	-	-	-	-	1	-	-	-	-	1	-	1	-	-	-	-	10	9	1	2	4	3	1	-	1	1	-
Mean litter size:	0	.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0										
No. of males:	3	-	-	-	-	-	1	6	6	6	1	-	-	-	-	-	2	4	5	3	1	-									

The fertility of one male in semisterile line 33 was very low. During the test the male produced only eight litters. The mean litter size of the eight litters was 2.5. Three sterile males were found in semisterile line No. 29. The second section of this report will describe tests that have been made concerning sterility in this line.

An effort was made to determine if there were chromosomal differences in the spermatocytes of the semisterile animals and those of the fertile animals. The testes of the animals were prepared for microscopic study by the propionic-carminc squash method.* The first metaphase of meiosis was the stage selected as being best suited for study. The 20 haploid chromosomes may be counted with accuracy in many polar views of this stage. In five semisterile animals, each representing a different semisterile line, there were 19 elements instead of 20. One element, larger than the other 18, was ring-shaped. It appeared to be different from any of the other 18 chromosomes, and no similar configuration had been observed in fertile animals. It was decided that these were rings of four chromosomes (two homologous pairs). These have been found by Koller (*Genetics*, 29:247 - 263, 1944) in semisterile mice. They are reported to result from a translocation of chromosome segments between two chromosomes. When the chromosomes become paired in the pachytene stage of meiosis, the two chromosomes involved in the translocation and their two homologues form a cross-like configuration with chiasmata in each arm. At the diplotene stage, segments of the homologues move apart to become a ring-shaped configuration held together by the four chiasmata. The ring persists through the first metaphase stage. Reduction and division of the four chromosomes result in approximately one-half the gametes being deficient for certain chromosome segments. These gametes are lethal to the zygote. The remainder of the gametes are either normal or they carry the reciprocal translocation. In one semisterile line a chain of four chromosomes was found. Chains, instead of rings, are formed if one arm of the four-membered cross is lacking a chiasma. The other four semisterile lines will require more study before it can be determined whether or not translocations are present.

Only the six lines in which translocations have been found will be dealt with in the remainder of this report. The mean litter sizes and the analysis of variance among litter sizes of the six lines are given in Tables 11 and 12.

The analysis of litter size indicated that there was no more variation between the six lines than one might find in a single homogeneous population. This was the case among the semisterile animals as well as the

* This technique is described under a separate heading.

TABLE 11
 MEAN LITTER SIZES OF SEMISTERILE AND FERTILE MALES
 OF SIX LINES

	Line	No. of litters	Mean litter size (\bar{x}_F)	
Fertile males	1	20	8.45	
	13*	-	-	
	21	45	8.50	
	28	40	8.83	
	33	45	8.45	
	38	70	8.22	
* The only two males available in line 13 were semisterile.				
Semisterile males			Mean litter size (\bar{x}_{SS})	Fertility ratio $\frac{\bar{x}_{SS}}{\bar{x}_F} \times 100$
	1	63	3.51	41.5
	13	42	3.76	-
	21	39	3.41	40.1
	28	39	3.41	38.6
	33	26	3.23	38.2
	38	17	3.59	43.6

TABLE 12
 ANALYSIS OF VARIANCE AMONG LITTER SIZES

	Source of variation.	d.f.	Mean square
Fertile males	Among lines	4	3.273
	Among males within lines	5	6.367
	Among matings within males	30	3.332
	Among litters within matings	180	3.332
Semisterile males	Among lines	5	1.105
	Among males within lines	6	4.038
	Among matings within males	36	1.601
	Among litters within matings	178	1.602

TABLE 13

POOLED LITTER SIZES

	No. of litters	Mean litter size
Fertile males	220	8.39 ± 0.123
Semisterile males	226	3.49 ± 0.084
Fertility ratio		41.64 ± 1.174

Fiducial limits for the fertility ratio are 39.3:44.0 in the 95 per cent confidence interval.

fertiles of the six lines. The differences in the fertility ratios in the lines are probably random differences and the means and fertility ratios derived from pooled data would be more informative of the extent of reduction of fertility in the semisteriles. These data are given in Table 13.

The fertility ratio derived from the pooled data indicates a reduction of fertility in the semisteriles greater than 50 per cent. This is also shown in each of the individual lines. Snell (Genetics, 31:157 - 180, 1946) tested several semisterile lines of mice. The semisteriles produced 1496 litters with a mean litter size of 3.12. The fertile group produced 790 litters with a mean litter size of 6.86. The fertility ratio was 45.48. Snell points out that there is an indication that at least some translocations in the mouse cause "a reduction in fertility to something less than 50 per cent." Hertwig (Z. i. A. V., 79:1 - 27, 1940) and Koller (1944) reported similar results.

In two generations 70 males of the six lines have been tested. Thirty-six were fertile; 32 were semisterile. Two failed to produce young. These two males were in line No. 33, and were offspring of a male that produced only eight litters, with a mean litter size of 2.5, even though he was mated to four females for a period of six months. The testes of one of the males were removed and slides were made. All maturation stages were present and sperm were found in abundance. A ring of four chromosomes was found in several metaphase stages.

Propionic-Carmine Squash Technique

The tubules of the testes are fixed for 24 hours in 1 part butyric acid: 3 parts 95 per cent ethyl alcohol. Short sections of the tubules are taken from the fixative and placed on a glass slide in a drop of 45 per cent propionic acid saturated with carmine. A cover slip smeared with a thin film

of albumen is placed on the slide. The blunt end of a dissecting needle is used to tap the cover slip lightly until the cells are well scattered beneath the cover slip. The cells are squashed with thumb pressure on the cover slip. The preparation is placed under the microscope and examined for the desired stages. If the stages are found in sufficient numbers, the preparation is made permanent. The slide is placed in a shallow dish containing 1 part acetic acid: 1 part ethyl alcohol (95 per cent). The cover slip will float away from the slide in a short length of time. The cover slip and slide are transferred to another dish containing 1 part butyl alcohol: 1 part ethyl alcohol (95 per cent). After 3 minutes they are transferred to a dish containing butyl alcohol. From 3 to 5 minutes is sufficient time in the butyl alcohol; then the cover slip is mounted on the slide in a drop of balsam. The preparation should be allowed to dry for 24 hours before observing it under the microscope.

Male Sterility in a Semisterile Line of Mice

(Wickham)

Three sterile males were found in the F_2 generation of a male that had been exposed to 1000 r of whole body X radiation in acute dosage. The sterile males were offspring of semisterile female No. 29 of the F_1 generation.

The fertility of all available male and female offspring of female No. 29 were tested. Of the 19 males tested, 14 were sterile; 2 were semisterile; 3 were fertile. Each sterile male was kept with four females for at least 3 months after they had reached the age of sexual maturity. Plugs were found in the females but no young were produced. Eight female offspring of female No. 29 were tested. None was sterile.

To further investigate the sterility, animals of the F_3 generation were tested. Twenty male and twenty female offspring of fertile males were tested. None was sterile. Twenty male and twenty female offspring of semisterile males were tested. None of the females was sterile; one male failed to produce young.* At least seven male offspring from each of the eight females were tested. Four of the females produced sterile males. Fifty-eight female offspring of the four females that produced the sterile males were tested. None was sterile. At present it is known that at least 20 of the females are producing sterile males.

* The testes of the male were sectioned, and a smear was made of the vas deferens. Sperm were viable and abundant in the smear. All stages of maturation were present and apparently normal.

It is of some importance to know whether or not fertile females as well as semisterile females produce the sterile males. The generation in which the steriles first appeared was produced by a semisterile female. In the next generation a fertile female produced sterile males. * Female No. 212 had eight litters with a mean litter size of 6.87. Twenty-three of her male offspring were tested. Ten of the males were sterile; thirteen were fertile. The fertiles produced 15 to 19 litters per male, with mean litter sizes ranging from 6.9 to 8.0.

Eighty male offspring of those females that produced sterile males have been tested. Forty-one were sterile; 39 produced young. The testes of some of the sterile animals have been sectioned and stained for microscopic study. Apparently the animals have the basic cells for the maturation of sperm, but for some reason the spermatocytes fail to reach the secondary stages of spermatogenesis. Early prophase and middle prophase stages are abundant, but later stages have not been observed.

Apparently some of the females in this line are carrying a sterility factor that is expressed in approximately half their male offspring. The consistency of the data suggests sex linkage. Additional tests will be made concerning the sterility factor.

* Probably there are other fertile females that produced sterile males. The litter sizes of these females are rather low, even in the fertile animals. Female No. 187 had eight litters with a mean litter size of 5.0. Thirteen of her male offspring were tested; 5 were sterile, 8 produced 8 to 10 litters per male with a range of mean litter sizes from 7.0 to 8.3. Female No. 189 produced 10 litters with a mean litter size of 4.5. Eighteen of her male offspring were tested; 10 were sterile; 8 produced 8 to 10 litters per male with a range of mean litter sizes from 7.1 to 8.3.

PATHOLOGY AND PHYSIOLOGY

PATHOLOGY AND PHYSIOLOGY

Jacob Furth (Leader)

M. C. Woods	R. R. Bigelow*
A. C. Upton	K. W. Christenberry**
J. B. Kahn	W. D. Gude
R. H. Storey	Peggy Ledford
J. R. Thomson	Mary M. Knoohuizen
Evelyn Gadsden	Frances Gamble
Mary H. Ross	I. L. Campbell †

Unsuccessful Protection Experiments Against Radiation Injury With Lymphocytes

(Campbell, Ross)

This experiment was undertaken to determine whether the injection of lymphocytes into irradiated animals would offer protection against the effects of radiation. The experiments were so designed as to obtain living lymphocytes in their natural medium and introduce them into genetically compatible hosts. The results, which were essentially negative, will be published as an ORNL report.

The lymph of mothers of an inbred line of rats was tapped and given to half of her babies of the same litter, the other half received the same volume of Tyrode solution. The lymph-injected animals died with acute radiation syndrome during the first 3 weeks (average of 13.2 days) post-irradiation. The Tyrode-injected animals died with acute radiation syndrome after an average of 12.1 days postirradiation.

Large numbers of lymphocytes were injected in rats whose lymphocyte count was depressed with X rays to determine by simple blood counts and differential counts whether the injected lymphocytes would circulate in the blood and, if so, how long.

It was found that only a relatively small number of the injected lymphocytes circulated in the blood of the irradiated animals shortly after injection, and even most of these left the circulation within 1 to 2 hours.

Under the conditions of these experiments one would expect that at least the introduced lymphoblasts would survive and multiply in the new

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host and thus exert a protective function. Should the monophyletic view of hemopoiesis be true, one would expect restoration of the levels of other types of leukocytes. These experiments fail to support the view that the lymphocytes exert protection against radiation damage and that the lymphocytes are mother cells of other white cells. Thus, protection by shielding the spleen as described by Jacobson is probably due to a substance related to another cell.

Permeability Problems of Ionizing Radiation

(Storey, Woods, Bigelow)

The discovery that the lymph of X-rayed animals (dogs, rats, rabbits, mice) is bloody led to a series of investigations on the lymph. The first of these is concerned with the approximation of the lymph volume and is in process of publication (Storey, Moshman, and Furth, *Science*, 114:665-667, 1951). The second part, dealing with the cellular composition of the lymph (Ross), will soon be published. The third part deals with the physiology of albumin interchange between blood and lymph in normal and X-rayed animals and is now being concluded (Storey and Woods).

In these studies information is being gathered on the rate of albumin clearance from the blood, albumin metabolism, the ratio of albumin concentration between blood and lymph, and the lymph volume in X-rayed and normal animals. From the technical standpoint these determinations proved exceedingly tedious. They yield, however, fundamental physiological information in this poorly known area in both normal and X-rayed animals.

The rapid entry of intravenously injected albumin into the lymph in X-rayed animals has been confirmed. The lymph space has been found to be variable and the relative albumin concentration of the lymph is frequently increased in X-rayed dogs. The metabolism rate of injected albumin was found to be essentially unchanged by irradiation.

Further Studies on the Anemia of Irradiation

(Kahn, Storey)

Studies summarized in the last report, now in process of publication, indicate that the anemia which follows X-ray doses near the LD_{50} is a self-aggravating process and is a result of a profound change in capillary permeability. Experiments are now being conducted to determine the effects of various X-ray doses above the LD_{50} on the production of the hemorrhagic

syndrome in rabbits, and to attempt to correlate this with the well-known thrombocytopenia occurring after midlethal X-ray doses. Such a correlation was suggested by the recent findings of Cronkite, et al. (Am. J. Roentgenol. Radium Therapy, in press) and Dillard, et al. (Proc. Soc. Exptl. Biol. Med., in press), emphasizing the role of thrombocytopenia in the hemorrhagic syndrome. It may well be that the two are interrelated.

After exposure of rabbits to whole-body X irradiation with 600, 1200, 2400, and 4800 r, the following parameters of radiation effect are being studied: weight, hematocrit, plasma volume, erythrocyte mass, WBC, RBC, and thrombocyte counts. Although this experiment is still in progress, the following tentative conclusions seem justified:

Rabbits receiving 2400 and 4800 r lack hemorrhagic features and suffer an entirely different type of death from those receiving 800 to 1200 r. The hemorrhagic tendency, on the contrary, is marked at autopsy in the rabbits receiving 1200 r, occurring between the eighth and twelfth day. There is a pronounced fall in RBC, hematocrit, and thrombocytes, and the lymph nodes and lymphatics are grossly bloody. The rabbits receiving 2400 or 4800 r all die 2 to 4 days after irradiation. Even when moribund, these animals have near normal RBC and thrombocyte counts. The pathological findings are usually pulmonary congestion and edema, and frequently massive bacterial infection with abscesses in the lung. Thus, both in time of death and hematological and pathological findings there is a marked difference between animals receiving doses near the LD₅₀ and those receiving more massive doses. The earlier findings on the importance of anemia and diversion of blood into lymph spaces apply only to the former group.

Chronic Effects of Slow Neutron Exposure

(Upton, Christenberry, Thomson)

These experiments were begun over 2 years ago, and about one-half the animals are still alive. These will be followed until natural death.

This large-scale project has been mentioned repeatedly in our quarterly reports. Cataracts were the first chronic change to appear. The smallest cataract-inducing dose is about 16 r of X rays, or a comparable dose of slow neutrons (at this dose level approximately 50 per cent of mice are positive for opacities at 11 to 14 months after exposure). Opacities of the lens when viewed with the slit lamp make their appearance 2 to 3 months after exposure in the animals receiving the larger doses, and become intensified with time (a few illustrative observations are presented in Table 14). Among the old nonirradiated mice cataracts of a different type are not uncommon, and these tend to obscure the radiation-induced

TABLE 14
 PROGRESSION OF OPACITIES OF THE LENS IN MICE EXPOSED
 TO SLOW NEUTRONS AND X RAYS

Dose and type of irradiation*	No. of mouse	Sex	Successive slit lamp examinations: degree of cataract (0 to +++) and months after exposure													
			2	3	4	5	6	7	8	9	10	11	12	13	14	15
80 N	4681	F	+	+									+++			
80 N	4643	M					++						+++			
80 X	3402	M											++			+++
80 X	4631	F					+						++			
20 N	4237	M								+					++	
20 N	4238	M								+					++	
20 X	4619	F					+						++			
20 X	3416	M											0			+
5 N	2667	F		0								+				
2.5 N	4733	M								+			+			
2.5 N	4597	F							+				+			
2.5 X	4422	M									0		+			
2.5 X	4742	M							+				+			
0	4645	M					0	0					0			
0	4606	F					0						0			

* N = exposure to slow neutrons in the Oak Ridge reactor at a flux of 0.9×10^9 per sq cm per sec. with an estimated 4 to 8 r of high energy γ per minute (See Christenberry and Furth, Proc. Soc. Exptl. Biol. Med., 77:559 - 560, 1951.). The coefficient (80, 20, etc.) indicates the duration of exposure in minutes.

X = exposure to X rays at a rate of 6.4 r per minute. For further details, see Christenberry and Furth, Proc. Soc. Exptl. Biol. Med., 77:559 - 560, 1951.

opacities (Christenberry and Furth, Proc. Soc. Exptl. Biol. Med., 77: 559-560, 1951).

A great increase of the leukemia incidence was noted at 8 to 14 months of age in the irradiated groups. These leukemias were mainly mediastinal lymphomas. Now, as the animals grow older, leukemias of all types appear in all groups, including the controls, but these are for the most part not mediastinal. Careful typing, now in progress, will require considerable histological study. Lung tumors are frequent among these mice, and they are being followed with respect to their number and character. The majority appear benign, a few are malignant. Ovarian tumors and pre-tumors are present in mice exposed to 32 r of X rays or equivalent and higher doses of neutrons. Miscellaneous tumors (hepatomas, soft tissue tumors, and bone tumors) occur in all groups. These will require careful histological typing as well as a statistical analysis to determine whether irradiation has increased their frequency.

Studies on the Pathological Influence of C^{14}

(Woods, Gamble)

The effects of C^{14} on neoplasia induction, longevity, etc. in mice are being studied under varying conditions of age and physiological state which will favor maximal body incorporation. Following exposure, the animals are being allowed to live normally until death, at which time autopsy and histological examinations will be correlated with findings in the controls groups.

Two sets of females from RF stock are being studied (Table 15).

No neoplastic changes are evident up to 10 months following exposure to glycine-2- C^{14} , or within 7 months following exposure to $NaHC^{14}O_3$.

TABLE 15

Exposed to:	Age (weeks) at exposure	Number	
		C^{14} -Injected	Controls
Glycine-2- C^{14}	8 to 9	68	121
$NaHC^{14}O_3$	3 to 4	49	97
	5 to 6	13	27
	9 to 12	56	120

Animals are being selected at random from each C^{14} -injected group at intervals following exposure, and a distribution analysis is made of the C^{14} content of selected organs and tissues. These data will be supplemented with radioautographic studies.

Preliminary findings on sacrificed animals which were injected with glycine-2- C^{14} show that 0.2 - 0.4 per cent of the activity to which they are originally exposed remains after 6 to 10 months in the tissues selected for analysis. One femur and the bones of the "thoracic cage" account for 65 - 70 per cent of this retained activity, with the bulk of the remainder being in about equal percentage distribution in femoral muscle, spleen, lungs, kidney, and liver. A variable, but often highly significant, activity appears in abdominal fat, lymph nodes, thymus, and submaxillary glands. It is surprising to find that the blood (including the erythrocyte) has an appreciable activity after 6 months.

Although it is well established that C^{14} from $NaHC^{14}O_3$ is preferentially retained in bone, the above findings were not expected on the basis of reports in the literature. To substantiate the preliminary findings, another group of mice will be exposed to larger doses of glycine-2- C^{14} under the conditions previously used, and the sites and forms of incorporation will be more definitely ascertained.

On the Mechanism of Leukemogenesis by Irradiation

(Upton, Thomson, Ledford)

Earlier experiments have shown that the leukemias induced in mice by irradiation are predominantly thymic in origin, and that thymectomy prevents the X-ray induction or spontaneous occurrence of leukemia in mice genetically susceptible to leukemias of thymic origin. It was thought that an agent which would bring about atrophy of the thymus might likewise prevent or inhibit the induction of leukemia by X rays.

To test this assumption mice of the RF stock were given cortisone in conjunction with irradiation, as outlined in Table 16. Some were irradiated before and some after the cortisone treatment. The controls included siblings receiving the vehicle only or X rays only. These experiments were begun on December 6, 1950, and a preliminary analysis of the results to date is shown in Table 16.

TABLE 16

EFFECT OF CORTISONE UPON THE INCIDENCE OF LEUKEMIA IN X-IRRADIATED AND NONIRRADIATED RF MICE (PRELIMINARY DATA)

Treatment*	Total no. of mice in group	No. of mice dying	No. dying with leukemia	Incidence of leukemia (per cent)
Cortisone only	139	2	2	1.4
Cortisone vehicle only	159	1	1	0.6
Irradiation after cortisone vehicle	122	20	20	16.4
Cortisone after irradiation	130	13	13	10.0
Cortisone before irradiation	131	14	13	9.1

* Females and males were distributed in approximately equal numbers within each group. X irradiation (350 r) was carried out at 8 to 12 weeks of age. Cortisone was given in subcutaneous injections of 1.0 mg each on 3 successive days, beginning either 1 week before or 2 to 3 weeks after irradiation. Cortisone vehicle (1.5 per cent benzyl alcohol in physiologic saline) was injected subcutaneously in comparable amounts (0.04 cc) on 3 successive days at 8 to 12 weeks of age, or beginning 1 week prior to irradiation.

This table shows that the leukemia incidence in the control mice receiving vehicle is thus far 0.6 per cent, those receiving cortisone alone 1.4 per cent, those irradiated and given only vehicle 16.4 per cent, and those receiving both cortisone and X-ray treatment 9 - 10 per cent. These data suggest that cortisone treatment retards the induction of leukemia by X rays. Since these experiments were set up, Kaplan et al. have reported comparable results in a similar investigation.

Another experiment was designed to test the possibility of preventing, by treatment with cortisone, the onset of leukemia in a strain of mice (AK) highly susceptible to spontaneous leukemia. The results are shown in Table 17.

Table 17 shows that the leukemia incidence in the controls is thus far 28.3 per cent as compared to 10.7 per cent in siblings treated with cortisone. As the table indicates, most mice of these groups are still alive. The plan calls for observing them until natural death.

TABLE 17

INFLUENCE OF CORTISONE ON THE SPONTANEOUS DEVELOPMENT
OF LEUKEMIA IN AK MICE (PRELIMINARY DATA)

Treatment*	Total no. of mice in group	No. of mice dying	No. dying with leukemia	Incidence of leukemia (per cent)
Cortisone	112	13	12	10.7
Control	67	19	19	28.3

* Cortisone was administered subcutaneously in three injections of 1.0 mg each on consecutive days at 8 to 16 weeks of age. The mice in this study were males.

In the course of these experiments it was noted that the cortisone treatment as given was not intense enough to bring about a lasting atrophy of the thymus. Therefore, a third experiment is being set up in which repeated injections of cortisone are given to bring about a more sustained atrophy of the thymus.

Whether or not these observations are "academic" or applicable to man remains to be seen. It is noteworthy, however, that stress brings about atrophy of the thymus and that the leukemia-lowering action of cortisone was evident even when cortisone was given after irradiation.

A Transmissible Splenomegaly of Mice with
Anemia and Leukopenia

(Upton, Ledford)

In an earlier report attention was called to a disease, isolated in mice, which offered an opportunity to study problems related to hypersplenism and erythropoiesis. This is characterized by marked splenomegaly, anemia, leukopenia, and extramedullary hematopoiesis; it has been transmitted to related mice through a series of subpassages, by intraperitoneal injection with particles of involved spleen. A strain of mice has been inbred which appears highly susceptible to this condition, and attempts are now being made to determine its pathogenesis and mode of transfer.

I^{131} -Induced Pituitary Growth

(Furth; Burnett, Radiation Protection group; Gadsden, Gude, Ledford)

Pituitary growths were induced by thyroid-destructive doses of I^{131} (200 - 400 μ c). These growths proved readily transplantable in mice similarly treated with I^{131} , but not in normal mice, with one exception. By successive transplantations of I^{131} -induced tumors several strains of graftable pituitary growths were established. All these are remarkably alike. They cause tremendous hyperplasia of the ovaries and uteri of young mice, indicating the discharge of gonadotropic hormones (both FSH and LH). The gross appearance of these organs bears a striking similarity to mice receiving urine of pregnant women. Some of these observations are being published (Furth and Burnett, Proc. Soc. Exptl. Biol. Med., 78:222-224, 1951).

More recent studies indicate that these tumors also discharge large quantities of TSH. This was demonstrated by Dent who injected tumor fragments into tadpoles which rapidly underwent metamorphosis. TSH can also be readily demonstrated in the blood and tumors of animals bearing grafted pituitary growths. A hitherto unknown and puzzling endocrine effect seen in these mice is a tremendous hyperplasia of the extrahepatic biliary tracts. Experiments in progress suggest that these pituitary growths can be controlled to some extent by administration of thyroprotein.

Several experiments are in progress designed to determine the character of these tumors and the factors involved in neoplasia induction by I^{131} . From the standpoint of neoplasia induction by ionizing radiation, these pituitary tumors represent a special type though akin to ovarian growths, induced by total-body irradiation. In these organs the radiating energy exerts its neoplastic action not by a direct effect on the cells rendered neoplastic but entirely by an indirect mechanism. Its action on the thyroid is probably analogous to surgical thyroidectomy and the neoplasms arise as a consequence of the hormonal disturbance (lack of TH) in an organ (hypophysis) which in itself is not irradiated and is known to be highly resistant to the direct action of ionizing radiations.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson (Leader)

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Oxidative Reactions in the Propionic Acid Fermentation

(Carson, Delwiche, Phares, Long, Gwin)

Previous studies (ORNL-989) on the propionic acid fermentation demonstrated that Propionibacterium pentosaceum contains enzymes which are typical of a tricarboxylic acid cycle. The presence of aconitase and isocitric dehydrogenase was shown by the conversion (in cell juices) of citrate to α -ketoglutarate with the uptake of one-half mole of oxygen per mole of citrate utilized; α -ketoglutarate was isolated and identified. A wide variety of substrates were oxidized with the uptake of molecular oxygen; addition of coenzyme A accelerated oxidations by acetone-dried cells.

Present studies demonstrated that acetone-dried cells were capable of carrying out the following reaction ("condensation reaction"):



Isotope experiments were performed with "resting cell suspensions" (aerobically grown cells) on labeled acetate, ethanol, and pyruvate. Citrate, α -ketoglutarate, succinate, pyruvate, propionate, malate, and acetate were isolated, purified and degraded to individual carbon fragments. Some typical results are presented in Table 18. These results rather clearly fall in line with predictions based upon current concepts of the tricarboxylic acid cycle. In addition, the distribution of labeling found in the α -ketoglutarate is an excellent example of the Ogston effect (asymmetry of the citric acid-enzyme complex), as illustrated in the following equations.

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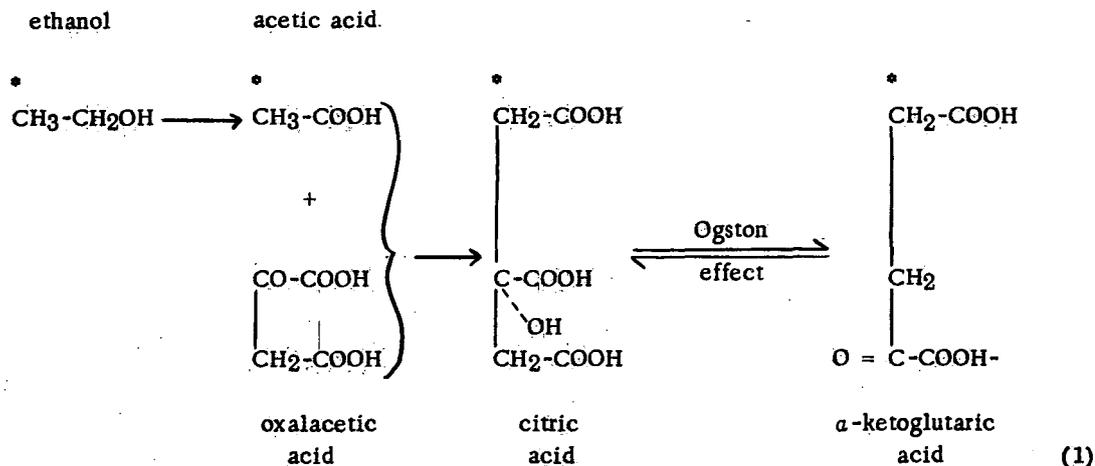


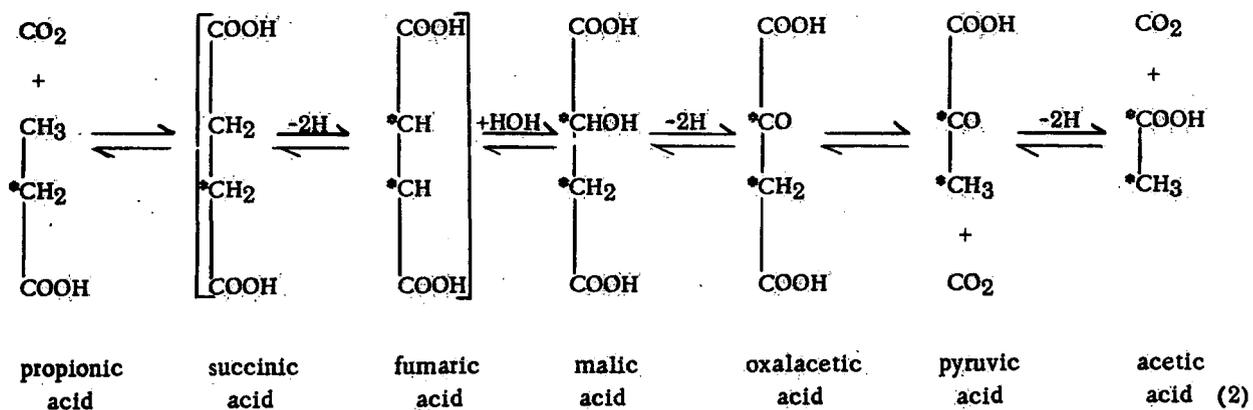
TABLE 18

LABELING OF α -KETOGLUTARATE AFTER OXIDATION OF ETHANOL-2-C¹⁴ BY PROPIONIBACTERIUM PENTOSACEUM IN THE PRESENCE OF SODIUM ARSENITE

	Relative specific activity*
5 COOH	1
4 CH ₂	93
3 CH ₂	} 3
2 C=O	
1 COOH	3

* Specific activities calculated as per cent of total specific activity in α -ketoglutarate molecule

More complex isotope experiments conducted with labeled pyruvate and acetate have confirmed and extended these observations and have led to an elucidation of the probable pathway of propionate oxidation. It appears as if the reactions by which propionate is oxidized involve reversal of the succinic decarboxylase reaction, followed by oxidation to acetate via fumarate, malate, oxalacetate, and pyruvate. According to this hypothesis, propionate oxidation would involve symmetrical molecules (succinate and fumarate); results of tracer experiments have made this hypothesis quite likely. α -Labeled propionate was oxidized by P. pentosaceum, and the end-product, acetate, isolated and degraded. The theory demands that the acetate be labeled in both carbons (equally) and indeed this result was obtained.



It thus appears quite likely that propionate is oxidized via symmetrical acids.

The present results, as well as those reported in ORNL-989, have allowed us to draw together a unified concept concerning the propionic acid fermentation, and which is being prepared for publication in *Bacteriological Reviews*.

BIOCHEMISTRY

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

W. E. Cohn (Leader)

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Composition and Structure of Nucleic Acids

(Cohn, Volkin, Khym, Jones)

Further information concerning the structure of the nucleic acids has been obtained with the use of various enzyme systems.

The pyrimidine diphosphates (Quarterly Report ORNL-989) obtained from snake venom digests of ribonucleic acids (RNA) have been isolated in solid form and characterized as the 3',5'- and/or 2',5'-diphosphates of nucleosides, cytidine, and uridine.

Quantitative determinations of the end products in hydrolysates of yeast and calf liver RNA digested with a purified snake venom phosphodiesterase have been carried out. The results are summarized in Table 19.

It can be seen that the principle products are: (1) the 5'-nucleotides of all the bases, (2) cytidine and uridine diphosphates, and (3) adenosine and guanosine. The calf-liver digest, which was carried out for a much longer period of time, shows the presence of about 1.7 μ M of each nucleoside as a result of phosphatase action on the nucleotides, as well as inosine instead of adenosine as a result of deamination.

Purine nucleosidase from calf liver and pyrimidine nucleosidase from cell-free preparations of Escherichia coli have been partially purified. The use of the latter enzyme in conjunction with a phosphatase (intestinal or prostatic) results in the quantitative liberation of ribose from pyrimidine nucleotides, a procedure which is refractory to chemical methods. Ion-exchange analysis for sugars reveal that RNA digested with a mixture of phosphatase and purine-pyrimidine nucleosidases contained the theoretical amount of ribose, with no detectable amounts of other sugars. As a by-product in the purification of bacterial pyrimidine nucleosidase, a specific

* Research Participant

TABLE 19

RNA	Phosphorus(μM)		Nucleosides(μM)					Nucleotides (μM)				Diphosphates (μM)		Organic P not recovered* (% of total)		
								Cyt.		Ury.		Ade.	Gua.		Cyt.	
	Total	Inorg.	Cyt.	Ury.	Ade.	Gua.	Ino.	5'	b	5'	b	5'	5'		Cyt.	Ury.
Yeast	87.0	3.6	0.3	0.8	4.1	3.5	0	8.6	1	9.0	0	7.3	13.7	4.3	3.3	34
Liver	51.5	6.8	1.9	1.7	0	5.9	3.3	5.6	1.9	2.5	0.8	3.5	8.1	4.2	2.9	18

* Probably represents polynucleotides

cytosine deaminase has been isolated. This enzyme catalyzes the reaction, cytosine-uracil, but does not deaminate cytidine or cytidylic acid.

A nuclease from calf spleen has been isolated which shows a different mode of action on nucleic acids from the pancreatic ribo- or desoxyribo-nucleases. Investigation of the products of nucleic acids hydrolyzed by this enzyme are in progress.

The Isotope Effect in the Urease-Catalyzed Hydrolysis of Urea-C¹⁴

(Doherty; Collins, Chemistry Division)

In 1948, Daniels and Meyerson reported a "reverse" isotope effect in the urease-catalyzed hydrolysis of urea containing tracer amounts of urea-C¹⁴. They reported that the carbon dioxide first evolved contained more carbon-C¹⁴ dioxide than that evolved toward the end of the reaction.

Although "reverse" isotope effects are not unknown, the magnitude of the effect reported by Daniels and Meyerson prompted this reinvestigation.

Our data from 20 separate experiments have now failed to confirm the results of Daniels and Meyerson. They show, qualitatively at least, that the hydrolysis of C¹⁴-labeled urea proceeds with a normal, and not a "reverse" isotope effect; that is, the carbon dioxide first evolved contains less C¹⁴ than that evolved toward the end of the reaction.

In this procedure, samples of $M/0.2$ urea-C¹⁴ (containing $0.176 \mu\text{C}^{14}/\text{mM}$) in $M/0.25$ tris(hydroxymethylaminomethane)-phosphate buffer were treated with urease and allowed to react at 28°C with stirring for varying periods of time. The separate reaction mixtures were quenched with aqueous lactic acid solution, and the carbon dioxide samples evolved were measured manometrically. The gas samples were transferred to ion chambers and their C¹⁴ content was determined.

Although experiments on any given day yield data through which a smooth curve may be drawn when total radioactivity evolved as carbon-C¹⁴ dioxide is plotted against per cent of reaction, it has not been possible from day to day to obtain points which lie on the same curve. This may be due to different reaction inhibition periods, different degrees of deactivation of enzyme, or temperature effects on the enzyme solution prior to reaction.

The data from one experiment are plotted in Fig. 14.

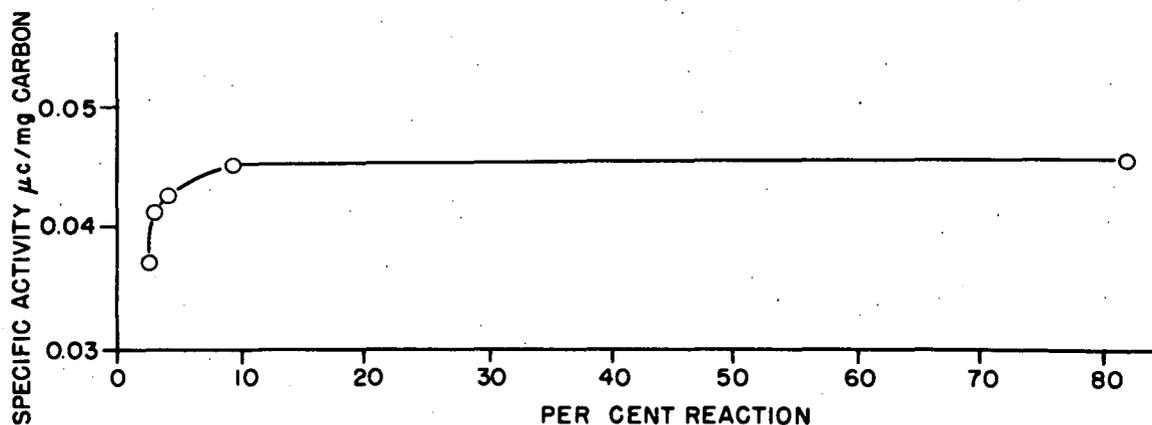


FIG. 14

Further experiments are necessary to achieve standard conditions before this effect can be evaluated precisely. The present results however, indicate qualitatively that a normal isotope effect occurs in the urea- C^{14} -urease reaction.

Enzyme-Substrate Equilibria

(Vaslow)

The thermodynamic properties of four systems of three different types have been measured thus far. The systems, their type, and values of the thermodynamic functions at pH 7.5 are given in Table 20. This pH is close to that of maximum activity of the enzymes.

TABLE 20

System	Type	ΔF	ΔH	ΔS
Chymotrypsin-acid *	Enzyme-substrate	-2000	-56000	-11.4
Chymotrypsin-ketone **	Enzyme-inhibitor	-2400	-4700	-7.9
Carboxypeptidase ***-acid	Enzyme-inhibitor	-2280	-4700	-8.8
Chymotrypsinogen-acid	Zymogen-substrate	-2200	0	+8.5

* N-acetyl-3:5-dibromo-L-tyrosine

** Derived from 1 by replacing the carboxy OH with a methyl group.

*** Carboxypeptidase has been shown to be polyvalent, hence, these are apparent and not actual values.

The inhibitors show behavior similar to that of the substrate but the rate of increase with pH of negative entropy and enthalpy are not as large. The binding with chymotrypsinogen, which has no catalytic activity, shows increased freedom (i. e. , positive entropy) rather than the strain which is found in the catalysts.

The system N-acetyl-3:5-dibromo-D-tyrosine — chymotrypsin is being measured.

Biological Energy-Transfer Systems

(Strehler)

Work has been continued on the use of the firefly luminescent system as a tool for measuring metabolic intermediates. It has been found possible to measure ATP and ADP in the presence of each other. This has been made possible by using purified myokinase prepared according to the method of Kalckar, Colowick, et al. , with the modification that sulfuric acid rather than hydrochloric acid is used in preparation. This change is necessitated by the fact that chloride ion is inhibitory to firefly organ luminescent extracts.

Additional information on some physical properties of firefly luciferin has been obtained. This includes the determination of this compound's infrared absorption spectrum, the determination by microtitration of its neutral equivalent and pKa and the polarographic half-wave potential of

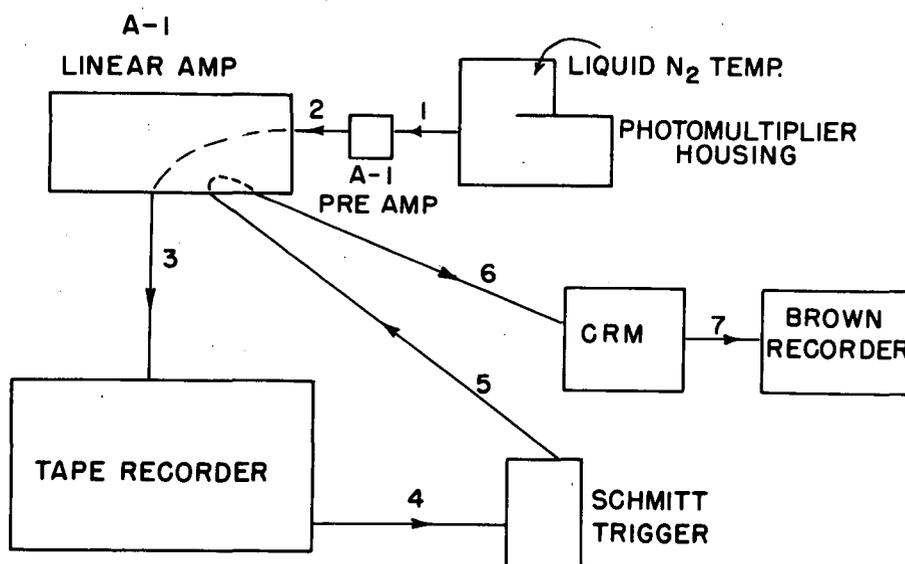


FIG. 15

this compound, one of its degradation products, and a variety of related compounds. The infrared absorption spectra of some twenty related compounds have also been determined. Many of the physical properties of this compound are similar to those of riboflavin, indicating a possible relationship to this B vitamin.

A method has been devised, in conjunction with J. Davidson of the Instrument Dept. for measuring rapid rises and decays in luminescence curves. The method consists of recording on a tape recorder the individual pulses from a quantum counter previously reported and then playing back the recorded pulses at $1/30$ or $1/60$ of the recording speed, triggering the A-1 linear-amplifier-pulse height selector with a Schmitt relay and integrating the low speed counting rate on a differential-integral counting rate meter and using a Brown to record the counting rate. This permits the use of the Brown recorder for the recording of decays and rises with a time constant as short as $1/10$ second. The apparatus was built to record the shape of the rise in luminescence of green plants after they are illuminated, in conjunction with a phosphoroscope now in construction. Fig. 15 graphically illustrates the method of slowing down decay curves in use.

PLANT PHYSIOLOGY

G. R. Noggle (Leader)

L. P. Zill

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Herbert Jonas*

M. Eleanor Schumacher

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Partition Chromatography of Organic Acids

(Noggle, Schumacher)

As pointed out in the last Quarterly Report ORNL-989, it would be desirable to have available a unified scheme for separating and isolating the organic acids, amino acids, and carbohydrates present in plant tissue. During the past quarter, additional work has been done to develop such a scheme. Up to the present time, however, no completely satisfactory results have been obtained. The method of extracting acidified dried plant tissue with ether gave excellent results for the organic acids. When the tissue was then extracted with 80 per cent ethanol, it was found that the previous acid treatment made it impossible to obtain a reliable measure of the carbohydrate fractions present in the original tissue. Sucrose was hydrolyzed during the acid-ether extraction and part of the glucose and fructose was extracted along with the organic acids. Pentosans, present in the untreated tissue, were also hydrolyzed by the acid-ether treatment and free pentoses appeared in the 80 per cent ethanol fraction.

If a complete separation of the organic acids, amino acids, and carbohydrates of plant tissue is required, the practical solution appears to be the analysis of separate aliquots of the original material. This does not work a hardship when one has available 5 to 10 g of tissue. However, in many tracer studies, the amount of tissue available is generally small and some compromise must be made regarding the completeness of separation.

Additional studies have been carried out on the identification and separation of the organic acids present in plants. Partition chromatography of tissue extracts has revealed the presence of a number of acids which have not been identified. Samples of isocitric, glutaric, adipic, cis-aconitic, quinic, tricarbollylic, and pyrrolidone carboxylic acids have been run under standardized conditions but some of the acids present in tissue extracts remain unidentified. This problem remains under investigation.

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Biosynthesis of Sugars

(Noggle, Zill, Cheniae, Schumacher)

It has been shown (ORNL-989) that the waste liquor for the Leuconostoc mesenteroides fermentation of sucrose contained sucrose, glucose, fructose, and several other carbohydrates of unknown identity. One of these was tentatively identified as a pentose. Since a sample of the original sucrose used in the fermentation was not available at that time, it was not known whether this pentose was an impurity in the sucrose or synthesized by the Leuconostoc during the fermentation. A sample of the original labeled sucrose along with a sample of the carrier sucrose was obtained from Dr. N. J. Scully of the Argonne National Laboratory and analyzed by the borate method. Neither sucrose samples contained any pentoses so it must be concluded that the pentoses were synthesized during the fermentation. Experiments are under way to reveal the identity of these pentoses.

Quantitative Analysis of Sugars in Plant Extracts by Ion Exchange

(Noggle, Zill, Cheniae, Schumacher)

Sugar beet leaves were extracted with boiling 80 per cent ethanol. Following removal of the alcohol, the solution was deproteinized and deionized. The solution was analyzed for reducing sugar and total sugar after acid hydrolysis by the Somogyi method. An aliquot was passed through the ion-exchange column (Khym and Zill, ORNL-1026) and the separated sugars determined colorimetrically with the anthrone reagent. The data are shown in Table 21. Reducing sugar by the Somogyi method showed 395 mg while the sum of glucose and fructose by ion exchange was 369 mg. Nonreducing sugar by the Somogyi method was 113 mg while the sucrose (nonreducing) from the ion-exchange column was 157.2 mg. The high value for sucrose from the ion-exchange column cannot be explained at present. Some sucrose may have been destroyed during the acid-hydrolysis treatment and resulted in a low total sugar value. Additional work is under way to study the application of the ion-exchange technique to the quantitative analysis of sugar mixtures.

TABLE 21

Sugar	Somogyi Method	Ion Exchange
	mg	mg
Total Sugar	508	-
Reducing Sugar	395	-
Glucose	-	336.8
Fructose	-	32.2
Sucrose	-	157.2

Biosyntheses of Plant Glycosides

(Chambers)

The term, glycoside, is applied to a compound which on hydrolysis yields a sugar and one or more other products. The glycosides are widely distributed in plants but their mode of formation or metabolic function is little understood. The present investigation was started to gain some insight as to how plants synthesize glycosides and concerning their metabolic role.

Preliminary work has been carried out on Chimaphila maculata (spotted wintergreen), a member of the Ericaceae. This plant is reported to contain arbutin, a glycoside which contains hydroquinone and glucose. A paper chromatographic method has been developed for the separation and detection of arbutin. Preliminary experiments have indicated that Chimaphila contains a substance which is believed to be methyl arbutin. Since methyl arbutin is unobtainable commercially, a known plant source of the material is being extracted to prepare it in pure form in order to check its presence in Chimaphila. Work is also under way to synthesize arbutin containing C^{14} .

In regard to the separation and analysis of glycosides in general, it has been found that arbutin, salicin, and amygdalin are held on an ion-exchange column and behave in a manner similar to the sugars when eluted with tetraborate solutions. This ion-exchange procedure holds great promise as a preparative method and as an analytical method for handling the glycosides.

Ion-Exchange Separation of Carbohydrates

(Zill, Cheniae)

The separation and analysis of sugar mixtures by means of the ion-exchange chromatography of borate-sugar complexes continues to yield information which more firmly establishes the method as a valuable tool for the investigation of diverse biochemical problems. Concurrent with the accumulation of information concerning the behavior of borate-sugar complexes on ion-exchange columns, has been the gradual understanding of the theoretical basis for their elution order and equilibrium reactions.

Up to the present time, certain of the disaccharides have been impossible to synthesize and have been obtained only with difficulty from trisaccharides which contain them as part of their more complex molecule. Representative of such a preparation is that of turanose from the trisaccharide melezitose. A clean and simple preparation of such a sugar has been effected by the ion-exchange method and consists essentially of the following steps:

1. The melezitose is hydrolyzed with dilute sulfuric acid giving a mixture of glucose, turanose, and possibly a small amount of residual melezitose.
2. The sulfuric acid is removed either by the use of a strong-base anion exchanger or by precipitation as barium sulfate.
3. The solution is concentrated to a small volume (ca 10 ml), made 0.01 M in potassium tetraborate, placed on a strong-base anion exchanger and eluted with 0.015 M potassium tetraborate. Under these conditions of elution, the following volume to peak values are obtained (all for 0.015 M $K_2B_4O_7$):

Melézitose	195 ml
Turanose	1250 ml
Glucose	3000 ml

The preparation of melibiose from the trisaccharide raffinose may be carried out in the same manner. The volume to peak values for this preparation are as follows (the fructose being the monosaccharide split from the raffinose yielding melibiose):

Raffinose	400 ml	0.005 <u>M</u> $K_2B_4O_7$
Fructose	1250 ml	0.015 <u>M</u>
Melibiose	7000 ml	0.015 <u>M</u>

From the volume to peak values for the two preparations given, it is seen that a quantitative resolution is possible in both cases. The preparation of gentiobiose from gentianose is also being investigated.

As stated in the last Quarterly Report ORNL-989 the melibiose-borate complex was extremely difficult to elute (ca 7000 ml volume to peak value for 0.015 M $K_2B_4O_7$). Although time has not permitted the further investigation of this behavior (by polarimetric means) another observation has been made in the case of gentiobiose which corroborates this behavior. The volume to peak value for this sugar was ca 5000 ml (0.015 M $K_2B_4O_7$) which is again much greater than those of the other disaccharides which have been studied. Since both melibiose and gentiobiose possess a 1-6 linkage, they are capable of forming a furanose ring, a configuration which has been shown to be particularly capable of forming a borate complex (the presence of cis-hydroxyl groups still being necessary). Further work will be required for the elucidation of this problem. It should be mentioned that the gentiobiose contained a quantity of fructose and a lesser quantity of an unknown hydroxy compound which was released upon regenerating the column, factors which favor its preparation by the method described above.

Removal of Borate from Purified Sugar Samples. The removal of borate has been accomplished in a manner quite different from that first used (see ORNL-989). This has been carried out as follows.

1. The pooled aliquots of a single sugar from a column run are freed of the sodium cations by passage through a column of Dowex-50.
2. The solution is evaporated to dryness under vacuum.
3. Absolute methyl alcohol is added and the solution again taken to dryness under vacuum. Methyl borate, a volatile ester, is removed by this distillation step. Step 3 is repeated at least two more times. The final residue is taken up in distilled water giving a solution of sugar.

Although removal of the borate has been successful by this method, it is hoped that it may be somewhat simplified by the use of a catalyst (such as sulfuric acid) which may give complete removal of borate with one distillation in the presence of methyl alcohol.

Separation of Sugar Alcohols. Three of the four naturally occurring sugar alcohols (sorbitol, dulcitol, mannitol) have been available for the investigation of the ion-exchange separation of their borate complexes. The fourth hexitol, iditol, is unavailable, as far as we know. There is

no known method which has been successful in separating the sugar alcohols up to the time at which the present method was introduced. It is therefore of large importance that such a separation may be achieved by means of ion exchange. The volume to peak values (for 0.015 M $K_2B_4O_7$) which have been obtained are:

Sorbitol	1700 ml
Dulcitol	3000 ml
Mannitol	3800 ml

Although a complete separation of the three hexitols was not obtained in a preliminary column run, the separation was sufficiently high to ensure a quantitative resolution upon establishment of the proper eluting agent (as in the case of a fructose-mannose mixture). The large enhancement of the conductivity of boric acid by the hexitols is reflected in the comparatively large volume to peak values obtained.

Growth of Callus Culture in Presence of Radioisotopes

(Ball)

Radioautographs made upon NTB plates reveal that there is an accumulation of P^{32} and S^{35} in the meristematic regions of the callus. These regions of intense cell division are at the margins of the tissue mass and around the tracheid groups inside the culture.

C^{14} added to the culture medium as radioactive sucrose has a different pattern of accumulation from the above two radioisotopes. There is accumulation in the marginal meristematic cells, and also a more general distribution throughout the callus. There appears to be no special accumulation in the cambium around the tracheid groups, while a pronounced accumulation occurs in the differentiating tracheids.

Earlier studies (Ball, Growth, 14:295-325, 1950) of this callus culture have given some evidence that the tannin-filled cells tend to divide more slowly and have less potential in the regeneration than the clear cells. The radioautographs show that there was apparently an equal accumulation of the radioisotopes by both types of cells. This may be considered evidence that each of the types of cells has an equal rate of ion accumulation, and to that degree, an equal metabolism.

There may be so much accumulation of the radioisotopes in the meristematic cells of the callus culture that certain cells are killed. These results have been obtained by adding 1.5 μ c of radioisotope per milliliter of culture medium. With the death of a meristematic cell, it was found

that adjacent meristematic cells continue dividing and cause the culture to grow beyond the necrotic area. This behavior may indicate either an unequal accumulation of radioisotope in different meristematic cells or an unequal tolerance to their effects.

During the space of 2 months most of the cultures receiving P^{32} and S^{35} were killed. Most of the cultures receiving C^{14} survived after an equal period of growth. The toxicity of phosphorus could be accounted for by the strength of its radiation. The lack of toxicity of C^{14} could similarly be accounted for by the weakness of its radiation. The sulfur, however, having a radiation strength but little more than that of C^{14} , could not be toxic due solely to strength of radiation. Its toxicity, in contrast, may be due to its high accumulation in the callus.

Growth of Callus Culture in Various Carbohydrates. The original shoots, grown from burls, contained glucose, levulose, and sucrose. The callus culture when grown upon either sucrose or glucose was found to contain glucose, levulose, and sucrose. The callus appeared able to convert sugars within itself. A series of cultures were set up upon media containing various sugars. After 2 months of growth, the callus was extracted for sugars. In order to determine whether the enzyme system converting the sugars was intra- or extracellular, the medium upon which the tissue had grown was also extracted for sugars (Table 22).

It appears that the enzymes which convert the sugars are extracellular in most cases and diffuse into the agar medium. In addition, however, the callus culture absorbs considerable quantities of the "foreign" sugar (in the case of raffinose and lactose) into its tissues and there also carries on the conversion.

Other sugars, e. g. , mannose, maltose, galactose, arabinose, xylose, and ribose are also being studied in the same manner. The cultures containing them are not yet ready for analysis.

TABLE 22

Description of culture	Sugars found by chromatograms
Sequoia shoots from burl planted Sept. 1, 1951	glucose, levulose, sucrose
Sequoia callus, 3% sucrose	glucose, levulose, sucrose
Sequoia callus, 3% glucose	glucose, levulose, sucrose
Sequoia callus, 3% raffinose	raffinose, levulose, sucrose, glucose
Sequoia callus, 3% lactose	glucose, levulose, sucrose, lactose
Sequoia callus, 3% levulose	glucose, levulose, sucrose
Agar medium containing 3% levulose on which Sequoia callus had grown Sept. 5 to Oct. 26	glucose, levulose, sucrose
Sequoia callus 3% dextrin	glucose, levulose, sucrose
Agar medium containing 3% dextrin on which Sequoia callus had grown Sept. 5 to Oct. 29	glucose, levulose (?), sucrose (?)
Sequoia callus 3% inulin	glucose, levulose, sucrose
Agar medium containing 3% inulin on which Sequoia callus had grown Sept. 5 to Oct. 30	no sugars
Sequoia callus 3% cellobiose	glucose, levulose, sucrose
Agar medium containing 3% cellobiose on which Sequoia callus had grown Sept. 5 to Oct. 31	

BIOPHYSICS

GENERAL PHYSIOLOGY

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 E. B. Darden, Jr. Patty Jean Mathias
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Erythrocytes

(Stewart, Mathias, Sheppard)

Following the studies on the use of ion-exchange methods to prepare blood tissue extracts for determinations of acetylcholine by the frog rectus abdominis method we have returned to a critical investigation of the factors influencing the reproducibility of the method. After studying such effects as toxic contaminants produced during the pretreatment, and the influence of temperature and both solution concentration on relaxation of the muscle, we have finally arrived at a set of conditions in which a stable sensitivity to acetylcholine following exposure of the muscle to blood extracts was achieved. These observations have confirmed the rectus muscle preparation as a satisfactory instrument for acetylcholine determinations, but have shown the complete unreliability of the method unless used under a very carefully controlled set of conditions.

Effect of Intense Local X Irradiation of Muscle on its Water and Electrolyte Content

(Wilde, Sheppard)

Studies on inulin and radiosodium distribution in the foreleg of rats following the exposure of the limb to intense local radiation showed that the increase in water inulin space and sodium space were all closely alike. This indicates that the early postirradiation disturbance is essentially a gain in interstitial water.

Vascular Phase of the Disappearance of K^{42} from the Blood of Dogs

(Overman, Wilde, Sheppard)

These studies described in the previous report (ORNL-989) were concluded during the past quarter. Both experimental and theoretical conclusions were in fair agreement on the following points.

* AEC Postdoctoral Fellow

1. During the period immediately following the sudden injection of a small quantity of labeled material into the circulation (if we study the concentration of the label at different points) events upstream are repeated downstream but delayed by the mean transit time between the injection and sampling points. A reduction in scale occurs which is determined by the mean value of the exchange rates in the intervening space. Progressive smearing of the time relations occurs due to the dispersing action of the variable circulatory paths.

2. For the circulation as a whole the relation between the concentration of circulating isotope and the time has two components, periodic and aperiodic. The periodic component will depend on the rapidity of the original injection, on the over-all dispersing action of the vascular tree, and on the rate of blood flow. The aperiodic component will be a quasi-exponential function whose initial rate of decline will yield the over-all mean rates of exchange between the circulation and the tissues.

From these considerations the injected material will move initially through the circulation as a wave. This wave will be rapidly suppressed and broadened, quickly returning on itself. The combined waves soon merge into a distribution of activity such that the concentration, although not uniform around the circuit, declines everywhere by the same fraction in a given interval of time.

3. The initial concentration of the aperiodic component of the solution can be obtained by extrapolating the semilogarithmic plot back to zero time. If observations are made close to the site of injection, the ordinate intercept value will be the blood volume dilution, i. e. , the amount of label injected divided by the blood volume. If observations are made downstream, a correction of the intercept must be made for the mean transit time and loss of tracer by intervening exchange with the intervening extravascular pools.

4. Initially there will be a circulation limitation effect in the spatial distribution of the labeled material; substances disappearing more rapidly will tend to be found in the extravascular pools farther upstream. This effect will not influence the rate of decline of the aperiodic component, since substances which have less time in which to pass out of the vascular circuit during one traversal are nevertheless more rapidly recirculated. Following the initial distribution of the label in the extravascular compartments, there will be a redistribution by backflow of tracer as all body compartments tend either rapidly or slowly to approach uniform specific activity.

Effects of Infrared in Modifying Aberration Frequency

(Kirby-Smith, Daniels)

The study of the effects of infrared radiation in combination with X and γ radiation on Tradescantia pollen has been completed. Repeated infrared irradiation of dry pollen at temperatures in the 0° to 25°C range have resulted in no significant changes in aberration frequency. Additional experiments on X- and γ -irradiated pollen posttreated with infrared during pollen tube growth has also shown no change in chromosomal aberration rate. This is in contrast to the finding (Swanson and Hollaender Proc. Natl. Acad. Sci., 32: 295-302, 1946) that, if the inflorescences of Tradescantia are irradiated with X rays and near infrared, the chromosome breakage is doubled over X rays alone.

A study of the effect of β rays on Tradescantia pollen is in progress.

Radiological Physics

(Darden, Kirby-Smith)

The collection of data on the absorption of P^{32} β rays in thin homogeneous layers of tissue-like material has been completed and the results will be presented shortly.

Assistance is being continued in the calibration of phosphorus bakelite β -ray plaques for the grasshopper neuroblast project. The considerable increase in intensity required in the current phase of this work has necessitated the installation of additional shielding in the γ source room for protection in handling these sources.

The 300-curie γ -ray source has been reinstalled following some initial difficulties requiring its temporary removal. The work of calibrating this source and the intermediate 10-curie source will be undertaken shortly.

A sandwich type exposure chamber for the β irradiation of pollen with phosphorus plaques has been constructed and put into operation. The sample is distributed between two sheets of rubber hydrochloride so that about 96 per cent of the measured surface intensity of the plaques reaches the biological material in between. The uncertainty of exposure time due to manipulating the sources is 2 seconds.

PHOTOSYNTHESIS

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

The effect of intermittent exciting light on the phosphorescence of Chlorella has been studied. When intermittent light is used to motivate photosynthesis, it is found that at low intensities there is no effect, but at high light intensities the rate of photosynthesis is always faster at the higher frequencies of flashing. The phosphorescence, as might be expected, shows no effect of intermittent light at low intensity, but at high intensity the phosphorescence is reduced as the frequency increases - just the reverse of photosynthesis. This result shows again that photosynthesis and phosphorescence are in competition.

Ultraviolet light has been found to have a quenching effect on the fluorescence of Chlorella pyrenoidosa. Decay curves show a rapid quenching immediately after radiation, followed by a more gradual decay lasting several hours. Investigation will be made at different dose levels and with other algae.