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QUARTERLY PROGRESS REPORT

FOR PERIOD ENDING AUGUST 10, 1952

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BIOLOGY DIVISION QUARTERLY PROGRESS REPORT for Period Ending August 10, 1952

Alexander Hollaender, Director

E. J. Slaughter, Technical Editor

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REVIEW OF RESEARCH PROGRESS

Alexander Hollaender

Cytogenetics. — In further investigations on the effect of oxygen on Paramecium, a clear-cut effect of oxygen tension on the X-ray sensitivity of genetic changes is evident. Tradescantia is proving its reliability as an experimental measure for the quantitative investigation of radiation effects. Previously reported findings on a combined effect of ultraviolet and γ radiation on Tradescantia chromosomes have been verified, and several interesting new points noted. Detailed investigations of several types of curvatures induced in grass roots by ultraviolet radiation brought out that one of these can be counteracted by auxin. Further evidence for sister-strand crossing over in maize chromosomes was found. There are increasing indications that oxygen has no effect on the number of chromosome breaks produced by X rays, but rather the effect is on the healing of chromosome pieces. Good evidence for this has also been found in Drosophila. In careful testing of the X-ray sensitivity of Escherichia coli during the different phases of growth, radioresistance was found to increase rapidly with the progression of the lag phase and to decrease during the earlier log phase; a return to normal sensitivity came in toward the end of the log phase. E. coli was found to leak adenosinetriphosphate after exposure to massive doses of X rays.

Mammalian Genetics and Development. — "The investigation of the X-ray-induced mutations in mice has been extended to dosages of 1000 r and is now being investigated at the 300-r level. It is too early to come to any conclusions in regard to these tests. Prenatal mortality, both through termination of pregnancies and through death of individuals within surviving pregnancies, is exceedingly high following irradiation of preimplantation stages. Irradiation during postimplantation stages causes little or no termination of pregnancy and considerably fewer deaths of individuals than does treatment during the preimplantation period. Furthermore, within the postimplantation period, susceptibility to prenatal killing decreases fairly rapidly with embryonic age. The effect of irradiation at various embryonic stages on neonatal viability does not parallel that on prenatal viability. Thus the highest rate of neonatal death follows irradiation on days 9 1/2 and 10 1/2 postconception, whereas irradiation during preimplantation stages, which has such drastic consequences prenatally, does not affect the viability of those animals which survive to term."

Pathology and Physiology. — Studies on the platelet transfusion during the hemorrhagic phase of radiation injury in the rat brought out that there is a diminished hemorrhagic tendency in animals injected with platelets after X irradiation. An enhanced spreading of Evans' blue, which can be attributed to an increase of permeability of the dermal connective tissues, was observed after exposure to X radiation. Experiments have shown that radiothyroidectomy has a marked enhancing effect on a 24-hour retention of radioiodine and that pituitary tumors grafted in the leg muscle of mice have an enhancing effect on the uptake of radioiodine.

Biochemistry. — The structure of nucleic acids is still undergoing investigation through the use of new tools developed with the application of ion-exchange methods. The Plant Physiology group, in their work in separation of sugars by this method, has been successful in separating different types of heptuloses.

Biophysics. — The presence of four Research Participants and their three assistants during this quarter has intensified the activity of the General Physiology group. An interesting method was developed for following the time relation of the release of radiopotassium from the heart muscle during contraction. Iodine metabolism was studied on Anuran larvae; the same techniques were also applied to similar studies in mammals. Studies on the production of acetylcholine in human red cells and investigation of isolated cell components, especially in regard to the nucleic acid content, have been expanded. There has been further verification of the earlier studies on the comparison of the effects of X, γ, and β rays on Tradescantia. The ratio of these three effects, which has been checked on pollen tubes and microspores, is about 1:0.75:0.5.

Conclusion. — I should like to point out that a considerable portion of this quarterly report has come from the work of Research Participants. It should also be mentioned that all projects under investigation in this Division are not discussed in each quarterly report. Many problems require a longer period of time for progress to a reportable stage.

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications. — In addition to the 32 articles (25 papers and 7 abstracts) listed below which have been published during the quarter, 59 articles are in press. Of the latter number, 40 are full-length papers, one is a book-length symposium, and 18 are abstracts.

AUTHOR (S)	TITLE OF ARTICLE	PUBLICATION
Atwood, K. C. and G. E. Stapleton	Lethal mutations and the bactericidal action of ionizing radiation	Naturwissenschaften, 39: 330-331, 1952
Baker, W. K.	Species and speciation from the standpoint of the geneticist	J. Tenn. Acad. Sci., 27: 235-240, 1952
Burnett, W. T., Jr., M. L. Morse, A. W. Burke, Jr., and Alexander Hollaender	A study of the reduction of the X-ray sensitivity of Escherichia coli by sodium hydrosulfite and certain other inorganic sulfur compounds	J. Bact., 63: 591-595, 1952
Furth, Jacob, E. L. Gadsden, and W. T. Burnett, Jr.	Autonomous transplantable pituitary tumors arising in growths dependent on absence of the thyroid gland	Proc. Soc. Exptl. Biol. Med., 80: 4-7, 1952
Giles, Norman H., Jr.	Recent evidence on the mechanism of chromosome aberration production by ionizing radiations	Symposium on Radiobiology (Oberlin, 1950) pp. 267-284, 1952 John Wiley & Sons, Inc.
Giles, Norman H., Jr.	Studies on the mechanism of reversion in biochemical mutants of Neurospora crassa	Cold Spring Harbor Symposia Quant. Biol., 16: 283-313, 1951
Hollaender, Alexander, W. K. Baker, and E. H. Anderson	Effect of oxygen tension and certain chemicals on the X-ray sensitivity of mutation production and survival	Cold Spring Harbor Symposia Quant. Biol., 16: 315-326, 1951
Hollaender, A. (edited by)	Symposium on radiation microbiology and biochemistry	J. Cellular Comp. Physiol., 39, Suppl. 1, 1952
Hollaender, Alexander	Physical and chemical factors modifying the sensitivity of cells to high energy and ultraviolet radiation	Symposium on Radiobiology (Oberlin, 1950) pp. 285-295, 1952, John Wiley & Sons, Inc.

AUTHOR (S)	TITLE OF ARTICLE	PUBLICATION
Hollaender, Alexander	Biology Division Quarterly Progress Report for Period Ending May 10, 1952	Project Report ORNL-1297
Khym, J. X. and D. G. Doherty	The analysis and separation of glucuronic and galacturonic acids by ion exchange	J. Am. Chem. Soc., 74: 3199, 1952
Kimball, R. F.	Genetic effects of radiation	Ann. Rev. of Nuclear Sci., Vol. I, 1952
Kimball, R. F. and Nenita Gaither	The role of externally produced hydrogen peroxide in damage to Paramecium aurelia by X rays	Proc. Soc. Exptl. Biol. Med., 80: 525-529, 1952
Lloyd, E. A. and David G. Doherty	2,4-Dinitrophenylhydrazones of some hexoses and pentoses	J. Am. Chem. Soc., 74: 4214, 1952
Noggle, G. R.	Review on Advances in Carbohydrate Chemistry, Vol. 5. Ed. by Claude S. Hudson and Sidney M. Cantor, Academic Press, Inc., New York	Quart. Rev. Biol., 27: 230-231, 1952
Nutting, L. A. and S. F. Carson	Lactic acid fermentation of xylose by Escherichia coli. I. Fermentation studies	J. Bact., 63: 575-580, 1952
Nutting, L. A. and S. F. Carson	Lactic acid fermentation of xylose by Escherichia coli. II. Tracer studies: Evidence for $C_2 - C_1$ condensation	J. Bact., 63: 581-589, 1952
Phares, E. F., E. H. Mosbach, F. W. Denison, Jr., and S. F. Carson	Separation of biosynthetic organic acids by partition chromatography	Anal. Chem., 24: 660, 1952
Pomper, Seymour	Purine-requiring and pyrimidine-requiring mutants of Saccharomyces cerevisiae	J. Bact., 63: 707-713, 1952
Russell, W. L.	X-ray-induced mutations in mice	Cold Spring Harbor Symposia Quant. Biol., 16: 327-336, 1951
Russell, W. L.	Mammalian radiation genetics	Symposium on Radiobiology (Oberlin, 1950) pp. 427-440, 1952, John Wiley & Sons, Inc.
Schwartz, Drew	The effect of oxygen concentration on X-ray-induced chromosome breakage in maize	Proc. Natl. Acad. Sci. U. S., 38: 490-494, 1952

AUTHOR (S)	TITLE OF ARTICLE	PUBLICATION
Stapleton, G. E., D. Billen, and A. Hollaender	The role of enzymatic oxygen removed in chemical protection against X-ray inactivation of bacteria	J. Bact., 63: 805-811, 1952
Stapleton, G. E., Alexander Hollaender, and F. L. Martin	Mechanism of lethal and mutagenic action of ionizing radiation on Aspergillus terreus. I. Relationship of relative biological efficiency to ion density	J. Cellular Comp. Physiol., 39, Suppl. 1: 87-99, 1952
Stapleton, G. E. and Alexander Hollaender	Mechanism of lethal and mutagenic action of ionizing radiations on Aspergillus terreus. II. Use of modifying agents and conditions	J. Cellular Comp. Physiol., 39, Suppl. 1: 101-113, 1952

ABSTRACTS

Anderson, Norman G.	The effect of various enzymes on nuclei isolated from rat liver	J. Tenn. Acad. Sci., 27: 198, 1952
Carlson, J. Gordon	Changes in the physical nature of the mitotic spindle during cell division	J. Tenn. Acad. Sci., 27: 119, 1952
Cohn, W. E. and Elliot Volkin	The products of ribonucleic acid hydrolysis and enzymolysis	Sec. Intern. Congr. Biochem., July, 1952, p. 199
Darden, E. B., Jr. and C. W. Sheppard	Depth dose relation for biological materials exposed to beta rays	J. Tenn. Acad. Sci., 27: 210-211, 1952
Gaulden, Mary Esther, Katherine Loemker, and Edgar B. Darden, Jr.	Fragmentation of chromosomes in grasshopper neuroblasts by beta rays and X rays	J. Tenn. Acad. Sci., 27: 211, 1952
Gaulden, Mary Esther, Marjorie Nix, and Edgar B. Darden, Jr.	Effects of beta rays and X rays on mitotic rate of living grasshopper neuroblasts	J. Tenn. Acad. Sci., 27: 211, 1952
Hunt, Ernest L. and Prentis Baker Huff	Iodine distribution in chick embryos	J. Tenn. Acad. Sci., 27: 204, 1952

Scientific Society Lectures and Traveling Seminars. — The season for Traveling Seminars has come to a close during the quarter, with only fourteen lectures having been offered. One paper was presented at a scientific society — at the Internation Biochemistry Congress in Paris.

Arnold, W. A.	Plant Physiology Dept. University of Tennessee Knoxville	The Quantum-Yield Problem in Photosynthesis
	Carnegie Institution of Washington, Stanford	Phosphorescence and Fluorescence of Green Plants
	Stanford University, Stanford	Same as above
	Hopkins Marine Station Pacific Grove	Same as above
	University of California Los Angeles	Same as above
	University of Southern California, Los Angeles	Same as above
	California Institute of Technology, Pasadena	Same as above
	University of Utah Salt Lake City	Same as above
	Oklahoma Agricultural and Mechanical College, Stillwater	Same as above
Cohn, W. E. and E. Volkin	Second International Congress of Biochemistry, Paris, France	The Products of Ribonucleic Acid Hydrolysis and Enzymolysis
Conger, A. D.	North Carolina State College Raleigh	Biological Effects of Slow Neutrons from an Atomic Reactor
	Blandy Experimental Farm University of Virginia Charlottesville	Same as above
Hollaender, A.	University of Rochester Rochester	Microbiological Effects of Radiation
Kimball, R. F.	Indiana University Bloomington	The Influence of Oxygen Tension and Substrate Production in the Medium on Action of X Rays upon *Paramecium aurelia*
Schwartz, Drew	Emory University Georgia	The Interaction of Nuclear and Cytoplasmic Factors in the Inheritance of Male Sterility in Maize

Visiting Lecturers. The Division has been fortunate in having the following five prominent investigators as guest lecturers on the Biology Seminar Program.

Dr. U. Fano	U. S. Bureau of Standards Dept. of Commerce Washington, D. C.	Present Information on the Distribution of Radiation Energy in Exposed Materials
Dr. Mark W. Woods	Laboratory of Infectious Diseases National Microbiological Inst. National Institutes of Health Bethesda, Maryland	Hereditary and Pathogenic Properties of Mitochondria
Dr. Frederick S. Brackett	National Institutes of Health Bethesda, Maryland	Studies on Photosynthesis
Dr. Savino A. D'Angelo	Department of Anatomy Jefferson Medical College Philadelphia, Pennsylvania	Mechanisms of Pituitary-Thyroid Gland Interplay in the Vertebrate Organism
Dr. M. M. Wintrobe	Department of Medicine Salt Lake City General Hospital University of Utah Salt Lake City, Utah	Factors and Mechanisms in the Production of Red Corpuscles

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball (Leader)
A. D. Conger
K. C. Atwood
A. H. Doermann
Drew Schwartz
Seymour Pomper
C. P. Swanson*
R. T. Brumfield*
Nenita Gaither
Mary Kathryne King
F. H. Mukai
Betty B. Hill
Dorothy W. McKee
Rachel C. Cheniae
L. Roberta Lovelace*
Anna J. Bridgman*

Effect of Oxygen Tension on Genetic and Nongenetic Effects of X Rays on Paramecium aurelia

(Kimball, Gaither, King)

In previous reports (ORNL-989, ORNL-1167) it was stated that low oxygen tension during irradiation led to an increased division delay and nongenetic death but to a decreased mutation rate. The data on mutation at that time were mainly for low doses of a few kiloroentgens, whereas the data for the nongenetic effects were for high doses, between 70 and 350 kr. More recently, more data have been collected on genetic effects at high doses (measured as percentage survival for 4 days after autogamy) and on division delay (measured as rate for completing six divisions expressed as a percentage of the control rate). In each experiment, paired data were obtained by exposing a group of animals at high and one animal at low oxygen tension simultaneously to the same dose of soft X rays. The effect of the difference in oxygen tension was measured by the ratio of the dose at high oxygen tension necessary to give a certain effect to the dose at low oxygen tension needed to give the same effect. Thus a ratio greater than unity means that the radiation produced a greater effect at high than at low oxygen tension.

The dose ratio was estimated on the assumption that the percentage decreased exponentially with dose. This assumption is nearly true for the genetic effect but is considerably in error for the division-rate effect. The latter shows a "multiple-hit" type of curve, but the form of this curve is difficult to estimate in the individual experiments. The assumption of an exponential relation gives ratios which depart from unity more than the true ratios.

*Research Participant

The data for the values of the dose ratio for all experiments at high doses, including those reported in ORNL-989 and ORNL-1167, are given in Table 1. In addition, some details of the experimental procedures

TABLE 1

DOSE RATIOS FOR THE EFFECT OF OXYGEN TENSION OF RADIATION-INDUCED DIVISION DELAY AND GENETIC EFFECTS IN PARAMECIUM

Experiment	Gases used	Exposure method	Dose ratio for division delay	Dose ratio for genetic changes
51.42	N_2 & O_2	*	0.8	—
51.58	"	*	0.5	—
51.63	2% O_2 in He & air	*	0.5	—
51.68	"	*	0.7	1.2
51.70	"	*	1.0	1.1
51.73	"	*	1.0	—
51.77	"	*	1.3	—
51.83	"	*	1.2	1.8
51.89	"	*	1.0	—
51.92	"	*	1.2	—
51.102	N_2 & O_2	**	0.8	1.6
51.108	"	**	0.7	—
51.113	"	**	0.8	—
51.114	"	**	1.4	—
52.66	"	**	0.5	—
52.67	N_2 & air	**	1.3	—
52.68	"	**	1.3	1.6
52.69	N_2 & 20% O_2 in N_2	**	1.1	1.2
52.70	"	**	1.2	—
52.71	"	**	2.6	—
		Mean	1.04 ± 0.11	1.42 ± 0.12

* Animals exposed in small drops on mica under mineral oil (method described in ORNL-989)

** Animals exposed in a hanging drop over a gas chamber

are included. It can be seen that, for division delay, the dose ratio varies considerably and values on both sides of unity are found. This variation is, of course, magnified by the method used to obtain the ratio. At the present, there is no convincing evidence that the average ratio departs significantly from unity. However, there is a suggestion that the ratio is smaller for those experiments in which pure oxygen was used. Further work will be needed to investigate this difference.

The dose ratio for the genetic effect at high doses is significantly greater than unity. However, it is significantly smaller than the value for low doses (2.60 ± 0.39) found in previous experiments. It appears to be

larger than the ratio for division delay. Using only the six experiments in which both effects were studied, a difference, significant at the 5 per cent level, is found.

In summary, there is no clearly established effect of oxygen tension on X-ray-induced division delay in Paramecium although there is some suggestion that a very small effect exists. If it does exist, it seems probable that both low and high oxygen tension decrease the delay. On the other hand, there is a clearly established effect on X-ray-induced genetic changes although the magnitude of the effect is less at high doses.

Effects of Various Treatments on Tradescantia

(Conger)

An increasing interest is being shown in the use of the pollen-tube technique for experimental cytology, particularly since Tradescantia pollen has been found in this laboratory to be suitable for experiments with a fair number of different treatments. Pollen has been grown in the past for various physiological studies on tube-growth rate and tube length, but more extensive studies have been discouraged by death of the tubes prior to the pollen-tube division. A paper is being prepared on the use and technique of growing pollen tubes for experimental cytology. A description of the culture technique will be reserved for later, but Table 2 demonstrates the variety of treatments that the pollen can endure. This is a considerable extension of the only treatments which have been reported previously, namely, X rays (Swanson), ultraviolet (Swanson, Fabergé, and Lovelace), and liquid air (Fabergé).

Studies on Effects of Combined Radiations on Chromosomes of Tradescantia

(Swanson)

Experiments have been initiated to test the effect of temperature on the induction of an infrared effect, with Co^{60} being used as a source of ionizing radiation rather than the customary X rays. So far, temperatures of 3.5°, 9.5°, and 18.0°C have been used, and results have been obtained. Differences in effectiveness of the infrared are apparent at the different temperatures, but more intermediate temperatures must be utilized before the relations can be clearly visualized. One conclusion, however, can be drawn from the data. Unlike X rays, γ rays do not give rise to increases in isochromatid aberrations when combined with infrared as a pretreatment. Whether this can be attributed to differences in ion density of the paths

TABLE 2

TOLERABLE TREATMENTS FOR TRADESCANTIA POLLEN GRAINS AND POLLEN TUBES*

Condition	Range	Comment
	A. On Dry Pollen Tubes	
Temperature	To +40°C, 1 hour	Will survive, but variable
	To -180°C (liquid air)	Pollen enclosed in a sealed chamber
Moisture	"0" per cent, to 8 hours	Dried over desiccant at normal pressure
	100 per cent relative humidity, 1 hour	Saturated air; liquid water on pollen is lethal
Gases		
O_2, N_2, He, Co	100 per cent, to 2 hours	O_2 breaks chromosomes; N_2 for >1 hour begins to kill
O_3	1 per cent, 5 minutes	O_3 breaks chromosomes
HCN (in O_2, N_2)	To 10 per cent, 1 hour	(0.1 per cent HF in O_2 or N_2 kills)
Pressure, vacuum	To 10 atm, any inert gas, 0.1-0.01 mm Hg, 1 hour	10^{-5} - 10^{-6} mm kills in 15 minutes or less
Age (before sowing pollen on slide)	0-10 hours	Fresh is best; variable germination after first day; sometimes viable to sixth day if kept cool and dry
Radiation		
X, γ, neutron, α	To 1500 r	Larger doses tolerable; growth slower with increasing dose
Ultraviolet		
2537 A	To 10^6 ergs/cm^2	>10^6 ergs/cm^2 kills
>2537 A	To 10^8 ergs/cm^2	
Infrared	Large doses	
	B. On Growing Pollen Tubes	
Temperature	12°-30°C, 8 hours	—
Gases (O_2, N_2 flow)	To 8 hours	Gas must be saturated with water vapor
X, γ, ultraviolet, infrared	Various doses	

*Pollen has been grown successfully to pollen-tube division after all the treatments listed.

remains to be determined, and a comparative study of the effects of γ rays, hard and soft X rays, and neutrons on infrared-treated chromosomes is to be made.

Solar radiation should have an infrared component of a spectral quality, and in sufficient magnitude, to cause increases in chromosome breakage when combined with the ionizing radiations. This was tested by giving influorescences a 5-hour exposure at a temperature of 19°C, followed by 100 rep of γ radiation. A 50 per cent increase in chromatid deletions and a 100 per cent increase in exchange aberrations were obtained, thus bearing out an earlier contention (based on experiments done at The Johns Hopkins University) that solar radiation could increase chromosome sensitivity to the ionizing radiations provided that the temperature of the cells was maintained at a temperature below 20°C.

Curvatures in Timothy Roots Induced by Ultraviolet Radiation

(Brumfield)

It was reported earlier (ORNL-807) that curvatures are induced in timothy (Phleum pratense) roots when exposed unilaterally to ultraviolet (2537 A). Negative curvatures (away from the source) are induced by exposure to a dosage of 8 ergs/mm^2/second for 2 minutes. Four-minute exposures to the same intensity result in two curvatures. A "positive" curvature (toward the source) occurs about 20 minutes after exposure, its center being about the same distance (1 mm) behind the root apex as the negative curvature induced by the 2-minute exposure. A negative curvature develops later, about 80 minutes after exposure and is nearer the root apex (0.4 mm) than the positive one.

The hypothesis that these curvatures are mediated through the inactivation of auxin has been under test for the past quarter. Audin (indoleacetic acid), added exogenously prior to irradiation, has been found to modify the curvatures induced. In general, treatment with auxin prior to 4-minute exposures results in weaker positive curvatures and stronger negative curvatures than those found in untreated controls. Treatment with auxin (down to 0.01 mg/liter) temporarily depresses the rate of root growth, and it is known that curvatures in plant organs are due to unequal growth on opposite sides of the organ. Thus it appears likely that the effect of added auxin in modifying the induced curvatures may be due to its effect on growth rather than as a supplement to auxin inactivated by ultraviolet.

The effect of auxin on the elongation of cells at different distances from the root apex is now being studied.

Evidence Supporting Sister-Strand Crossing Over in Maize

(Schwartz, Chenaie)

In a previous report (ORNL-989) a mechanism was described whereby an X-ray-induced ring chromosome involving almost the whole of chromosome 6 is converted into a stable rod chromosome. This conversion arises as a result of crossing over between the ring and its homologous rod chromosome. Dicentric chromosomes are formed which appear as bridges in anaphase (Fig. 1). In the absence of chromatid interference the four

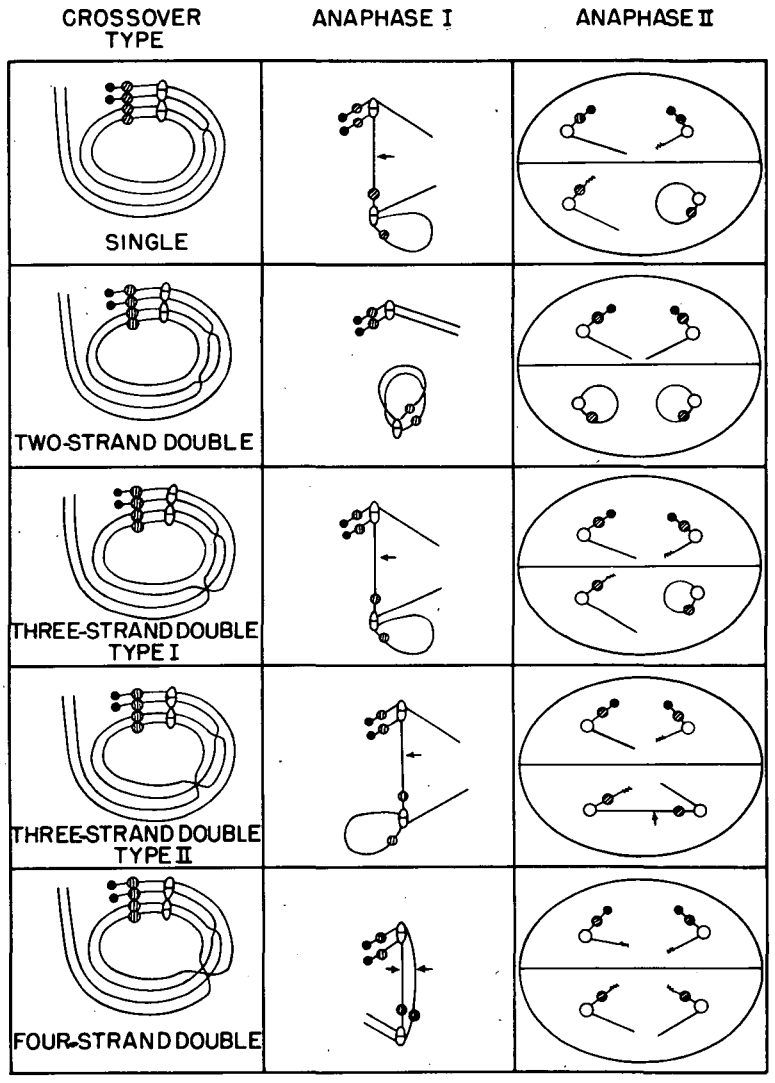

FIG. 1 Anaphase Configurations in Maize as Evidence for Sister-Strand Crossing Over

double crossover types should occur with equal frequencies, i. e., one two-strand: one three-strand (type I); one three-strand (type II); one four-strand. Two of the double crossover classes can easily be distinguished: type-II three-strand doubles which form single bridges in both A_I and A_{II} and four-strand doubles which form double bridges in A_I. Equal frequencies of single bridges in A_{II} and double bridges in A_I would indicate the absence of chromatid interference.

The data as given in Table 3 indicate that single bridges in A_{II} are

TABLE 3

MEIOTIC ANAPHASE CONFIGURATIONS IN PLANTS HETEROZYGOUS FOR A RING AND A ROD

	Anaphase I				Anaphase II (daughter cell pairs)			
	Single bridge	Double bridge	No bridge	Total	Single bridge	Double bridge (dicentric ring)	No bridge	Total
No.	368	81	171	620	166	47	262	475
Per cent	59	13	28	100	35	10	55	100

approximately three times as frequent as double bridges in A_I. A thorough analysis of the data has shown that this discrepancy from the expected 1:1 ratio cannot be explained by chromatid interference and that the frequency of single bridges in A_{II} is not a true measure of the frequency of type-II three-strand double crossovers. Type-II three-strand doubles are responsible for only 13 per cent of the A_{II} single bridges. Some other mechanism must be operating which is responsible for the remaining 22 per cent of A_{II} bridges. This mechanism is sister-strand crossing over. Such crossing over has been postulated previously to explain the occurrence of dicentric double-sized ring formation from monocentric rings (McClintock, Cold Spring Harbor Symposia Quant. Biol., 9: 72, 1941).

A single nonsister crossover between the rod and the ring gives rise to a single bridge in A_I only. However, if this crossover is associated with a sister-strand crossover between the ring chromatids, a bridge in A_{II} as well will result. A sister-strand crossover between the rod chromatids will not form an A_{II} bridge. It is not possible to estimate from the data the number of sister-strand crossovers which occur per bivalent except that there must be one or more to account for the high frequency of A_{II} bridges. One sister-together with a nonsister-strand crossover will give 50 per cent bridges in A_I only and 50 per cent in A_I and A_{II} if there is an equal probability that the former will occur in the ring or the rod. Likewise, a large number of sister-strand crossovers per bivalent will give

50 per cent of each type if there is an equal probability of an odd or an even number occurring in the rod and the ring since even numbers of sister-strand crossovers will cancel each other.

It has been calculated from the data that the frequency of single nonsister crossovers is 33 per cent. As a result of sister-strand crossing over, half of these, or 16.5 per cent, will form single bridges in A_{II}. Thus, 29.5 per cent single A_{II} bridges would be expected on the basis of one or many sister-strand crossovers per bivalent—16.5 per cent from the single and 13 per cent from the double nonsister crossovers. The observed value was 35 per cent. This difference is not statistically significant.

The term "sister-strand crossing over" as used here refers simply to an exchange of chromatin between sister chromatids and does not imply any relation to nonsister-strand crossing over in time and manner of occurrence, interference, etc. The evidence which has been accumulated against sister-strand crossing over from studies on attached-X chromosomes in Drosophila rules out only sister-strand crossing over, which is analogous to nonsister crossing over and shows chiasma interference.

EFFECTS OF RADIATION ON RATE OF MITOSIS

Mary Esther Gaulden (Leader)
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Effect of Oxygen on the Induction by X Rays of Dominant Lethals

(Baker, Von Halle)

Previous work in maize (Schwartz, Proc. Natl. Acad. Sci. U. S., 38: 490, 1952) and in Drosophila (Baker and Edington, Genetics, in press) casts some doubt as to whether the proper explanation of the oxygen effect on chromosome breakage by X rays lies in the assumption that more breaks are produced at higher oxygen concentrations than at lower tensions. From this work it seemed plausible that the oxygen effect is not a matter of differential breakage but is caused by an increased probability of broken ends rejoining when irradiation is performed in lower concentrations of oxygen as compared to higher levels. Since dominant lethals in Drosophila melanogaster are caused mainly by chromosome breakage and since an adequate theory has been developed to explain the behavior of chromosomes involved in dominant lethals, further light might be shed on the oxygen-effect problem by a study of this material.

Three- to four-day-old virgin males of the Oregon-R strain were given various doses of X rays (250 kvp, 30 ma, 260 r/minute) while being maintained at a constant temperature (25 °C) in an environment of either air, 5 per cent O_2 (balance N_2), or pure N_2. After treatment, the males were mated individually to females of the same strain. Twenty-four hours later the males were removed, and the females were allowed to deposit eggs on specially prepared media. The frequency of dominant lethals was determined by making hatch counts of the eggs, 24 hours after they were laid. The experiments were so designed that the dominant-lethal frequency could be obtained under the six conditions of sperm age (between time of irradiation and time of fertilization) given in Table 4.

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†ORINS Fellow

TABLE 4

EXPERIMENTAL DESIGN

Experimental type	Length of time (hours) from		
	Irradiation to insemination	Insemination to fertilization	Irradiation to fertilization
Ia	0-24	0-48	24-48
Ib	0-24	24-72	48-72
IIa	24-48	0-48	48-72
IIb	24-48	24-72	72-96
IIIa*	24-48	0-48	48-72
IIIb	24-48	24-72	72-96

*Type III differs from type II in that the males used in the latter experiments had ejaculated previously to produce the type-I experiments. Males used in the type-III experiments were held without mating for 24 hours after irradiation.

The results of this study involving over 92,000 eggs are shown graphically in Fig. 2. Each of the experimental points has been corrected for the control frequency of dominant lethals which was obtained from experiments run simultaneously with the irradiated group. It is to be noted that the a and b subgroups of each experimental type have been lumped together. This is justified since it was found that the dominant-lethal frequency does not differ significantly in these subgroups. There are two important facts that become evident from these data. In the first place the dose-reduction value from air to nitrogen in the case of the type-I experiments is about 1.5, whereas in the type-II and type-III experiments it is only 1.2. Both these values are much smaller than those previously reported (ca. 2.5) for the oxygen effect on irradiated chromosomes. Second, it is evident that sperm retained in the male from 24-48 hours after irradiation in air and 5 per cent oxygen contain fewer dominant lethals than those retained for a maximum time of 24 hours. However, this effect is not observed with the males which were exposed in a nitrogen environment.

Neither of these facts can be adequately explained on the basis that more initial chromosome breaks are produced in oxygen than are produced in nitrogen. The low dose-reduction factor cannot be attributed to the uniqueness of Drosophila chromosomes because the production of translocations in this form shows a marked oxygen effect (Baker and Edington, loc. cit.). A logical explanation lies in the fact that only a portion of the dominant lethals are caused by nonunion of broken ends, the remainder

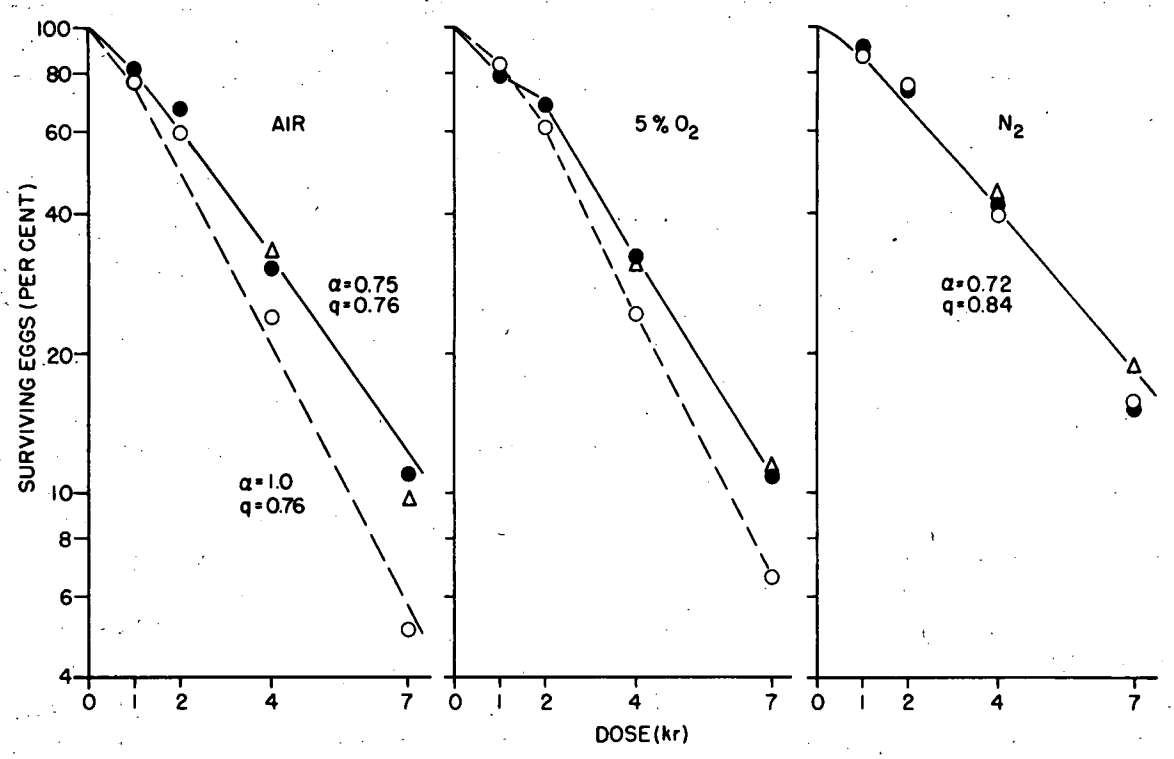

FIG. 2. Dominant-Lethal Frequency in Drosophila Exposed to X Rays in an Environment of Air, 5 Per Cent Oxygen (Balance Nitrogen), or Pure Nitrogen

	Sperm Batch	Age
○	1	0-24
●	2	24-48
Δ	1	24-48

being the result of rejoinings which produce inviable zygotes (e.g., asymmetrical interchanges and large interstitial deletions). Therefore, if irradiation in nitrogen favors the rejoining process, then these rearrangements should be relatively more frequent, thus making the apparent oxygen effect smaller. The second fact mentioned provides even stronger evidence for rejecting the hypothesis of differential breakage. On this hypothesis the difference in frequency of dominant lethals induced in the sperm batches used in experiments I and II would have to be attributed to a decreased sensitivity of the latter sperm at the time of irradiation. That this is not the case is shown by the type-III experiments in which the males were not allowed to ejaculate any sperm for the first 24 hours following irradiation. Thus the sperm used by these males in insemination of females (from 24-48 hours after irradiation) were exactly the same as the sperm used in the type-I experiments. However, the frequency of dominant lethals induced

in these sperm (type III) is the same as that found in males which previously ejaculated (type II). Furthermore, a decreased sensitivity should have affected the nitrogen-treated flies as well as those exposed under the other conditions, but this was not found to be true. It appears that some "recovery" process takes place quickly in males treated in nitrogen but only after a measurable period of time in air.

Once again, these results become explicable on the basis that nitrogen increases the probability of broken chromosome ends rejoining. In Drosophila it is known that no reunion of broken ends to form new arrangements occurs until fertilization, but some investigators have postulated that restitution of broken chromosomes may take place in the sperm prior to syngamy. Since this restitution process evidently is a function of time, little difference would be expected in the frequency of dominant lethals recovered in the two sperm batches of different ages which had been treated in nitrogen, but a measurable difference between the batches exposed in air would be expected if nitrogen favors the rejoining of broken ends, and the only type of rejoining possible before fertilization is restitution.

There are two features of this study which bear further elucidation. From the data it would appear that restitution takes place only in the sperm chromosomes while the sperm is retained in the male. Although work of other investigators has suggested that this is probable, further verification seems in order. In addition, the value obtained for 5 per cent oxygen is much closer to that of air than to that of nitrogen. This suggests that the oxygen-concentration curve for dominant lethals may have a different shape from that of the curves for translocations and recessive lethals.

RADIATION PROTECTION

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Postirradiation Loss of Adenosinetriphosphate from Escherichia coli B/r

(Billen, Stapleton; Strehler, Brigham - Biochemistry)

The effects of X rays on adenosinetriphosphate (ATP) metabolism of Escherichia coli B/r have been investigated in a continuation of our studies on the X-ray-induced physiological changes in the bacterial cell. The ATP was assayed for by use of the firefly luminescent system previously reported (ORNL-889).

The following results were obtained:

1. Exposure to 60,000 r did not alter the initial ATP content of the cells (Table 5 and Fig. 3).

TABLE 5

FORMATION OF ATP BY X-IRRADIATED ESCHERICHIA COLI B/r INCUBATED IN NUTRIENT BROTH PLUS GLUCOSE*

Incubation time (minutes)	ATP content (arbitrary units) Irradiated cells (60,000 r)	Control cells
0	9	11
30	29	22
60	48	56
90	75	67

*In this experiment, 1.0-ml culture samples were used, and the cells were not separated from the medium at the end of the incubation period.

*Consultant

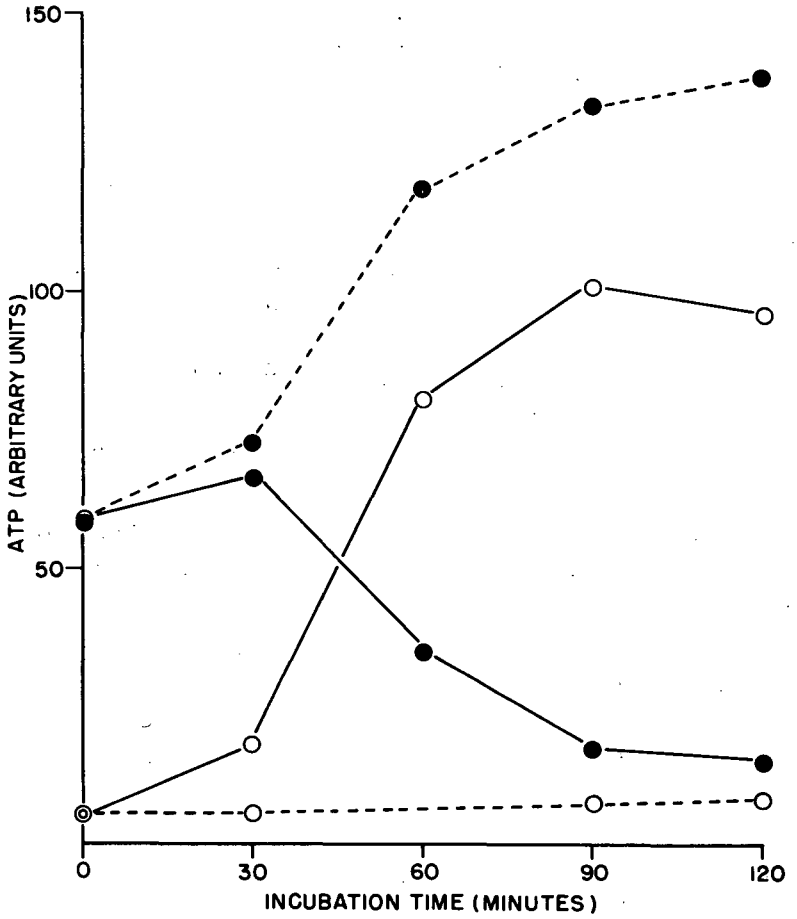

FIG. 3. Distribution of ATP following Incubation of X-Irradiated Escherichia coli B/r in Nutrient Broth plus Glucose

●---● - control cells
●—● - irradiated cells (60,000 r)
O---O - supernatant from control culture
O—O - supernatant from irradiated cell culture

2. The irradiated cells, when incubated at 37°C in a nutrient broth containing glucose, appeared to be capable of forming ATP at a rate approximately equal to that of the control cells (Table 5).

3. Irradiated cells, separated from the incubating medium, showed a decreasing ATP content with increasing incubation time (Fig. 3). An analysis of the supernatant from these cultures revealed an increasing concentration of ATP with increasing time of incubation. No such loss of ATP was noted from the nonirradiated cells.

4. The chemical nature of the medium in which the bacteria were incubated subsequent to irradiation had striking effects on both ATP formation and its distribution between cells and medium. Irradiated cells incubated in M/15 phosphate buffer plus 1 per cent glucose showed a loss of ATP similar to that observed in nutrient broth plus 1 per cent glucose (Table 6). No ATP was found in the supernatant when irradiated cells were incubated in phosphate buffer alone for periods up to 2 hours. The addition of either pyruvate, succinate, or glutamate as a substitute for glucose caused the irradiated cells to lose ATP at rates that varied with metabolite added (Table 6).

TABLE 6

EFFECT OF VARIOUS METABOLITES ON THE X-RAY-INDUCED LOSS OF ATP BY ESCHERICHIA COLI B/r

	Total ATP content (arbitrary units)					
	Cells (min incub.)			Supernatant (min incub.)		
Metabolite added*	0	30	90	0	30	90
Glucose	252	552	484	5	31	342
Pyruvate		710	300		27	1935
Succinate		216	216		13	27
Glutamate			400			108

*Final concentration of metabolite in M/15 phosphate buffer (pH 6.8) was 1 per cent.

5. The rate of loss of ATP by the irradiated cells incubated in nutrient broth plus glucose was decreased if the temperature was lowered below 37°C. Irradiated cells incubated in phosphate buffer plus glucose exhibited a lag in ATP loss, the duration of this lag increasing as the temperature was decreased below 37°C (Table 7).

6. The amount of ATP lost by irradiated cells during 90 minutes incubation at 37°C was found to increase with increasing X-ray dose (Table 8).

7. On the basis of evidence obtained by turbidity measurements, microscopic observations, and cell counts the possibility that gross lysis of the irradiated cells is responsible for the loss of ATP is eliminated. However, the evidence does not eliminate the possibility of a subtle form of lysis or structural change rather than a true permeability change in the cell membrane being the cause of the ATP leakage.

TABLE 7

EFFECT OF (INCUBATION) TEMPERATURE ON ATP LOSS BY X-IRRADIATED ESCHERICHIA COLI B/r

Temperature (°C)	Length of time required for first evidence of a leakage* (minutes)
22	270
27	150-180
32	60-90
37	30

*Cells incubated in M/15 phosphate buffer at pH 6.8 plus 1 per cent glucose.

TABLE 8

EFFECT OF X-RAY DOSE ON ATP LOSS BY ESCHERICHIA COLI B/r

Dosage (r)	Viable cells/ml in sample	Total ATP content of supernatant* (arbitrary units)
0	71×10^7	9.5
1,000	61×10^7	10.0
2,000	55×10^7	11.5
5,000	45×10^7	21.0
15,000	112×10^6	44.0
30,000	266×10^5	98.0
60,000	23×10^4	145.0

*The ATP was determined following 90 minutes' incubation of the cells in a medium of nutrient broth plus glucose at 37°C.

The data suggest that the loss of ATP by irradiated E. coli is a secondary effect of the radiation damage. Two lines of evidence support such an hypothesis: (a) The loss of ATP does not occur immediately after exposure and indeed is undetectable for relatively long periods of time if the cells are incubated at temperatures below 37° C in a phosphate-buffer-glucose solution. (b) The degree of metabolic activity apparently affects the loss of ATP.

It is difficult to determine at the present time whether the loss of ATP is a result of or is a contributing cause of the death of X-irradiated E. coli.

Because of the importance of ATP in assimilatory and other endergonic activities of living cells, its loss could be a vital factor in the physiological changes induced by X irradiation which lead to death of the cell. Further investigations may reveal that other materials equally as important for cell viability and normal physiological functions are lost from the cell.

X-Ray Sensitivity of Escherichia coli during Different Phases of Growth

(Stapleton)

Numerous investigators have reported that young bacterial cells are more susceptible or more sensitive to a variety of deleterious treatments, agents, and conditions than are mature cells. The consensus seems to be that the place of maximal sensitivity is the logarithmic phase of the growth cycle. It was possible to demonstrate that sensitivity to ionizing radiation follows the same pattern as that found for most other potentially damaging treatments.

If a small inoculum of Escherichia coli B/r is transferred from a mature culture (nutrient broth, 0.8 per cent at 37°C with 18 hours of incubation under constant aeration) to fresh medium at the same temperature, the usual multiphasic growth curve is obtained. The lag phase is about 90 minutes, and the mean generation time is about 25 minutes under these conditions. When cells are removed, at various intervals of time, from this growing culture, centrifuged, washed, and resuspended in M/15 phosphate buffer (pH 6.8) in equilibrium with air and irradiated at ice-bath temperature, the survival at a constant-X-ray dose varies with age of cells as shown in Fig. 4. During the lag phase, an increase in resistance is observed which goes through a maximum at the end of the lag phase or the beginning of the logarithmic phase. As the logarithmic phase progresses, the resistance of the cells decays at a rapid rate, paralleling the rate of division, until at the end of the logarithmic phase the cells are found to be more sensitive than elsewhere in the growth cycle. The cells return to the initial resistance during the maximum stationary phase. The reversion to initial sensitivity during this phase would be expected, since the original inoculum was obtained from a previous culture in this phase.

To gain some insight into the cause for these observed variations, it was deemed necessary to know something of the nature of the survival curves for the cells of different ages. Washed cell suspensions of this organism were grown as before and exposed to increasing X-ray doses. The fractions of cells surviving various doses of X rays are shown in Fig. 5. The points plotted are averages for five or six experiments and are subject to about 10 per cent error. It is clear from this figure that at

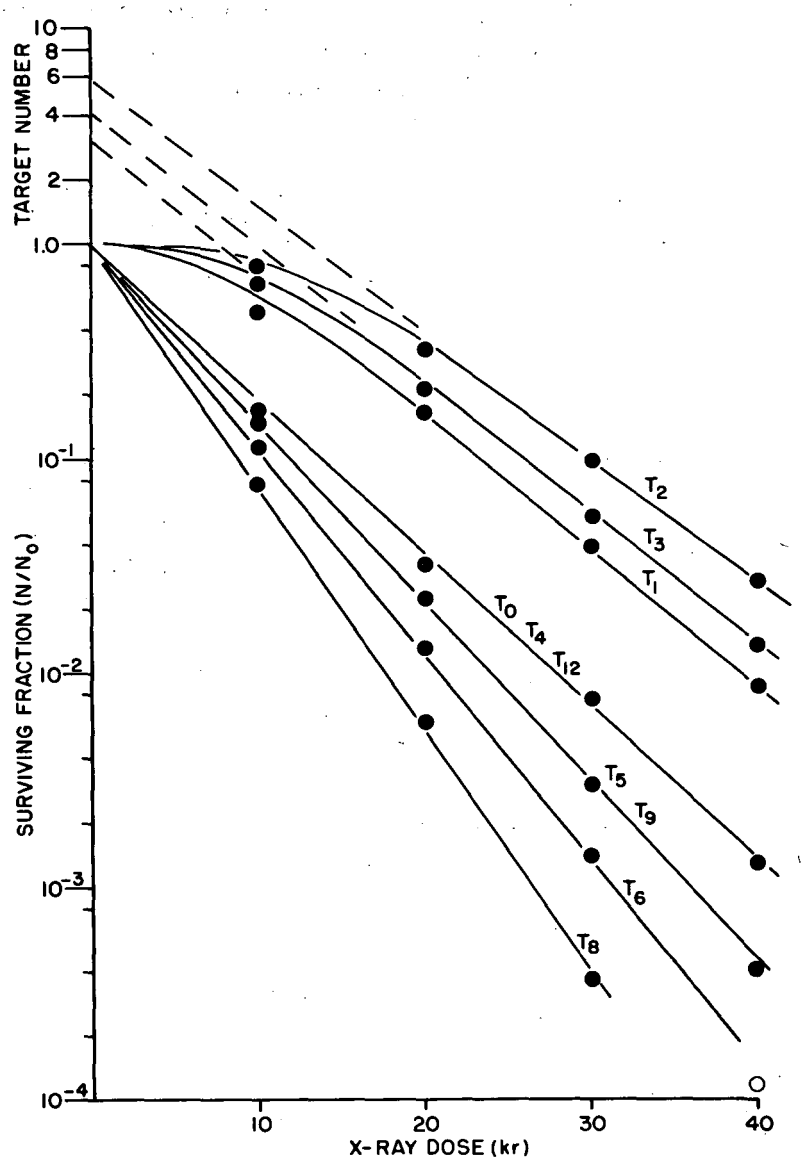

FIG. 4. Survival of Cells of Various Ages As a Function of X-Ray Dose. The age of the cells is indicated at T_1, T_2, etc., representing cells of 1, 2, etc., hours of age. The target number for the cells is shown by extrapolation of the exponential portion of the survival curves as described in the text. Dose rate was 2000 r/minute.

T_0 (time of inoculation) the log survival is an exponential function of X-ray doses. However, during the lag phase the survival curves tend to become more and more sigmoid up to T_2 (2-hour cells). The slope of the curves changes very little. At 4 hours, the cells again yield an exponential survival curve.

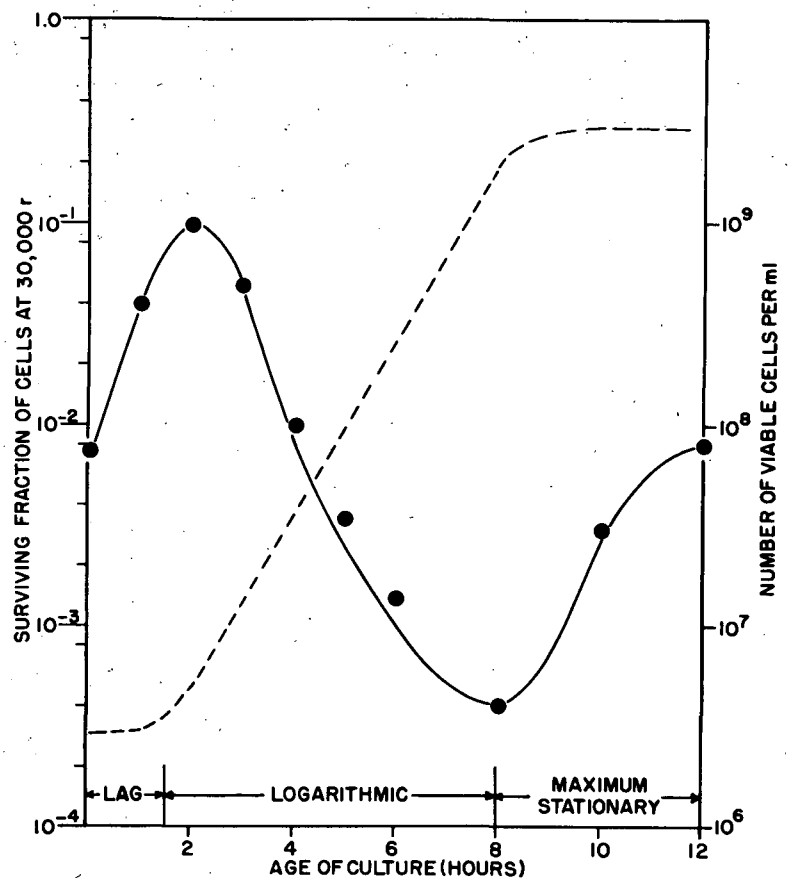

FIG. 5. Survival at a Constant Dose of X Rays As a Function of the Age of the Culture.

Dose rate in all experiments was 2000 r/minute.
— — — growth curve
——— surviving fraction at 30,000 r for cells at various ages indicated on the abscissae

It is of interest that, at all later stages, exponential survival curves are obtained; however, the slopes of the curves increase up to 8 hours, at which time cells are approximately twice as sensitive as were cells at zero time. After this time the slopes of the curves decrease until the original sensitivity is obtained at about 12 hours.

In the light of the cytological observations which have been made on E. coli by Robinow and Knoll and Zapf as well as those of the author, it is tempting to attribute the increased resistance of the lag-phase cells to the multinucleate conditions of these young cells. Actually the average number of nuclear bodies as demonstrable by Robinow's osmic acid-HCl-Giemsa technique compares favorably with the target number determined by extrapolation of the first-order portion of the sigmoid survival curves. Atwood

and Normand have shown similar correlation of nuclear number and target number as measured by this technique with Neurospora conidia. It must be remembered that, since the increase in target number can be correlated also with increase in volume of these cells during the lag phase, increase in amount of any sensitive material which occurs concomitant with the increase in cell volume or nuclear number would be expected to alter similarly the survival curves. Similarly, if the lag-phase cells are in reality multicellular forms, their inactivation would be expected to be other than exponential.

It would appear that increases in slope of survival curves as found for those cells between 4 and 12 hours of age cannot be explained on the basis of further reduction of the nuclear number. When these sensitive cells are incubated at reduced temperature after irradiation, the survival is not essentially different from that obtained for cells at T_0 or T_{12}, whereas at 37 °C the survival is significantly different. This finding suggests that part of the increased sensitivity may be due to an increased thermolability of some vital systems in cells at this stage of the growth cycle. It is of interest that several investigators have shown increased temperature sensitivity for nonirradiated bacteria during this phase of the culture cycle.

Other possible explanations present themselves for both increased and decreased resistance to radiation, some of which are subject to test and will make up a portion of the future research of this group.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

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Mary B. Cupp
Gloria J. Jasny
Elizabeth M. Kelly
Mary H. Major
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Radiation-Induced Mutations in the Mouse

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

It has been stated (ORNL-1244) that since it proved possible with the method used to obtain a reliable induced mutation rate for the mouse at the 600-r dose level (ORNL-989), it seemed desirable to use the same method in an attempt to test whether or not the relation between dose and mutation rate is linear for irradiated spermatogonia in the mouse. A new experiment using a 1000-r X-ray exposure was accordingly started. Several mutations have already been obtained in this experiment. It will, however, probably take a year or two to accumulate and test an adequate number of mutations for a meaningful comparison with the 600-r mutation rate. The results obtained so far give some indication of a departure from linear increase in mutation rate with increase in dose. Although there is only an indication of this at the present time, the curve for mutation rate and dose is of such vital importance in estimating human hazards that it seemed mandatory to begin an experiment at another dose level immediately, rather than to wait for the final outcome of the 1000-r experiment. The dose chosen was 300 r. Irradiations with this dose were begun during the present quarter. This experiment has additional importance in that it carries mutation-rate determination down to a dose level closer to the doses that must be considered in evaluating the genetic hazards of radiation in man.

Effects on Viability of Prenatal Irradiation of the Mouse

(Russell, Russell, Major)

Results of several experimental series dealing with a variety of effects of prenatal irradiation in mice have been presented in past quarterly reports (ORNL-150, ORNL-220, ORNL-318, ORNL-244, ORNL-457, ORNL-644, ORNL-727, ORNL-807, ORNL-889, ORNL-989, ORNL-1244). Only one of these experiments (ORNL-644, ORNL-889) dealt with mortality per se,

but mortality data have been obtained incidentally in most of the other series. Since relatively large numbers are required to provide meaningful information on mortality, it now seems of interest to pool the pertinent data from a number of separate experiments.

Prenatal mortality. — Prenatal mortality may be manifested in the termination of pregnancies and in the death of individuals within surviving pregnancies. The former may be the result of direct radiation death of every embryo in a uterus, or direct radiation death of a number of embryos sufficiently large to exert an adverse effect on the viability of the rest of the embryos in the uterus, or radiation effect on the mother which prevents continuation of the pregnancy.

Table 9 shows the proportion of copulations resulting in observable

TABLE 9

PERCENTAGE OF COPULATIONS RESULTING IN PREGNANCY IN CONTROLS AND FOLLOWING IRRADIATION WITH DIFFERENT DOSES AT DIFFERENT STAGES IN THE PRENATAL DEVELOPMENT OF THE MOUSE

Dose (r)	Observation	Yield of litters following treatment on indicated postcopulation day					
		1/2 - 4 1/2		5 1/2 - 8 1/2		9 1/2 - 13 1/2	
		Pregnancy not diagnosable				Pregnancy diagnosable	
		No. ♀♀ treat.	% w. litt.	No. ♀♀ treat.	% w. litt.	No. ♀♀ treat.	% w. litt.
0	Term	18	72.2	18	72.2	10	100
100	Term	15	46.7	1	100	6	83.3
200	Term	20	45.0	29	69.0	24	87.5
300	Term	—	—	9	0.0	27	96.3
400	Term	—	—	—	—	4	100
0	10 1/2, 13 1/2*	77	79.2	—	—	—	—
200	10 1/2, 13 1/2*	77	55.8	—	—	—	—

*Postcopulation

pregnancies following irradiation with different doses at different stages in the prenatal development of (C57BL x NB)F_1 mice (three experimental series pooled). It may be noted that complete interruption of pregnancy often follows irradiation during preimplantation stages (days 1/2 - 4 1/2) but probably rarely as a result of later irradiation. This is shown in the almost 100 per cent yield from females treated with 200, 300, or 400 r in stages when pregnancy can generally already be diagnosed externally (days 9 1/2 - 13 1/2) and is no less apparent from comparison with controls of those females irradiated with 200 r at a time when it is unknown whether

copulation was fertile (days 5 1/2 - 8 1/2). It should be mentioned that findings not reported in detail here indicate that the loss of entire litters in the groups irradiated on any of days 1/2 - 4 1/2 inclusive occurs before implantation and accounts for the death of about 29 per cent of the irradiated embryos.

Table 10 shows the average size of litters coming to term following irradiation with different doses at different stages in the prenatal development of the mouse. Six separate experimental series have been pooled to give a total population of 419 litters (2667 young). (It should be pointed out here that Table 10, in contrast to Table 9, includes several genetic strains, distributed unevenly among groups, with probable differences in radiation response. It is therefore suitable only for showing broad trends.) Although litter-size data at term are scanty for preimplantation stages (days 1/2 - 4 1/2), a separate experiment involving dissection of uteri and not reported in detail here (for preliminary reports see ORNL-644, and ORNL-889) indicates that, with 200 r, 58 per cent of the individuals in surviving pregnancies die prenatally and that all deaths occur considerably before the halfway point in gestation. In contrast, irradiation of postimplantation stages with 200 r permits an over-all average litter size of 5.94 ± 0.27 at term, i.e., causes a decrease of only 11 per cent from the control mean of 6.69 ± 0.18 ($t = 2.43$). This over-all slight depression is due primarily to the significantly reduced litter sizes of groups irradiated near the beginning of the period, namely, on days 6 1/2, 7 1/2, and 8 1/2 (reduction for these three is 27 per cent; $t = 4.46$). The 300-r data substantiate the 200-r series in again showing sensitivity to prenatal killing of individuals only in the early part of the postimplantation period. Doses of 100 r and below apparently do not affect prenatal viability at all when administered between days 5 1/2 and 12 1/2.

Neonatal mortality. —A pooling of six experimental series (involving different genetic strains) is represented in Fig. 6. This shows that, although 100 r and below has no effect on survival at birth, 200 r applied on any one of days 7 1/2 - 11 1/2 inclusive causes neonatal mortality, with a sharp peak for days 9 1/2 - 10 1/2 (75 and 67 per cent, respectively). Raising the dose to 300 r increases natal mortality in general, the curve paralleling the 200-r curve and the peak reaching 100 per cent (days 9 1/2 and 10 1/2). These data indicate that the slope of the dose-mortality curve for any given stage is steep around 50 per cent lethality. Mortality is markedly dependent on the stage irradiated, even when stages are separated by only 24-hour intervals. For the data presented in Fig. 6, the LD_{50} at birth for irradiation on days 1/2 to 8 1/2 postconception is > 200 r, on days 9 1/2 and 10 1/2 postconception is < 200 r, on day 11 1/2 postconception is between 200 and 300 r, and on days 12 1/2 to 15 1/2 postconception is > 300 r.

TABLE 10

AVERAGE SIZE OF LITTERS COMING TO TERM FOLLOWING IRRADIATION WITH DIFFERENT DOSES AT DIFFERENT STAGES IN PRENATAL DEVELOPMENT OF THE MOUSE

Day irradiated (postconception)	0 r		25 r		50 r		75 r		100 r		200 r		300 r		400 r	
	No. litt.	Litt. size	No. litt.	Litt. size	No. litt.	Litt. size	No. litt.	Litt. size	No. litt.	Litt. size	No. litt.	Litt. size	No. litt.	Litt. size	No. litt.	Litt. size
1/2			—	—	—	—	—	—	3	6.0	1	3.0	—	—	—	—
1 1/2			—	—	—	—	—	—	2	4.0	1	2.0	—	—	—	—
2 1/2			—	—	—	—	—	—	2	5.0	1	1.0	1	2.0	—	—
3 1/2			—	—	—	—	—	—	—	—	4	4.0	—	—	—	—
4 1/2			—	—	—	—	—	—	—	—	4	6.3	1	7.0	—	—
1/2 -4 1/2			—	—	—	—	—	—	7	5.1	11	4.3	2	4.5	—	—
5 1/2			—	—	—	—	—	—	1	8.0	4	6.5	—	—	—	—
6 1/2			—	—	—	—	—	—	—	—	7	5.4	1	1.0	—	—
7 1/2			5	6.0	10	7.0	—	—	19	6.4	11	4.5	1	1.0	—	—
8 1/2			13	6.5	13	5.3	7	7.0	26	6.8	14	4.9	1	1.0	—	—
5 1/2 - 8 1/2			18	6.4	23	6.0	7	7.0	46	6.7	36	5.1	3	1.0	—	—
9 1/2			—	—	—	—	—	—	16	6.4	12	7.0	3	2.7	—	—
10 1/2			—	—	—	—	—	—	11	6.8	9	4.9	4	7.0	—	—
11 1/2			—	—	—	—	—	—	13	7.7	15	6.8	12	5.8	—	—
12 1/2			—	—	—	—	—	—	6	8.7	5	9.0	7	7.7	3	4.0
13 1/2			—	—	—	—	—	—	—	—	—	—	10	7.1	3	6.3
14 1/2			—	—	—	—	—	—	—	—	—	—	9	6.3	—	—
15 1/2			—	—	—	—	—	—	—	—	—	—	6	7.0	—	—
9 1/2 - 15 1/2			—	—	—	—	—	—	46	7.2	41	6.7	51	6.5	6	5.2
	122	6.7														

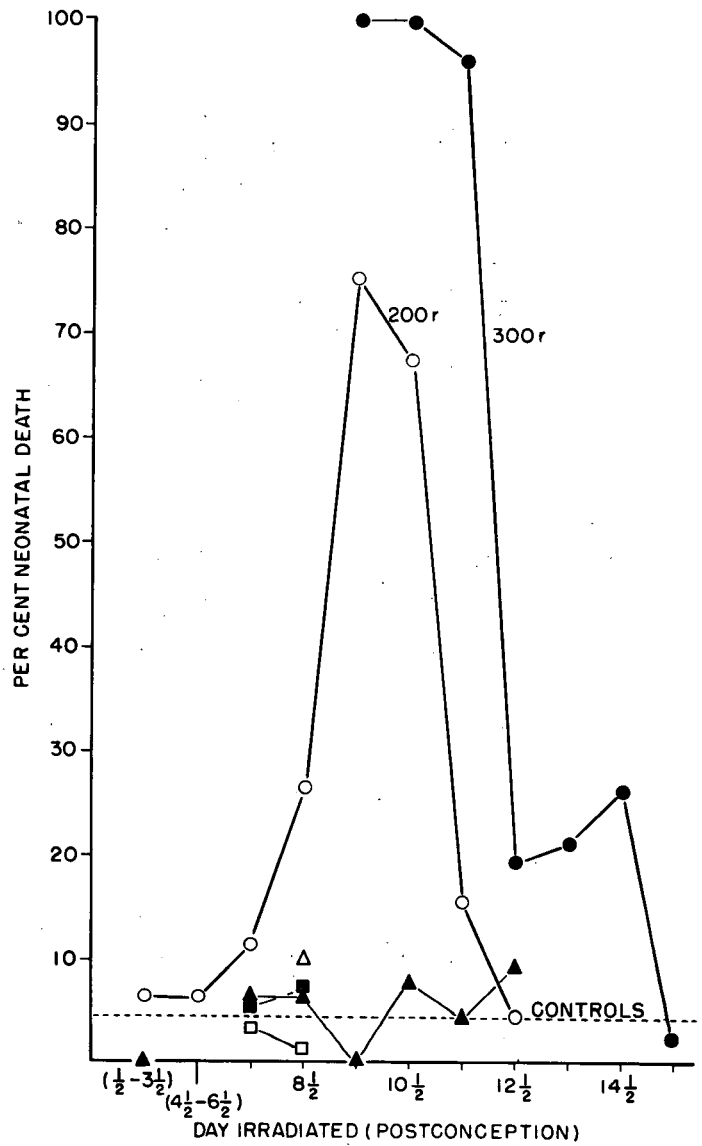

FIG. 6. Incidence of Neonatal Death following Irradiation with Different Doses at Different Stages in the Prenatal Development of the Mouse

● - 300 r Δ - 75 r
○ - 200 r ■ - 50 r
▲ - 100 r □ - 25 r

Summary. —Prenatal mortality, both through termination of pregnancies and through death of individuals within surviving pregnancies, is exceedingly high following irradiation of preimplantation stages. Irradiation during postimplantation stages causes little or no termination of pregnancy and considerably fewer deaths of individuals than does treatment during the preimplantation period. Furthermore, within the postimplantation period, susceptibility to prenatal killing decreases fairly rapidly with embryonic age.

The effect of irradiation at various embryonic stages on neonatal viability does not parallel that on prenatal viability. Thus the highest rate of neonatal death follows irradiation on days 9 1/2 and 10 1/2 postconception, whereas irradiation during preimplantation stages, which has such drastic consequences prenatally, does not affect the viability of those animals which survive to term.

The information presented in this report is roughly summarized in Table 11.

TABLE 11

EFFECT ON VIABILITY OF PRENATAL IRRADIATION OF THE MOUSE

	Day postconception irradiated			
	1/2 - 4 1/2	5 1/2 - 8 1/2	9 1/2 - 10 1/2	11 1/2 - 13 1/2
Effect on prenatal viability	++++	++	+	0 - +
Effect on neonatal viability	0	+	++++	0 - +

PATHOLOGY AND PHYSIOLOGY

PATHOLOGY AND PHYSIOLOGY

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Effect of Platelet Transfusion on the Hemorrhagic Phase of Radiation Injury in the Rat

(Woods, Gamble)

The ability of homologous platelets to decrease the numbers of erythrocytes appearing in the thoracic lymph of X-irradiated dogs has been detailed in a previous report (ORNL-1297). The experimental techniques have been successfully established in the Wistar rat, and the earlier observations have been confirmed with respect to the erythrocytes in the mesenteric lymph after platelet transfusion. Figure 7 includes the observations obtained in five experiments in which pairs of animals (matched for weight, age, X-ray exposure, and time after irradiation) were studied simultaneously. Fresh rat platelets were injected intravenously into the experimental animals in sufficient concentration to cause a marked rise in platelet level at a time when the level was greatly depressed by irradiation and a further drop was to be anticipated. The controls received an equal volume of the plasma in which the platelets were suspended. All animals were sacrificed after 18 hours' observation with the exception of pair No. 5(in this experiment, five attempted controls died within 3-6 hours postoperation). At autopsy a diminished hemorrhagic tendency was usually discernible in the platelet-injected animals, principally in lymph nodes.

Effects of X Radiation on the Permeability of Connective Tissue As Measured by the Dermal Spreading of Evans' Blue

(Upton)

Increased vascular permeability is one of the important acute effects of ionizing radiation; its pathogenesis, however, is obscure. In view of the regulatory role of the interendothelial cement in controlling capillary

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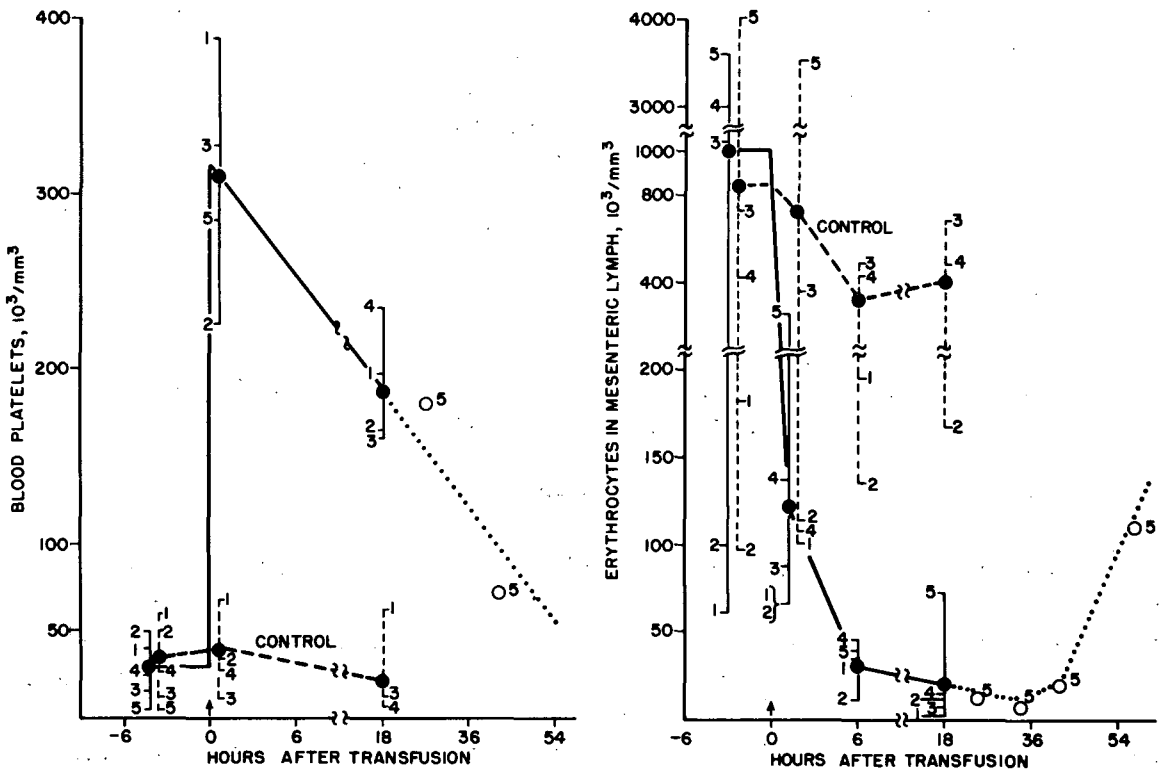

FIG. 7. Blood Platelet and Erythrocyte Levels in the Mesenteric Lymph of Platelet-Transfused Irradiated Rats. Transfusion 9-12 days postirradiation at 600-750 r. Figures beside each observation refer to the number of the experimental pair. Time intervals are arbitrarily plotted as follows: intervals 4-6 hours after platelet injection, observations within 30 minutes of plotted time; all other intervals, observations within 2 hours of plotted time.

—— (vertical) - range of observations in platelet-injected animals
– – – (vertical) - range of observations in plasma-injected controls
—— - interpolated mean curve in platelet-injected animals
– – – - interpolated mean curve in control animals
····· O - curve drawn on basis of single observations
● - average of observations

permeability, investigation of the effects of irradiation on this material was undertaken. Among the initial steps was a study of the modification by X radiation of the spreading of dye in the skin since the permeability of the dermis parallels in many respects that of the capillary wall and is dependent on the permeability of the intercellular ground substance.

The observations made thus far (Figs. 8 and 9) indicate that the permeability of the dermal barrier is markedly increased by whole-body irradi-

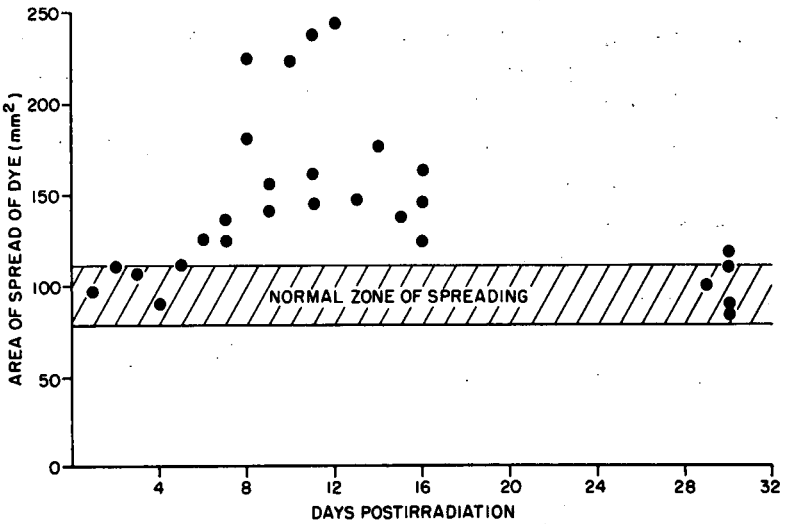

FIG. 8. Dermal Spreading of Evans' Blue in Female Rats after X Irradiation. Each point represents area of spreading of 0.5 ml of 0.5 per cent Evans' blue in distilled water 4 hours after intradermal injection. A separate rat was used to determine each point.

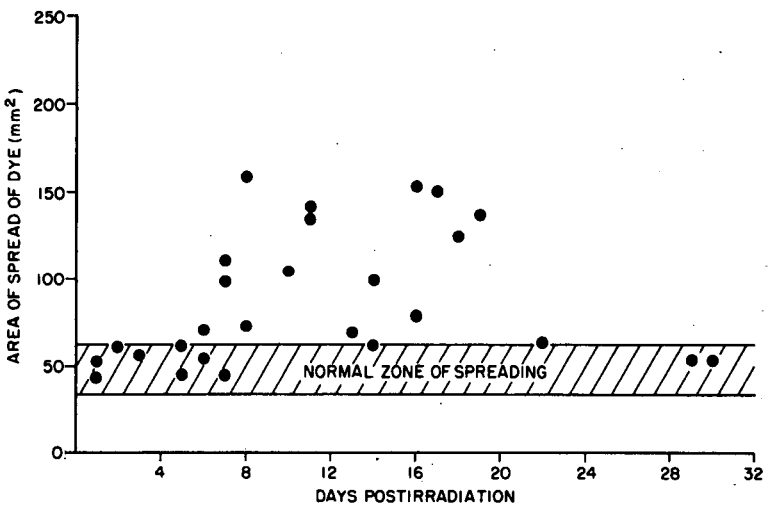

FIG. 9. Dermal Spreading of Evans' Blue in Male Rats after X Irradiation. Each point represents area of spreading of 0.5 ml of 0.5 per cent Evans' blue in distilled water 4 hours after intradermal injection. A separate rat was used to determine each point.

ation, beginning 5-7 days after exposure. This effect appears maximal during the second week postirradiation; by the thirtieth day there is evidence of recovery.

The finding of increased dermal spreading of dye postirradiation confirms earlier observations of Painter and associates (Proj. Report CH-3727), who established the heightened dermal spreading of Evans' blue in rabbits after local irradiation of the skin. The present study demonstrates that this change occurs after whole-body irradiation and coincides in time with the hemorrhagic phase of capillary permeability, as determined previously in this laboratory in rats of the same stock under identical experimental conditions. The enhanced spreading of dye can be attributed to an increase in the permeability of the ground substance of dermal connective tissue. The possibility of analogous changes in the interendothelial cement of the capillary will be investigated.

Qualitative Relation between Thyroid Function and Induction of Pituitary Tumors

(Burnett, Gadsden, Gude)

The thyroids of mice were destroyed or depressed with graded doses of I^{131}, and the susceptibility of these mice to grafts of pituitary tumors was compared with the histological appearance of the thyroid gland and its ability to synthesize thyroid hormone.

Figure 10 shows the results in one of seven experiments of this type,

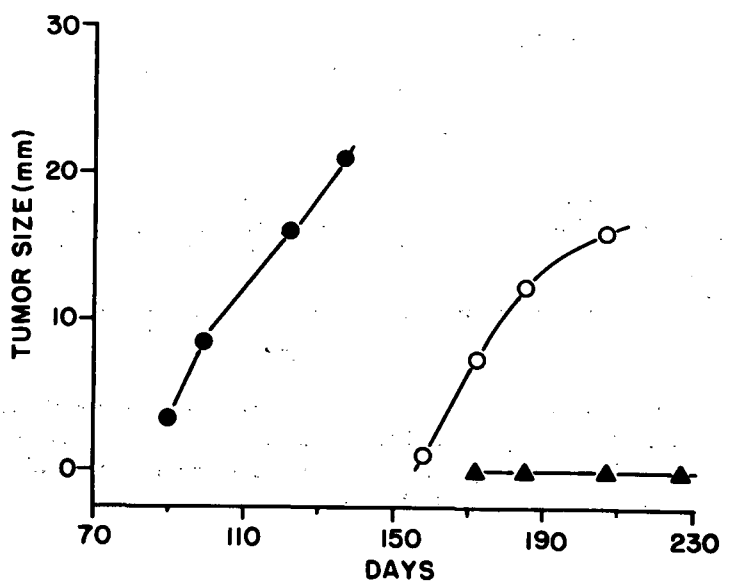

FIG. 10. Tumor Growth in Mice Pretreated with I^{131}.
● - 225 μc o - 75 μc ▲- 25 μc

all indicating that complete destruction of the thyroid function (~225 μc) is not necessary to render mice susceptible to grafts of pituitary tumors; a mere depression of thyroid function (~75 μc) will accomplish this. A survey of all experiments performed indicates a quantitative relation between depression of thyroid function and stimulation of growth of pituitary cells.

In order to quantitate thyroid function, tracer doses were given to mice whose thyroid was depressed or destroyed by I^{131}. Some of these mice carried grafted pituitary tumors; others served as controls. The I^{131} retention in the body and thyroid was followed for 5 days with results illustrated in Figs. 11-13.

At intervals of 24 hours the radioiodine remaining in the whole animal was estimated by the 100 per cent geometry γ chamber described by Jones and Overman. The radioiodine localized in the thyroid was estimated by a scintillation counter having a thallium-activated sodium iodide crystal.

Aluminum was interposed between the source and crystal so that only the γ rays of iodine were counted. The mouse was placed at a distance of 3.5 inches from the crystal with 2 inches of lead shielding with a cylindrical hole 3/4 inches in diameter. The error introduced by the fact that the activity in the entire thyroid region was also counted can be corrected for by laborious calculations, but this seemed unnecessary in the present study.

Having established that a 24-hour retention of tracer doses of I^{131} is adequate to evaluate thyroid function, numerous mice of the stated groups were given tracer doses and were killed after 24 hours, and the functional capacity and morphological appearance of the thyroid glands were correlated with ability of the gland to stimulate proliferation of pituitary-tumor cells. The results are summarized in Table 12.

The results of these experiments show further that radiothyroidectomy itself has a marked enhancing effect on the 24-hour retention of I^{131} and that pituitary tumors grafted in a leg muscle of mice have a further enhancing effect on uptake of radioiodine. Although this effect of radiothyroidectomy is transient, that of the pituitary tumors is enduring.

It had been thought that the radioiodine might localize in the tumors, resulting in a higher total retention particularly in those animals whose thyroid had been previously destroyed by radioiodine. Both estimations in vivo with the scintillation counter and measurements of the radioiodine in tumors made with the γ chamber at autopsy showed that the localization of radioiodine was no greater in tumors than in muscles. Throughout the experimental period a higher I^{131} retention was observed in normal mice bearing large tumor grafts than in those bearing minute tumor grafts, in

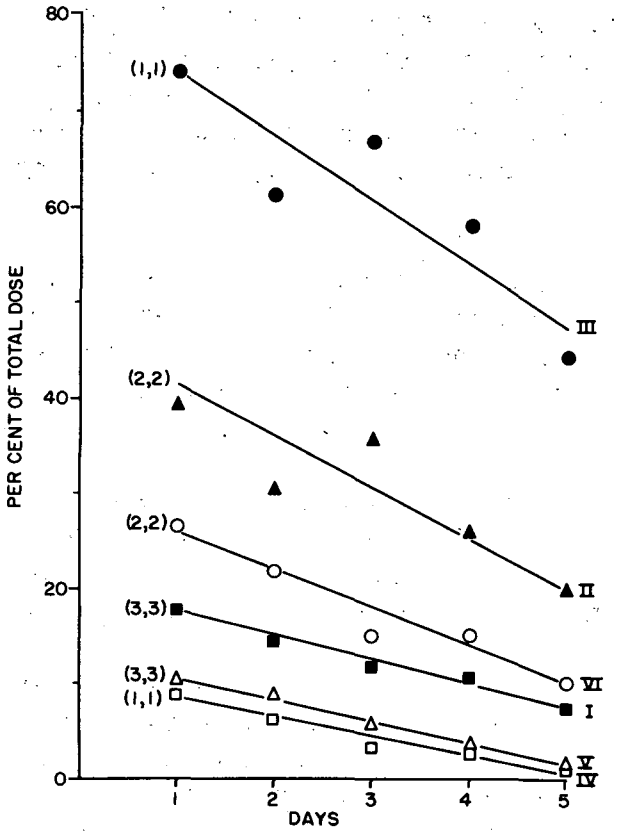

FIG. 11. Radioiodine Retained in Thyroid Region

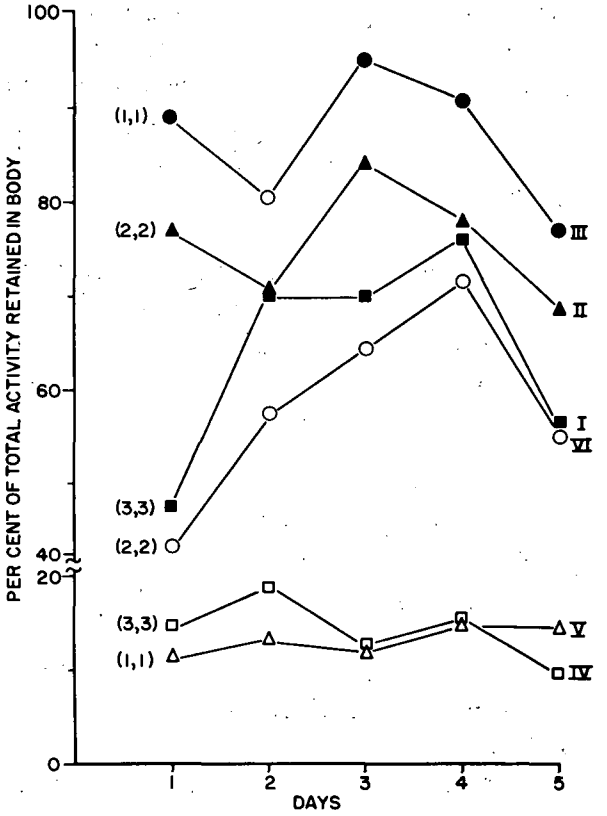

FIG. 12. Radioiodine Retained in Thyroid Region

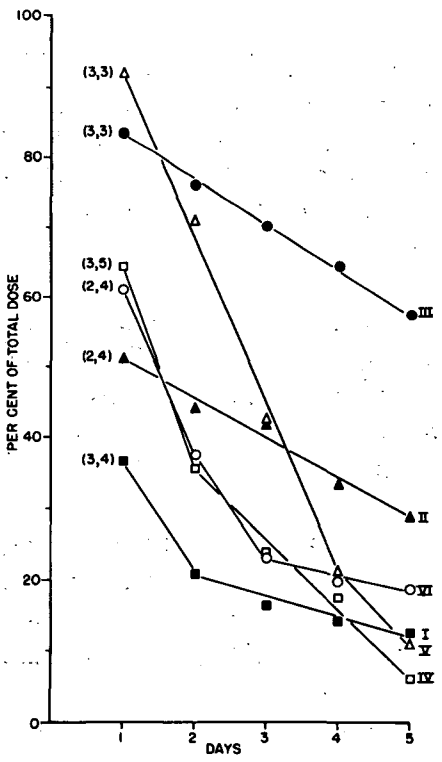

FIG. 13. Radioiodine Retained in Body

FIGS. 11-13. Radioactivity in Normal and Radioiodine-Pretreated Tumor and Nontumor-Bearing Male Mice following a Small Dose (~10 μc) of I^{131}. The first number in the parentheses indicates the number of mice in the group. The second number indicates the number of tests in the pooled data. In about half the cases, two consecutive doses were given to a mouse at an interval of 10 days.

I - normal nontumor-bearing mice
II - normal mice bearing minute tumor grafts
III - normal mice bearing large tumor grafts
IV - I^{131}-pretreated (~300 μc) nontumor-bearing mice
V - I^{131}-pretreated (~300 μc) mice bearing large tumor grafts
VI - I^{131}-pretreated (~ 25 μc) mice bearing minute tumor grafts

TABLE 12

SURVEY OF EXPERIMENTAL RESULTS CORRELATING TUMOR GROWTH WITH I^{131} PRETREATMENT, THYROID FUNCTION, AND MORPHOLOGY

Group	I^{131} pre-treatment (μc)	Tumor size (mm)	Thyroid		% of I^{131} retained in 24 hours		
						In thyroid	
			Size	Stimulation	In body	% Injected	% in body
I	—	—	Normal	Normal	29.6	17.4	62.3
II	—	1-3	Moderately to greatly enlarged	+++	73.0	68.1	92.2
III	—	10-30	Greatly enlarged	+++	73.7	67.7	91.8
IV	300	—	—	—	53.0	0.0	0.0
V	300	10-30	—	—	76.0	0.0	0.0
VI	25	1-10	Normal to slightly enlarged	+	49.1	31.8	61.4
VII	75	1-10	Small to slightly enlarged	+	48.5	34.7	71.4
VIII	75	10-25	Small	+	63.1	4.3	10.6

both the thyroid region and the entire body. This relation is obvious since the tumors exert this I^{131}-retaining effect by discharge of TSH. The presence of the latter has been ascertained in numerous bioassays made in cooperation with Dr. Evelyn Anderson (U. S. P. H. S., Bethesda) and Dr. J. N. Dent (University of Virginia) and will be more fully described later.

These investigations point to a quantitative relation between thyroid depression and stimulation of pituitary cells and indicate the inability of thyroid gland partially injured by I^{131} to undergo full homeostatic compensatory hyperplasia.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

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E. F. Phares Suzanne Berger
M. I. Dolin J. M. Adams*
Mary V. Long E. A. Delwiche**

Propionic Acid Fermentation

(Carson, Dolin, Delwiche, Phares, Long, Berger)

Studies have been continued on the problems of terminal metabolism of propionate and succinate by cell-free extracts of propionic acid bacteria. These extracts obtained by grinding with alumina have been found to activate both succinate and propionate in the presence of adenosinetriphosphate (ATP) as measured by the hydroxamic acid reaction. The addition of hydroxylamine after the incubation period as compared to its inclusion during the incubation period has resulted in the finding that the activation is occurring only during the presence of hydroxylamine, indicating the necessity for an acylacceptor. With the demonstration of a coenzyme-A dependence as described subsequently, the existence of the CoA-dependent ATP activation is strongly suggested. Treating the cell-free extracts with ion-exchange resin (Dowex-1) resulted in an almost complete elimination of the ability to activate both succinate and propionate. Activity could be completely restored by the addition of highly purified coenzyme A. Boiled extracts were assayed for coenzyme A by the use of the resin-treated extract. The extracts as prepared by the alumina grinding technique were found to contain approximately 150 units of coenzyme A per milliliter.

A comparison of propionic acid bacteria and Veillonella with Streptococcus faecalis as concerned the ability to activate acetate, propionate, and succinate revealed that S. faecalis, although capable of the activation of acetate and propionate, could not activate succinate. The comparative observation strengthens the hypothesis that the propionic acid bacteria (and Veillonella) do actively employ the ATP-CoA-dependent reaction as a main mechanism in the oxidation of propionate and in the anaerobic conversion of succinate to propionate and carbon dioxide.

In the experiments in which the carboxylic acid activation was measured by the hydroxamic acid method, it has been observed that formate apparently undergoes, to a limited degree, a similar type of activation.

*ORINS Predoctoral Fellow
**Research Participant

Investigations concerning transacetylase content were made by measuring sulfanilamide acylation. The data indicate that transacetylase is of a low order of activity. Sulfanilamide acylation with acetate, propionate, and succinate along with ATP occurred, thereby providing additional evidence for the existence of acyl-CoA intermediates, the existence of which was strongly indicated by the finding in the Dowex-treated preparations of the CoA-ATP-dependent activation of these fatty acids.

The systems described did not produce measurable amounts of carbon dioxide from succinate in the Warburg apparatus. In order to test the possibility that a CoA-dependent partial reaction was taking place without evolution of carbon dioxide but with formation of propionate, C^{14} labeled succinate was employed as a substrate. Succinic acid-2-C^{14} was incubated with the Dowex-treated cell extract without added coenzyme A and with substrate amounts of coenzyme A, an excess of ATP being present in both cases. Based on recovered activity in the propionic acid, the conversion of succinic acid was about 3 per cent in the reaction with no added coenzyme A and, contrary to expectations, much less in the case of added coenzyme A. The reaction is being studied further to determine the nature of this coenzyme-A effect.

α-Keto Acid Oxidation by *Streptococcus faecalis*

(Dolin, Long)

The investigation of α-keto acid oxidation, begun at the University of Illinois, is being continued in order to define further the separate steps involved and to determine the function of lipoic acid in this reaction.

Previous work (Dolin and Gunsalus, Federation Proc., 11: 203, 1952) has shown that soluble extracts from *Streptococcus faecalis*, 10C1, carry out the oxidation of pyruvate to equimolar amounts of acetyl phosphate, lactate, and carbon dioxide and of ketobutyrate to propionyl phosphate, α-hydroxy butyrate, and carbon dioxide. Lipoic acid is required for both these reactions with apopyruvate dehydrogenase cells. The cell-free extracts can be separated into two enzymatic components which, together with lactic dehydrogenase and transacetylase and in the presence of cocarboxylase, DPN, CoA, Mn, orthophosphate, and cysteine or glutathione, will reconstruct the dismutation system. The mechanism of the reaction was partially defined from the nature of these requirements and by comparison to an analagous system in *Escherichia coli*.

It has now been found in experiments, using synthetic DL-lipoic acid, that diacetyl oxidation by *S. faecalis* is a lipoic-requiring reaction, whereas aldehyde oxidation is not. This suggests that lipoic acid may be involved

in the splitting of the $\overset{O}{\overset{\|}{-C}}-\overset{O}{\overset{\|}{C}}-$ bond with the formation of active aldehyde. Both diacetyl and keto acid oxidation may be linked to ferricyanide reduction. Ferricyanide is known to oxidize thiols such as glutathione and cysteine rapidly and spontaneously. Since lipoic acid is a dithiol and since inhibitor studies (Gunsalus, Biology Research Conference, Oak Ridge, April 1952) show the dithiol portion of lipoic to be involved in keto acid oxidation, the ferricyanide experiments make it probable that lipoic acid is acting as a hydrogen transport catalyst. Moreover, since ferricyanide oxidizes a component of the acyl-transfer system (cysteine or glutathione) and probably the SH on CoA itself, acyl-CoA reactions must be by-passed in the ferricyanide system, which places the locus of lipoic acid function somewhere between cocarboxylase and CoA.

From the nature of the lipoic acid molecule, a further function could be in acyl-transfer reactions via the thio-acetyl linkage. A possible lipoic-requiring system would be the activation of fatty acids by ATP through a mechanism analogous to Lipmann's CoA-pyrophosphate reaction (Lipmann, Biology Research Conference, Oak Ridge, April 1952). This aspect of the reaction was investigated with both vacuum-dried cells grown to be deficient in lipoic acid and with cell-free extracts. The activation of acetate and propionate in such systems was independent of both CoA and lipoic acid.

BIOCHEMISTRY

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

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J. X. Khym
E. Volkin
Fred Vaslow
B. L. Strehler
M. Helen Jones
G. M. Cheniae
Elizabeth H. Brigham

Enzyme-Substrate Equilibria

(Vaslow)

Carboxyl derivatives of tyrosine show a maximum sensitivity toward chymotrypsin at about pH 6 in comparison to N-acetylated derivatives which show their maximum sensitivity between pH 7 and 8. The binding of dibromo-L-tyrosine has accordingly been measured to determine the differences in binding properties between tyrosine and N-acetyl tyrosine. The results again show that there is a correlation between the thermodynamic functions of binding and the enzyme activity. The tyrosine system reaches its minimum values of -4000 cal/mole and -9.5 eu for the heat and entropy of binding at pH 6 instead of pH 7.5 for the acetyl tyrosine system. Above pH 6 the binding and thermodynamic functions of binding for dibromo-L-tyrosine go rapidly to 0; the demonstration of such a change is not possible in those systems having minima at higher pH values. Although the interpretation of these differences in binding is not at all clear, this appears to be further proof that the quantities being measured are intimately involved in the catalytic process rather than being coincident with an acid ionization of any of the components of the system.

Nucleic Acid Structure

(Volkin, Khym, Jones)

Two new developments have come forth which should make the identification of the phosphoryl position of a and b mononucleotides possible. The first is the development of an ion-exchange method of separation of borate-complexed sugar phosphates (Khym, in press). The second is the isolation of an enzyme preparation first described by Komita (Tokyo J. Biochem., 25: 405, 1937), which produces intact ribose phosphate from purine nucleotides. Thus, by mild degradation of guanylic acid, two ribose phosphate isomers have been clearly separated by the ion-exchange technique, both of which are different from the 5' ribose phosphate. The two

isomers have been obtained from hydrolysis of the commerical nucleotide preparation and from degradation of the pure a or pure b compounds, a result to be expected on the basis of intermediate acid-catalyzed migration of the phosphoryl group. Preliminary experiments involving enzymic degradation of pure a and b guanylic acids indicate that the first ribose phosphate peak is obtainable from the a nucleotide, the second from the b. From consideration of the probable structure of the borate complexes of the 2' and 3' ribose phosphates, it seems probable that the more readily eluted compound is the 2' ribose phosphate, the second being the 3' isomer. It is our intention first unequivocally to identify the correspondence of the sugar phosphates with the proper isomeric mononucleotide precursor, then prepare enough of each of the sugar phosphate isomers to effect a chemical identification with respect to phosphoryl position.

Nucleotide Studies

(Cheniae, Doherty)

The conditions necessary for the methylation of ribonucleic acid were investigated in an effort to improve the yield of methylated product and to decrease the amount of degradation. The best procedure gave a product which on ultracentrifugal analysis, was shown to have a fraction with a molecular weight of 5000-10,000. Ion-exchange chromatography on Dowex-1 (Fig. 14) revealed that the major products of methylation were

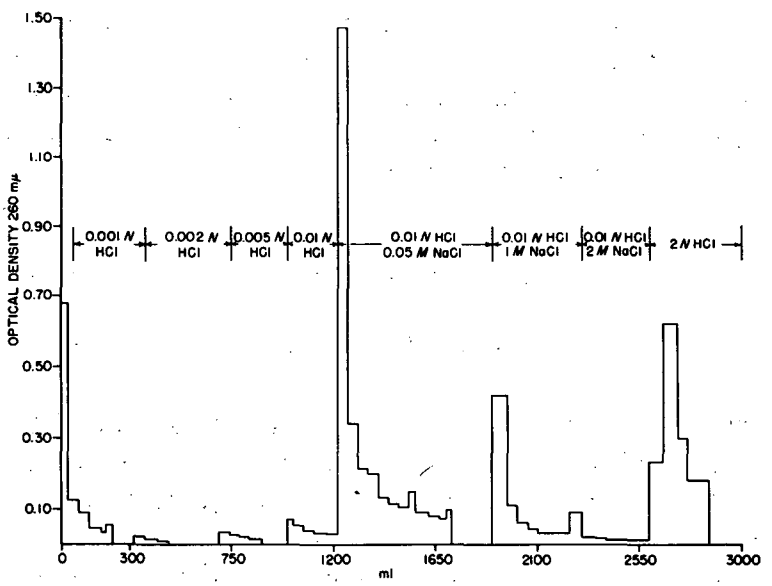

FIG. 14. Ion-Exchange Chromatography on Dowex-1 + Cl^-, Methylation of Ribonucleic Acid. Calf-liver RNA + Ag_2O + CH_3I

tetranucleotides or larger with about 20 per cent of the high-molecular-weight fraction. All the products had an ultraviolet absorption peak of 270 mμ compared to 260 for the starting material. Large-scale isolation of this large-molecular-weight fraction is in process at the present time. Although current theories of nucleotide structure propose that substituted nucleic acids should be resistant to alkaline hydrolysis, the methylated product mentioned was completely degraded to mononucleotides by alkali. Methylation of intact ribonucleic acid with diazomethane yielded products similar to the $AgO-CH_3I$ methylation.

Glyconyl Peptide Studies

A series of glyconyl peptides with various configurations were prepared in order to check their susceptibility to enzymatic action. Included in this work were the following aldonic acids: D- and L-galactonic, L-gulonic, D-xylonic, D-lyxonic, D- and L-erythronic, and D-threonic; and the following amino acids: tyrosine, glycine, phenylalanine, glutamic acid, leucine, and alanine.

PLANT PHYSIOLOGY

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Preparation of C^{14}-Labeled Sugars by Ion-Exchange Chromatography

(Noggle, Schumacher)

Previous reports (ORNL-989, ORNL-1026, ORNL-1167, ORNL-1244, ORNL-1297) have indicated the applicability of separating the borate complexes of carbohydrates by anion-exchange chromatography. Work on this problem has followed two main lines: (1) the isolation and preparation of C^{14}-labeled carbohydrates and (2) the quantitative analysis of carbohydrates in extracts of biological origin. The principles of the latter problem have been completed. The problem relating to the isolation and preparation of C^{14}-labeled carbohydrates is also completed, and the essentials are here reported.

Leaves of pokeweed (Phytolacca decandra) were removed from field-grown plants and placed in the dark for 40 hours. The leaves (70 g fresh weight) were then exposed to 2.5 mc of C^{14} as $C^{14}O_2$ in a chamber for 20 hours at a light intensity of approximately 700 ft-c. At the conclusion of the illumination period the leaves were cut up and dropped into boiling 80 per cent ethanol for 30 minutes. The alcohol was decanted from the leaves, fresh 80 per cent ethanol was added, and the leaves were then macerated in a Waring Blendor for 10 minutes. The macerate was filtered and washed with ethanol. The insoluble residue was extracted for starch (175 mg isolated, 1.7 μc/mg). The alcohol was removed from the alcohol extract, after which the solution was deproteinized, deionized, and made up to a volume of 100 ml. An aliquot of the sugar solution was analyzed by the ion-exchange procedure, and 61.5 mg of sucrose, 10.8 mg of raffinose, 67.1 mg of fructose, and 84.7 mg of glucose, plus at least two other carbohydrate fractions at present unknown, were found. One is suspected of being a saponin since this fraction frothed and foamed during its isolation. Another aliquot (20 ml) was then separated by anion exchange on a Dowex-1 column (4.9 cm^2 x 10 cm). The four major peaks (sucrose, raffinose, fructose, and glucose) were pooled separately. Each pooled sample was shaken with Dowex-50 (hydrogen form) to remove the potassium ions, leaving

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a solution of the sugar in boric acid. It is essential that the potassium ions be removed quickly because the sugars are unstable in alkaline solution of potassium tetraborate over long periods of time. Boric acid was removed by the following procedure: The solutions were concentrated to a volume of approximately 50 ml. At this stage, boric acid begins to crystallize out of solution. Absolute methyl alcohol was then added (100 ml), and the solutions were vacuum distilled (water pump) at 30°C. The methyl borate was redistilled and removed at this operation. After about three distillations with methyl alcohol all the boric acid has been removed and the sugar remains in the flask. The sugar was taken up with water, treated with a mixed anion and cation resin (Amberlite 120 and 400), and then filtered. Samples were then combusted for radioactivity measurements with the following results: sucrose, 1.89 μc/mg; raffinose, 0.90 μc/mg; fructose, 1.78 μc/mg; and glucose, 1.27 μc/mg.

Manipulation necessary to free the solutions of boric acid resulted in some loss of sugar. Recovery experiments have indicated that 92 per cent of sucrose, 90 per cent of glucose, and 85 per cent of fructose were recovered.

Isolated sugars were examined by paper chromatography, and autoradiographs of the papergrams indicated that the sugars were radiochemically pure.

Separation of Sedoheptulose, Sedosan, Glucoheptulose, Galaheptulose, and Mannoheptulose on Ion-Exchange Columns

(Noggle, Schumacher)

Experiments under way to investigate the role of heptuloses in plants made it desirable to study a number of different heptuloses that might be encountered. Through the courtesy of Dr. Nelson K. Richtmyer, samples of sedosan, glucoheptulose, galaheptulose, and mannoheptulose were obtained. The following report considers a number of properties of these heptuloses.

Paper chromatography. — The heptuloses were dissolved in water to give a concentration of 5 mg/ml. Sedosan (the anhydride form of sedoheptulose) was dissolved in 1 per cent hydrochloric acid and heated in a steam bath for 30 minutes. This gives a mixture of sedosan and sedoheptulose (80:20). The acid was removed with Dowex-3 (hydroxyl form), and the final concentration was 8 mg/ml of sedosan and 2 mg/ml of sedoheptulose. One-dimensional chromatography was carried out in the following solvents:

1. Phenol saturated with water

2. Ethyl acetate, acetic acid, and water (3:1:3), top layer

3. Butanol, pyridine, and water (5:3:1)

4. Benzyl alcohol, acetic acid, and water (3:1:3), bottom layer

The following spray reagents were used to detect the sugars:

1. Orcinol, 0.5 g; trichloroacetic acid, 15 g; and 100 ml of water-saturated n-butanol (Klevstrand and Nordal, Acta. Chem. Scand., 4: 1320, 1950)

2. Aniline hydrogen phthalate in n-butanol (Partridge, Nature, 164: 443, 1949) followed by

3. Orcinol in hydrochloric acid (Bidwell, Krotkov, and Reed, Can. J. Botany, 30: 291, 1952)

4. m-Phenylenediamine in aqueous ethanol (Chargaff, Levine, and Green, J. Biol. Chem., 175: 67, 1948)

5. Ammonium molybdate, hydrochloric acid, and ammonium chloride (Aronoff and Vernon, Arch. Biochem., 28: 424, 1950)

Table 13 gives the results of these chromatograms. Conventional R_f values are not given because, in most cases, the solvents were allowed to drip from the bottom of the filter paper to achieve a maximum separation of the different sugars.

Ion-exchange chromatography. — The borate complexes of the heptuloses were also studied with regard to their behavior on Dowex-1 anion resins. Before these studies were carried out it was necessary to determine the optical density of the colors formed by the anthrone and orcinol reagents. Table 14 gives these results.

The heptuloses (2 mg) were individually placed on a Dowex-1 resin column (0.85 cm^2 x 2 cm) and removed with 100 ml of 0.015 M sodium tetraborate followed by 0.030 M sodium tetraborate. These preliminary results indicate that the heptuloses can be separated from each other and quantitatively determined. The volume-to-peak-values for the sugars were: sedosan, 132 ml; sedoheptulose, 212 ml; galaheptulose, 260 ml; glucoheptulose, 320 ml; and mannoheptulose, 385 ml.

TABLE 13

Sugar	Distance moved from origin (mm)				Spray				
	Phenol	Ethyl acetate, acetic acid, water	Butanol, pyridine, water	Benzyl alcohol, acetic acid, water	Ammonium molybdate, HCL, NH_4Cl	Orcinol, TCA	Aniline phthalate	Orcinol then HCl	Phenylene diamine
Glucoheptulose	163	39	123	46	Blue	Blue	Brown	—	Pale brown
Galaheptulose	185	34	112	45	Blue	Blue	Blue	—	Brown
Mannoheptulose	150	35	126	41	Blue	Blue-green	Blue-green	—	Brown
Sedoheptulose	168	39	160	49	Blue	Blue	Blue	—	Brown
Sedosan	272	55	129	303	Blue	—	—	Blue	Fluorescent
Glucose	164	33	—	—	Blue	No color	Brown	—	Brown
Ribose	243	91	—	—	Blue	No color	Brown	—	Red
Fructose	199	—	152	—	Blue	Yellow	Brown	—	Brown

TABLE 14

Sugar	Concentration μg/ml	Optical density		Ratio, orcinol/anthrone
		Anthrone	Orcinol	
Galaheptulose	20	—	0.112	—
	40	0.068	0.262	3.88
	60	0.0969	0.398	4.12
	100	0.176	—	—
Glucoheptulose	20	—	0.113	—
	40	0.0655	0.248	3.98
	60	0.0757	0.380	5.02
	100	0.163	—	—
Mannoheptulose	20	—	0.111	—
	40	0.0862	0.250	2.90
	60	0.125	0.414	3.31
	100	0.254	—	—
Sedosan	20	—	0.166	—
	40	0.0928	0.352	3.80
	60	0.147	0.573	3.90
	100	0.237	—	—

Ion-Exchange Separation of Sugars

(Zill)

Samples of sugars in borate solution, representative of those which would be obtained from a column run were carried through the borate-removal procedure previously reported (ORNL-1297). The final samples were assayed spectrographically for boron (by C. Feldman of the Chemistry Division). The control sample containing no borate was found to have 1.2 μg of boron/ml. A single methyl-alcohol distillation of a glucose-borate sample resulted in a boron reduction of 830 μg/ml, and a second alcohol distillation reduced the boron concentration of 3.2 μg/ml. Further distillations gave no further decrease. As a safety factor a third distillation was carried out in all further experiments. Samples of raffinose, ribose, and sucrose carried through the procedure gave boron concentrations which were not significantly higher than the control sample. Sugar recoveries ranged from 70-90 per cent.

BIOPHYSICS

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Time Relation of the Release of Potassium (K^{42}) from Heart Muscle to the Contraction Event and Electrocardiogram

(Wilde, O'Brien)

An aspiration of long standing among physiologists and biochemists is to relate biochemical events in muscle metabolism to simultaneous mechanical and electrical events during the following muscular contraction. However, in mammalian muscle, the latter events are so rapid (of less than 0.08 second duration) and attended by such minute chemical changes that previously available microchemical analyses have been too slow and too insensitive to detect changes. Workers have been able to measure changes only after hundreds of contractions were summated without any relation to the various phases of the single contraction event itself.

In an attempt to surmount these difficulties, a preparation, turtle heart, has been selected since it has the slowest mechanical events found in commonly available material. The isolated perfused heart stands up well under experimental manipulation and, since the animal is cold blooded, the muscle may be slowed down further by lowering the temperature.

We have chosen potassium metabolism not only because potassium is important biochemically but because it is very abundant in muscle and would be expected to yield greater changes than less abundant chemicals.

Finally, the sensitive tool of radiochemistry is applied where a large amount of radioactivity is produced and detected in a small amount of potassium. Through the kindness of the Pile Operations group, it has been possible to expose potassium in the Low Intensity Training Reactor

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(LITR) to produce specific activities ten times that previously available (probably the highest K^{42} specific activity ever used). This presents problems of handling active materials that have been resolved in the "hot" laboratory of the Biology Division.

The heart is excised from a turtle pre-equilibrated with K^{42}. Non-radioactive physiological fluid is then perfused through the coronary vessels of the heart muscle itself. The outflow is caught on a rapidly revolving filter-paper strip which is cut into segments for counting. Iodine-labeled protein is added to the perfusion fluid so that, by determining the ratio of K^{42} to I^{131}, the minute amount of potassium per cubic centimeter can be determined during the course of a single cardiac contraction cycle. The method as applied to multiple contractions produced by repetitive stimulation and perfusion onto an endless belt was developed at Tulane University, and a paper describing it has been accepted for publication in SCIENCE. The feasibility of the method for a single contraction experiment and the release of potassium have been demonstrated here during the quarter. The elimination of several technical difficulties has been achieved. Pending re-establishment of the service of the temporarily interrupted LITR, investigations have been made of the distribution of travel times of perfusion fluid through the isolated heart.

Erythrocytes

(Sheppard, Mathias)

The production of acetylcholine is suspensions of hemolyzed human red cells has been investigated, and certain necessary conditions for the production of the ester have been established. By using diisopropylfluorophosphate instead of eserine as a cholinesterase inhibitor, the assay method can be used without the difficulties which arise due to the deleterious action of eserine on the frog muscle. Normal human red cells contain less than 0.03 μg of acetylcholine per cc of cells. Added acetylcholine is readily observed. In the presence of added cholinesterase inhibitor, no detectable increase was found after several hours' incubation at room temperature and at 38°C with or without added sugar. Hemolyzed cells will make a substance which satisfies all the criteria for acetylcholine listed in the previous report but only upon addition of choline chloride and an extract of acetone dried powder from rat brain. Thus acetylcholine turnover in normal human red cells is regarded as a process which is too slight to measure by our present techniques. Experiments are now under way to test the ability of red-cell hemolyzates to produce acetylcholine in artificial systems.

Studies on Iodine Metabolism

(Dent, Hunt)

In Anuran larvae. — A generalized attack designed to clarify the role of certain factors involved in the uptake and turnover of iodine in tadpoles of *Hyla versicolor* has been undertaken. The experiments thus far conducted are preliminary in nature but have produced data which indicate:

1. The amount of iodine taken up is dependent on the concentration available in solution, i. e., more iodine is absorbed in a given period of time from a solution containing 5 μc/ml than from a solution containing 1 μc/ml of carrier-free I^{131}.

2. Animals, at the period when the hind limbs are in an early stage of differentiation, take up from a solution containing 1 μc/ml of I^{131} 0.24 μc/g of wet weight in 10 minutes, 1.28 μc/g in 1 hour, and 8.5 μc/g in 24 hours at 28° ± 4°C.

3. In temperature-controlled experiments, animals in advanced stages of metamorphosis (just prior to forelimb emergence) take up in 24 hours the following amounts: at 10°C, 2.83 μc/g of wet weight; at 20°C, 2.92 μc/g of wet weight; and at 30°C, 5.56 μc/g of wet weight.

4. Starved animals kept at 20°C in an iodine-free salt solution eliminate within 24 hours 30 per cent of the radioiodine taken up during a preceding 24-hour exposure. Fifteen per cent of the original amount is lost on the second day, and another 15 per cent on the third day. Experiments are now in progress to determine the effect of feeding on this elimination.

5. Animals are being conditioned for 24 hours in solutions containing 0, 2.5, and 5 ppm of nonradioactive iodine, after which they are exposed to 1 μc/ml of radioiodine which has been added to these solutions. This experiment is imcomplete but so far indicates that the uptake of iodine is significantly higher in the carrier solutions.

6. Previous studies (Dent and Hunt, in press) have shown that an appreciable amount of iodine becomes localized in the pigmented layer of the eye. Some data have been obtained which indicate that the amount thus localized in young animals approaches one-half that localized in the thyroid. Attempts are being made to identify by electrophoretic paper-chromatographic means the compounds to which iodine is bound in the eye.

In chick embryos. — Preliminary results of a similar survey in cheick embryos indicate, in eggs injected with 50 μc of I^{131} at 8 days of incubation:

1. The first measurable concentration of iodine is found in the embryo on the tenth day of incubation.

2. From the tenth to the fourteenth day of incubation the iodine content of the embryo increases sharply, from less than 0.05 μc on the tenth day to about 4.0 μc on the fourteenth day (corrected for radioactive decay).

3. From the fourteenth to the nineteenth day the increase is gradual.

4. On the twentieth and twenty-first days on incubation there is another sharp increase, probably correlated with the absorption of the yolk sac and the drying up of the extraembryonic circulation.

5. Measurements on the thyroid glands and eyes of these animals, although still incomplete, indicate a straight-line increase in concentration, not marked by the sharp changes noted for the entire embryo.

Studies on Isolated Cell Components

(Anderson)

In any consideration of the role of the interphase nucleus in the economy of the cell, the permeability of the nuclear envelope must be considered as a possible limiting factor. Observations on the effects of certain highly charged substances (heparin, Paritol, protamine, and histone) and enzymes, whose substrates are not thought to be constituents of the nuclear envelope, show that these substances can produce drastic effects inside the isolated nucleus without apparent damage to the envelope. It has even been found possible to remove the nuclear contents almost entirely by a combination of enzyme digestion and treatment with nonionic media and still maintain the bleb-forming properties (previously described) of the remaining envelopes intact. These observations are the basis of a method for the isolation of rat-liver nuclear envelopes. Although considerable alteration in the properties of the nucleus may occur during isolation, the findings reported support the point of view that the envelope is permeable to substances of very high molecular weight.

Detailed observations on the effects of certain enzymes on isolated rat-liver nuclei in a phosphate-bicarbonate-sucrose medium at pH 7.1 were recorded. All observations were made with the phase-contrast microscope. Desoxyribonuclease caused rapid loss of nuclear image density, condensation of the nucleoli, a slight decrease in nuclear diameter, and a sharper definition of the nuclear envelope. The nucleoli generally exhibited Brownian movement. Effects were observed in 1-4 minutes. Ribonuclease caused the nucleoli to condense into small dense masses within 30 seconds. Slight condensations were observed elsewhere in the nuclear substance. Treatment with both nucleases resulted in the formation of numerous intranuclear particles in rapid Brownian movement. Trypsin and chymotrypsin caused a rapid expansion of the nuclear gel and rupture of the nuclear envelopes, which appeared to be more resistant to digestion than the nuclear contents.

The gradual clearing observed in incubated homogenates is believed to be due to the combined action of proteases and desoxyribonuclease. It is concluded that the envelope of the isolated nucleus is a porous structure and that it is not composed solely of the chromosomal sheaths of swollen or hydrated chromosomes.

A method has been devised for the mass isolation of Golgi bodies from liver homogenates. These bodies have been previously identified in such preparations but have not been isolated. The Feulgen plasmal relation for lipid aldehydes was found to be negative. A positive test was obtained, however, after ultraviolet irradiation and is believed to be due to the presence of linolenic, linoleic, or arachidonic acid.

Comparison of the Effect of X, Gamma, and Beta Rays on *Tradescantia*

(Kirby-Smith)

The experimental phases of the investigation are now completed. One additional experiment comparing 0.20-A X rays and 0.06-A X rays with Co^{60} γ rays on microspores was recently carried out, confirming earlier work. The relative effectiveness of 0.20-A X rays, 0.06-A X rays, and Co^{60} γ rays on chromosome breakage in *Tradescantia* microspores was found to be in the ratio of 1:0.75:0.5. Both pollen-tube and microspore results are being prepared for publication.

PHOTOSYNTHESIS

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

An emission spectrum, for the phosphorescence of Chlorella, that showed that the delayed light came from chlorophyll was given in the last quarterly report. This work has been extended to the blue-green alga Synechococcus cedorum. We find that, although the emission spectrum for phosphorescence is the same as in Chlorella, thus showing that chlorophyll emits the delayed light, the action spectrum for the blue-green is almost exactly that for phycocyamin. Thus for phosphorescence, the same as for photosynthesis, phycocyamin can transfer energy to chlorophyll.