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QUARTERLY PROGRESS REPORT

for Period Ending February 10, 1953

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BIOLOGY DIVISION
QUARTERLY PROGRESS REPORT
for Period Ending February 10, 1953

Alexander Hollaender, Director

E. J. Slaughter, Technical Editor

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REVIEW OF RESEARCH PROGRESS

Alexander Hollaender

Cytogenetics.—Sensitivity to X rays of the cytoplasmic particle, kappa, in *Paramecium aurelia* has been reported. This sensitivity has now been found to be easily influenced by oxygen concentration.

The genetic structure of bacteriophage is the subject of some interesting recent reports. X radiation seems to be an excellent elucidating agent in this problem.

Further analysis of heterochromatin and variegation in X-ray-induced chromosome rearrangements in *Drosophila* has shown that breaks occurring almost anywhere within the long heterochromatic region can be effective in suppressing the action of the *pe* gene which is located at the distal end of this region.

Formate metabolism studies initiated a short time ago in this laboratory indicate that, unlike isotopic glycine, formate labels protein in at least two positions with different apparent turnover rates.

Macromolecular particles consisting mainly of ribonucleoprotein have been obtained by high-speed centrifugation from extracts of *Escherichia coli*. The particle fraction so obtained shows enzyme characteristics similar to those found in the mitochondria of animal tissues. X irradiation of *E. coli* causes a marked decrease in the ribonucleic acid content of this particle fraction.

Radiation protection studies carried out with temperatures as low as that of liquid nitrogen showed a very wide variation in X-ray sensitivity of *E. coli*. Even at very low temperatures an oxygen effect was found.

The study of the X-ray effect in maize lends further support to the finding that oxygen removal is more effective in the process of healing up of chromosome pieces than in causing the initial chromosome breaks.

Mammalian Genetics and Development. —Data obtained on effects of 1000 r on mutations in the mouse strengthen the early evidence that at this dosage the mutation rate is somewhat lower than the expected rate. The first data obtained on the effect of 300 r indicated that the mutation rate may be somewhat higher than was expected on the basis of the 600 r rate.

However, these data are so preliminary that caution should be used in their evaluation. There is also indication, on the basis of 600- and 1000-r experiments, that there may be an effect on the viability of the first generation offspring. There is a striking effect of prenatal irradiation on the birth weight in the mouse. The mean birth weight plotted against the dose gives a linear relationship.

Pathology and Physiology. —The uptake and storage of iron by the mouse liver was investigated by means of radioactive iron. The incorporation of C^{14} formate in the blood elements and especially on platelets was studied in detail. The effect of total-body X irradiation on incorporation of C^{14} in platelets is being investigated.

The effect of fast neutrons in producing cataracts in mice has been discussed in several reports. It should be emphasized that the spontaneous rate of cataract formation in mice is extremely high and the increased rate by neutron induction should therefore be evaluated on this basis.

It was found that TSH content of the plasma of mice in autonomous tumors is considerably less than in the plasma of mice with dependent tumors. Mice living on a low iodine diet have increased iodine-retaining ability. A detailed review of the present status of conditioned and autonomous neoplasms is given.

Biochemistry. —A group of the Biochemistry Section now reports confirmatory evidence for the structure of adenylic acids a and b which have been isolated in this laboratory.

Bacterial luminescence has been shown to be obtainable from cell-free extracts of *Achromobacter fischeri*. It was found that diphosphopyridinenucleotide could activate the luminescence in bacterial extracts.

Plant Biochemistry. —Work on the biosynthesis of C^{14}-labeled sedoheptulose was expanded. A small project on the effects of infrared radiation on growth of wheat seedlings and potato sprouts has been initiated.

Biophysics. —Considerable additional work was done in the analysis of the β radiation emitted by P^{32} bakelite discs. Extensive work on neutron dosimetry in connection with the ORNL 86-inch cyclotron was initiated. A number of biological materials were tested for neutron sensitivity in this cyclotron. A small project on paramagnetic resonance is in progress. It was found that the effect of neutrons on aberration frequency in *Tradescantia* for varying energies is dependent over the range of neutrons tested. The work on erythrocytes was extended to other biological materials. Interesting results have been obtained in regard to the state of desoxyribonucleic acid in various rat tissues. It is now felt that nucleic acid in the native state is much more highly polymerized than was originally thought.

PRESENTATION OF RESEARCH RESULTS TO THE SCIENTIFIC PUBLIC

Publications. —Publication has been slow during the past quarter, many of the papers submitted being involved in delayed schedules of several journals. Sixty-one papers, including the "Symposium on Some Aspects of Microbial Metabolism," and thirty-three abstracts are in press. The Symposium is to appear as a March 1953 supplement to Journal of Cellular and Comparative Physiology.

The fourteen papers and three abstracts which have appeared during the quarter are listed.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Baker, W. K., and Charles W. Edington	The induction of translocations and recessive lethals in *Drosophila* under various oxygen concentrations	Genetics, 37: 665-667, 1952
Billen, Daniel, G. E. Stapleton, and Alexander Hollaender	The effect of X radiation on the respiration of *Escherichia coli*	J. Bact., 65: 131-135, 1953
Cohn, W. E., D. G. Doherty, and Elliot Volkin	The products of ribonucleic acid hydrolysis and their relationship to its structure	Symposium on Phosphorus Metabolism, ed. W. D. McElroy and Bentley Glass, Vol. II, The Johns Hopkins Press, 1952, pp. 339-354
Doermann, A. H., and M. B. Hill	Genetic structure of bacteriophage T4 as described by recombination studies of factors influencing plaque morphology	Genetics, 38: 79-90, 1953
Geckler, R. P, and R. F. Kimball	Effect of X rays on micronuclear number in *Paramecium aurelia*	Science, 117: 80-81, 1953
Giles, N. H., Jr., A. V. Beatty, and H. P. Riley	The effect of oxygen on the production by fast neutrons of chromosomal aberrations in *Tradescantia* microspores	Genetics, 37: 641-649, 1952
Hollaender, Alexander	Biology Division Quarterly Progress Report for Period Ending November 10, 1952	Project Report ORNL-1456

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Kimball, R. F.	The Science of Heredity by J. S. D. Bacon and What's All This About Genetics by Rona Hurst (Book Review)	Quart. Rev. Biol., 27: 390-391, 1952
Kimball, R. F.	Biological Effects of External Beta Radiations by R. E. Zirkle (Book Review)	Quart. Rev. Biol., 27: 418-419, 1952
Kimball, R. F., R. P. Geckler, and Nenita Gaither	Division delay by radiation and nitrogen mustard in Paramecium	J. Cellular Comp. Physiol., 40: 427-459, 1952
Pomper, S., and D. W. McKee	Flocculence in yeast	Science, 117: 62-63, 1953
Sheppard, C. W.	Symposium on Radiobiology - The Basic Aspects of Radiation Effects on Living Systems. James J. Nickson (ed.) (Book Review)	Chem. Eng. News, 30: 4881-4882, 1952
Strehler, Bernard L.	Photosynthesis — Energetics and phosphate metabolism	Symposium on Phosphorus Metabolism, ed. W. D. McElroy and Bentley Glass, Vol. II, The Johns Hopkins Press, 1952, pp. 491-506
Vaslow, Fred, and David G. Doherty	Thermodynamic study of some enzyme-inhibitor complexes of chymotrypsin. II.	J. Am. Chem. Soc., 75: 928-931, 1953

ABSTRACTS

Furth, Jacob	Recent experimental studies and current concepts on the etiology and nature of leukemia	Proc. Inst. Med. Chicago, 19: 95-102, 1952
Russell, W. L.	Irradiation and sterility (Reply to an article by Dr. I. C. Rubin)	J. Am. Med. Assoc., 150: 1619-1620, 1952
Sheppard, C. W.	Synthesis of dye dilution curves	Am. J. Physiol., 171: 767, 1952

Scientific Society Lectures and Traveling Seminars.—Many talks on subjects under investigation in the Biology Division have been given before scientific society meetings and on the Traveling Seminar program. Speakers and their subjects are listed.

Anderson, N. G.	Columbia University New York	Properties of the nuclear envelope
Arnold, W. A.	Knoxville College Knoxville	Problems connected with photosynthesis
Atwood, K. C.	Zoology Department Columbia University New York	The role of the nuclei in the response of Neurospora conidia to radiation
Baker, W. K.	University of Mississippi Oxford	The role of oxygen in modifying the effects of X rays on genetic material
_____	Rice Institute Houston	The relationship between heterochromatin and variegation in Drosophila
Billen, Daniel	Microbiology Dept. Tulane University New Orleans	Studies on radiation-induced changes in the physiology of bacterial cells
_____	Sigma Xi, Louisiana State University Baton Rouge	As above
_____	Biochemistry Dept. Louisiana State University Baton Rouge	As above
Burnett, W. T., Jr.	Emory University Georgia	Tracer studies in mineral metabolism
_____	University of Tennessee Memphis	As above
_____	Prairie View A & M College, Prairie View, Texas	As above
_____	Oklahoma A & M College Stillwater	As above
Carson, S. F.	Biochem. and Bact. Dept. University of Tennessee Memphis	Balance and functioning of aerobic respiratory cycles in anaerobic bacteria
_____	Dept. of Bacteriology University of Texas Austin	As above

_____	Dept. Biochem. and Microbiol., Tulane University, New Orleans	Balance and functioning of aerobic respiratory cycles in anaerobic bacteria
Cohn, W. E.	Mt. Sinai Hospital New York City	The separation of biochemically important substances by ion-exchange chromatography
Dolin, M. I.	Bacteriology Dept. University of Illinois Urbana	Diacetyl oxidation by the keto acid system of Streptococcus faecalis
Furth, Jacob	Research Society Baylor University Houston, Texas	Clinical pathology of irradiation injury
_____	University of Texas Galveston	Carcinogenesis by ionizing radiations in man and animals
_____	University of Maryland School of Medicine Baltimore	Experimental leukemia
Gaulden, Mary Esther	University of Virginia Charlottesville	Effects of radiation and chemicals on mitosis and cell structures as observed in living cells
_____	University of Mississippi Oxford	As above
_____	Texas A & M College Bryan	As above
_____	University of Arkansas School of Medicine Little Rock	As above
Hollaender, Alexander	Yale University New Haven	Protection against and recovery from radiation damage
_____	Harvard University Cambridge	As above
_____	Chester Beatty Res. Inst. London, England	Modification of radiation damage by treatment after exposure to X rays
_____	Radiotherapeutic Res. Unit Hammersmith Hospital London, England	As above

Hollaender, Alexander	Atomic Energy Res. Establishment, Harwell, England	Secondary effects of radiation on biological materials
_____	Hospital Staff The Johns Hopkins University, Baltimore	Effects of radiation at the cellular level
Schwartz, Drew	University of Illinois Urbana	Evidence for sister-strand crossing over in maize
Strehler, B. L.	Biochem. Dept., Duke University School of Medicine, Durham	Photosynthesis — ATP changes and energetics
_____	Botany Dept., University of North Carolina Chapel Hill	As above
_____	Chemistry Dept., University of Tennessee Knoxville	As above
Tolbert, N. E.	Biology Department University of Tennessee Knoxville	Two-carbon fragment from glucose shunt metabolism and photosynthesis
Hollaender, Alexander (G. E. Stapleton and D. Billen)	Faraday Society Cambridge, England	Modification of radiation damage by treatment after exposure to X rays
Cohn, W. E.	Am. Assoc. Adv. Sci. St. Louis	Biochemistry of nucleic acids
_____	Conf. Ion-Exchange Resins in Medical and Biological Research, New York Acad. Sci. New York	The separation of biochemically important substances of ion-exchange chromatography
Schwartz, Drew	Am. Soc. of Agronomy Cincinnati	The interaction of nuclear and cytoplasmic factors in the inheritance of male sterility
Cohn, W. E., and E. Volkin	Second International Congress of Biochemistry Paris, France	The products of ribonucleic acid hydrolysis and enzymolysis
Carson, S. F.	East Tenn. Sect., Am. Chem. Soc. Knoxville	The degradation of labeled compounds

Visiting Lecturers. —The Biology Division has been privileged to hear seminars given by the following prominent scientists.

Dr. Eugene Guth	Dept. of Physics Notre Dame University South Bend	Muscular contraction and rubber-like elasticity
Dr. Melvin H. Knisely	The Medical College of South Carolina, Charleston	Observations of phenomena in living capillary beds
Dr. R. W. Bates	National Institutes of Health, Bethesda	The physiology and chemistry of pituitary hormones, especially prolactine
Dr. Donald P. Costello	Dept. of Zoology University of North Carolina Chapel Hill	Discussion entitled: The carrier effects as a means of protection against radiation damage from P^{32}
Dr. Richard B. Setlow	Sloane Physics Lab. Yale University New Haven	Temperature dependence of the direct action of radiation
Dr. Milton L. Zucher	Biology Department The Johns Hopkins University, Baltimore	Quantitative pigment differentiation in single cells

Sixth Annual Biology Research Conference. —The 1953 spring conference sponsored by the Biology Division and supported by the Atomic Energy Commission will be held on April 20 and 21 in the East Lounge of Ridge Recreation Hall. The program on "Effects of Radiation and Other Deleterious Agents on Embryonic Development" follows.

Monday, April 20, 9:30 A.M.

Chairman — Viktor Hamburger, Washington University

James G. Wilson, University of Cincinnati College of Medicine
"Differentiation and the Reaction of Rat Embryos to Radiation"

Roberts Rugh, Department of Radiology, Columbia University
"Effects of Radiation on Amphibians"

James N. Dent and Ernest L. Hunt, Miller School of Biology, University of Virginia, and Emory University
"Tracer Techniques in Embryological Research"

Monday, April 20, 2:00 P.M.

Chairman — Sewall Wright, University of Chicago

Liane Brauch Russell, Biology Division, Oak Ridge National Laboratory
"The Demonstration, by Means of Prenatal Irradiation, of Critical Periods in the Development of a Variety of Characters in the Mouse"

Samuel P. Hicks, New England Deaconess Hospital, Boston
"Comparison of Effects of Radiation, Certain Hormones, and Radiomimetic Drugs on the Developing Nervous System"

Tuesday, April 21, 9:00 A.M.

Chairman — Paul Weiss, University of Chicago

Dietrich Bodenstein, Chemical Corps Medical Laboratories, Maryland
"Effects of Radiomimetic Substances on Embryonic Development, with Special Reference to Nitrogen Mustard"

Josef Warkany, Children's Hospital, Research Foundation, Cincinnati
"Disturbance of Embryonic Development by Maternal Vitamin Deficiencies"

Tuesday, April 21, 2:00 P.M.

Chairman — L. C. Dunn, Columbia University

Clarke Fraser, Department of Genetics, McGill University
"The Experimental Production of Cleft Palate with Cortisone and Other Hormones"

Walter Landauer, Department of Genetics, University of Connecticut
"Some Problems of Chemical Teratogenesis and Their Bearing on Physiological Genetics"

Summarizing Discussion.

Biology Division Speakers at Spring Meetings of Scientific Societies. — A number of papers on work in the Biology Division will be given at coming meetings of professional societies. At the March meeting of the American Chemical Society in Los Angeles, M. I. Dolin will present a paper on work done in cooperation with Hager and Gunsalus of the University of Illinois. In April the following persons will present papers at the Federation of American Societies for Experimental Biology in Chicago and the American Association for Cancer Research in the same city: T. T. Odell (coauthors, F. Gamble, and J. Furth), Jacob Furth (coauthors, W. T. Burnett, Jr., and E. Gadsden), N. G. Anderson (Tech. Asst., M. L. Anderson), A. C. Upton (coauthor, W. D. Gude), Alexander Hollaender (coauthor, G. E. Stapleton), S. R. Tipton (coauthor, G. St. Amand), J. M. O'Brien (coauthor, W. S. Wilde), J. R. Totter, J. X. Khym (coauthors, W. E. Cohn, E. Volkin, and D. G. Doherty), D. G. Doherty, E. A. Delwiche (coauthors, E. F. Phares, and S. F. Carson), M. I. Dolin, and A. C. Upton (coauthor, J. Furth).

At the Association of Southeastern Biologists, which meets in Chapel Hill, North Carolina April 16-18, the following will give papers: R. F. Kimball, Daniel Billen (coauthors, B. L. Strehler, and G. E. Stapleton),

J. G. Carlson (coauthors, N. Harrington, and M. E. Gaulden), R. T. Brumfield, E. L. Gadsden, and Georgia St. Amand (coauthor, S. R. Tipton).

Liane B. Russell will represent the Division at the Sixth Biology Research Conference in Oak Ridge, also in April. A. H. Doermann and G. H. Bowen will present papers at the Cold Spring Harbor Symposia on Quantitative Biology on June 8-16.

The program of the first annual meeting of the Radiation Research Society, June 22-24 in Iowa City will carry papers by Daniel Billen, G. E. Stapleton, K. C. Atwood, J. R. Totter, Jacob Furth (with A. C. Upton), and N. H. Giles (with F. J. de Serres, and A. V. Beatty).

CYTOGENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball (Leader) G. H. Bowen
A. D. Conger* Nenita Gaither
K. C. Atwood Mary Kathryn King
A. H. Doermann F. H. Mukai
Drew Schwartz Martha C. Chase
C. P. Swanson** Anita D. Galey
R.P. Geckler (Biology Division, AEC)

Studies on the Cytoplasmic Factor, Kappa, in Paramecium aurelia

(Geckler, Kimball)

The cytoplasmic particle, kappa, in Paramecium aurelia offers unique opportunity for study of effects of external agents upon a self-reproducing desoxyribonucleoprotein particle about which considerable information is available.

Paramecia containing kappa produce a substance, paramecin, which kills those lacking kappa (sensitives). Under appropriate conditions the concentration of kappa can be reduced to a point where some animals receive no kappa particles at division. Since kappa does not arise de novo, these animals, if isolated and allowed to multiply, give rise to sensitive clones whereas animals receiving one or more particles will ultimately give rise to killer cultures. From the proportion of sensitive clones derived in this way one can calculate the approximate mean number of kappa particles by the relations, $P = e^{-m}$, where P is the proportion of cultures which are sensitive, e the base of natural logs, and m the mean number of kappa particles.

A series of experiments, utilizing these principles, has been performed on the effects of hydrogen peroxide on kappa in variety-4 animals and on the influence of X radiation on kappa under conditions of hypoxia.

No destructive effects could be demonstrated with hydrogen peroxide in concentrations as high as 37 μg/ml with 10-minute exposures; use of larger amounts of this substance are impractical because of lethal effects on the animals. It is interesting to note that variety-4 animals can, apparently, tolerate higher concentrations of hydrogen peroxide than variety-1 animals which were used in previous studies of division delay.

*On leave of absence—Fulbright Fellowship
**Research Participant

The destruction of kappa by X rays is well known and has been demonstrated in several laboratories. It appeared to be of considerable interest to test whether an "oxygen effect" could be demonstrated for this phenomenon. The following experiment was therefore performed.

Animals containing a small mean number of kappa particles were divided into eight groups and placed in tubes through which the appropriate gases could be bubbled. Four tubes were bubbled with pure nitrogen and four with 20% oxygen-80% nitrogen. Bubbling was continued for 10-15 minutes, the tubes were sealed, and two tubes from each group irradiated with approximately 7500 r of X rays. Isolations of samples of 90 animals from each group were made, followed by a period of slow growth to permit those cultures containing any kappa particles to become killers. All cultures were then characterized as to whether they were killers or sensitives. Table 1 shows the results of this experiment. The difference between the groups irradiated in oxygen and in nitrogen was highly significant by the chi-square test. The dose-reduction factor is 2 if exponential decrease of particle number with dose is assumed.

TABLE 1

EFFECTS OF X RAYS ON KAPPA IN NITROGEN AND IN 20 PER CENT OXYGEN

Treatment	Total cultures tested	Fraction sensitive	Mean no. of kappa particles
N_2-7500 r	89	0.697	0.36
N_2-7500 r	89	0.742	0.30
O_2-7500 r	85	0.906	0.10
O_2-7500 r	82	0.902	0.10
N_2-0 r	90	0.278	1.28
N_2-0 r	90	0.322	1.13
O_2-0 r	89	0.281	1.27
O_2-0 r	89	0.292	1.23

X Radiation as a Tool for Investigating Replication of Genetic Material in Bacteriophage

(Doermann, Chase)

Previous quarterly reports (see ORNL-1297 and ORNL-1456) have dealt largely with the problem of elucidating the genetic structure of bacteriophage T4 by means of genetic recombination experiments. Sufficient information and genetic markers are now available to enable us to utilize

the latter for investigating phage replication. Evidence from many sources indicates that replication of phage material occurs during some noninfective state of the virus. Thus the first major problem is one of characterizing these replicating structures, which cannot be identified or enumerated by permitting them to form plaques. By using radiation as a tool it may, nevertheless, be possible to draw some inferences about the genetic organization of the replicating structures. The plan of the experiments requires that the amount of radiation required to register several lethal hits on intracellular phage constituents should not interfere appreciably with over-all phage synthesis in the cell. A cell may be infected simultaneously with several phage particles of a given genotype and with a single particle of a genotype differing in one, two, or three loci. If this cell is irradiated at an early stage, where replication has been prevented, it should be possible to discover whether the radiation inactivates the loci of the singly infecting particle independently or as a group. By using radiations of different energies, and by irradiating after different periods of growth, experiments should be possible which will be indicative of the organization of the genetic material and the multiplicity of individual loci at different stages. It should also be possible to make some inferences about the extent of genetic damage (in terms of map distances) of single quanta of various radiations.

Enough results with X radiation are now available to indicate that experiments of this type are feasible. Radiation of infected bacteria with doses used so far showed no appreciable effect on the burst size (average phage yield per infected bacterium). The first objective of the experiments was to find out whether X-ray-inactivated phage particles can contribute any of their genetic complement to a bacterium infected simultaneously with active phage. The result conclusively showed that they can. Bacteria were infected with a mixture of X-irradiated wild type (survival 13%) and untreated $\underline{r}_{48}\underline{tu}_{42b}$ (unlinked genetic markers). The mixture was such that most of the bacteria would be infected by at least one $\underline{rtu}$ particle, but that only 20% of the host cells would receive an X-ray-surviving wild-type phage. Actually 80% of the bacteria liberating any phage yielded phage containing the wild-type markers. A control experiment was performed using unirradiated phages only. The infections here were such that wild type was expected to occur in 39% of the bursts. A value of 44% was found. The experimental results contained additional qualitative evidence making inescapable the conclusion that X-ray-inactivated phage can contribute genetic markers. Among the bursts from the mixture containing irradiated wild type, seven contained significant numbers of one of the genetic markers of the irradiated type but none of the other (four contained $\underline{r}^+$ but no $\underline{tu}^+$ and three contained $\underline{tu}^+$ but no $\underline{r}^+$). No bursts of this category were found in the control experiment. Thus the phage which was irradiated appears to be different from the phage which was not, in that it occasionally contributes only part of its genetic complement. It is this result which in-

dicates that the technique should be able to aid in characterization of the genetic material of the phage particle during its noninfectious state.

A further experiment was performed in which a mixture, similar to that in the control experiment above, was adsorbed onto bacteria in the presence of cyanide, thus stopping the progress of the phage reproductive cycle at a very early stage. Irradiation of these bacteria gave results qualitatively similar to those mentioned; namely, that the genetic markers could be eliminated independently by X radiation.

Photoreactivation of Bacteriophage T2

(Bowen)

A bacteriophage particle is called active if it is capable of giving rise to a plaque when plated on nutrient agar together with sensitive host bacteria under standard conditions. Exposure of phage particles to ultraviolet radiation (2537 A) inactivates them in this sense. However, following adsorption of the inactive particles to sensitive bacteria, exposure to radiation of the violet and near-ultraviolet region causes a fraction of them to regain their activity, a phenomenon called photoreactivation.

Photoreactivation of phage was discovered by Dulbecco, who gave a rather complete general description of it (Dulbecco, J. Bact., 59: 329-347, 1950). A detailed study by Bowen (Doctoral thesis, California Institute of Technology, 1953) of the kinetics of photoreactivation demonstrated the existence of a dark-reaction step, described the interaction of the dark and light reactions under various conditions, and measured rate constants over the temperature range 0-45°C. It is possible to describe photoreactivation satisfactorily in terms of a simple reaction model. This line of attack is being continued and extended, with the objective of fully understanding the mechanism of photoreactivation and thereby learning more of the nature of the original ultraviolet-radiation damage.

The past quarter has been occupied with a general survey of various conditions affecting photoreactivation of bacteriophage T2r, particularly a reexamination of certain earlier findings.

Dulbecco stated that the number of active particles increased with the time of illumination, t, as the function $(1-e^{-kt})$, which suggested that a single event is necessary to complete the reactivation of any given particle. Over a comparatively narrow range of ultraviolet doses the rate of photoreactivation appeared to be independent of the dose. This "one-hit" repair of what must be a number of independent initial ultraviolet damages has seemed paradoxical.

Working with phage T2r adsorbed to washed cells of Escherichia coli B in phosphate buffer, at 36°C, we have investigated the dependence of photoreactivation on the ultraviolet dose for a range of doses in the ratio of 7:1 survival in the dark from 3×10^{-1} to 1×10^{-7}. The rate of reactivation clearly decreases with increasing dose and appears to be approximately inversely proportional to the dose. The simple exponential function mentioned above approximately describes the reactivation kinetics at all doses, but at the higher doses a small lag appears at the beginning of reactivation and systematically increases with increasing dose. The observed degree of approximation to one-hit kinetics and the dependence of the rate on the dose can be explained on the basis of the sum of various multiple-hit effects in a heterogeneous population of damaged particles, but it is not yet clear exactly how this should be done.

Measurements of the maximum rate of photoreactivation have shown a sharp change in its temperature dependence at about 18-20°C, with a very rapid decrease in rate at lower temperatures. For technical reasons this rate has always been determined from the initial rate of appearance of active particles, rather than from the over-all kinetics. It has been necessary to assume that the maximum obtainable amount of photoreactivation does not change with temperature. An experiment has now been performed in which the photoreactivation of the same preparation was followed to completion at both 36° and 4°C. The same maximum appears to be reached in both cases. Additional such experiments will be done, but there is little doubt that they will confirm the result that the maximum is independent of temperature. The assumption therefore holds true, and the explanation of the complex temperature behavior mentioned must be found elsewhere.

No means has ever been found to make possible photoreactivation of free phage; adsorption to bacteria is essential. However, photoreactivation proceeds well with resting bacteria suspended in buffer in the absence of external metabolites and in the absence of oxygen. No chemical means of affecting the reaction has previously been known. Dulbecco stated that 0.01 M cyanide was without effect. However we have just discovered that cyanide at this concentration does have an inhibitory effect on photoreactivation under some conditions. Experiments are now in progress to determine the nature of this effect and the point in the reaction scheme at which it is effective. This may provide at once a clue to the actual chemistry of photoreactivation and a tool for further research.

EFFECTS OF RADIATION ON RATE OF MITOSIS

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Heterochromatin and Variegation in X-Ray-Induced Chromosome Rearrangements

(Baker)

A cytological and genetical analysis has been completed on thirty-two different induced rearrangements which cause variegation of the eye pigmentation in Drosophila virilis (see ORNL-1167 for preliminary results). All rearrangements were induced in wild-type males. The variegation of peach and wild-type pigmentation in the eye is observed when the rearranged chromosome is heterozygous with the recessive allele of a gene, peach, located near the centromere of chromosome 5.

All the breakage points in each of the rearrangements have been located accurately on the salivary gland chromosomes, or, when the breaks were not visible, genetic tests have located them. At least one breakage point in each rearrangement is within the small region of heterochromatin at the proximal end of chromosome 5. Unequivocal genetical and cytological evidence indicated that the pe locus is normally located within the last four bands of heterochromatin seen in chromosome 5 in the salivary gland cells.

Contrary to previously published concepts of other investigators as to the factors which produce variegation of heterochromatic loci, the analysis of the rearrangements indicates that variegation is not only evoked when euchromatic chromosome regions are placed on one side or the other of the pe locus, but also that foreign heterochromatin located either proximal or distal to the gene will produce variegation.

It was found that, if wild-type males were given 5000 r of X rays and then mated to peach females, approximately 5% of the F_1 offspring carry rearrangements capable of producing variegated eyes. Practically all these rearrangements have a break proximal to the pe locus which is observed cytologically as a break between the last band of heterochromatin at the base of chromosome 5 and the centromere. This high break fre-

*Consultant

quency in such a small region of the salivary gland chromosome raises the question as to whether this region is particularly susceptible to X-ray-induced breakage. An analysis of an induced chromosome deletion including the pe locus indicates that the small region of heterochromatin as seen in the salivary gland cells occupies about two-fifths of the metaphase chromosome length in ganglion cells. Since the metaphase chromosome structure in the latter cell is cytologically comparable to the condition of the chromosomes in the sperm, the high break frequency would be reasonable if breakage in this region were equally likely per unit length. If the latter assumption is valid, it would appear that a chromosome break and subsequent transposition of foreign material to a point at some distance from the pe locus would still be effective in producing variegation. A direct test of this assumption has been made by cytological and crossing-over studies of three different 3:5 translocations (producing variegation) which have their breakage points in chromosome 3 within six salivary gland bands of each other and their breaks in chromosome 5 proximal to the locus of pe. From the relative lengths of the translocated chromosomes as seen at metaphase in the ganglion cells, it has been determined that the breaks occurred at measurably separated locations within the heterochromatin located proximal to the locus. Studies on the frequency of crossing-over between pe and the point of breakage in each of these rearrangements confirm the cytological findings although the crossover frequency is very low in this, as in other, heterochromatic regions.

It can thus be concluded that breaks occurring almost anywhere within this long heterochromatic region can be effective in suppressing the action of the pe gene which is located at the distal end of this region.

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

(Schwartz, Cheniae*)

In an earlier study (Schwartz, Proc. Natl. Acad. Sci. U. S., 38: 490-494, 1952) experiments were described which were conducted in an attempt to ascertain the mechanism of the oxygen effect on the radiosensitivity of maize chromosomes. The criterion used was the relative protection of low oxygen concentration against terminal deletions as compared to internal deletions. These experiments indicated that the effect of low oxygen tension is on the recovery process rather than on the initial breakage mechanism.

These experiments were repeated in the summer of 1952 using three linked markers on chromosome 9. These markers were: wx which de-

*No longer with ORNL

termines the composition of the starch in the endosperm, c which controls the formation of aleurone color, and bz which modifies aleurone color. The dosage used was 1000 r. With such a setup it is possible to distinguish with certainty mutant kernels arising from terminal and internal deletions.

The results of these experiments support the contention that oxygen acts on the recovery process. Approximately the same frequencies of internal deletions were obtained in oxygen- and nitrogen-treated pollen grains—0.54 and 0.52%, respectively. However, an eightfold difference was found for terminal deletions; 1.66% terminal deletions were obtained in air as compared to 0.20% in nitrogen. If irradiation in nitrogen serves to reduce the number of chromosome breaks, the frequency of internal deletions in the nitrogen treatment should have been reduced much more than the terminal deletions, since the former follows a two-hit curve while the latter increases linearly with dose.

The Effect of Oxygen Tension of X-Ray-Induced Chromosome Breakage in Onion Root Tips

(Schwartz, Galey)

Hayden and Smith (Genetics, 34: 26-43, 1949) studied the effect of oxygen concentration on the frequency of anaphase bridge formation in irradiated barley root tips. They found a 4.7-fold difference between the frequencies of bridges obtained from treatment in air and in a vacuum. These studies have been reported in the last quarter using onion root tips in experiments designed to compare the magnitude of the oxygen effect on single and double anaphase bridges. The root tips were irradiated with 300 r of X rays in air and nitrogen and fixed 48 hours after irradiation. Sax (Genetics, 26: 418-425, 1941) has shown that chromosomes act as single strands 48 hours prior to anaphase, and thus only the chromosomal types of aberrations appear. The double bridges are formed by dicentric chromosomes (two-hit aberrations) whereas the single bridges result from terminal deletions followed by fusion of the broken ends of sister chromatids.

The data are shown in Table 2. The results compare favorably with those reported for similar experiments in maize using genetic markers (Schwartz, Proc. Natl. Acad. Sci. U. S., 38: 490-494, 1952). The mosaic kernels in maize are to be compared with the single bridges in onion since both result from terminal deletions followed by sister-strand fusion. The "whole losses" kernels are a composite class including both one- and two-hit aberrations. The protection values obtained for the maize mutant types are included in the table for comparison.

TABLE 2

FREQUENCY OF ANAPHASE BRIDGE CONFIGURATIONS FOLLOWING IRRADIATION IN AIR AND NITROGEN

	Treatment	Total cells scored	Normal	Single bridges		Double bridges		Total bridges	
				No.	%	No.	%	No.	%
Onion	300 r in air	2184	2070	56	2.56	58	2.66	114	5.22
	300 r in nitrogen	2565	2521	7	0.27	37	1.44	44	1.64
			Air/nitrogen	9.48		1.85		3.18	
				Mosaics		Whole losses		Total mutants	
Maize			Air/nitrogen	9.9		2.7		3.2	

RADIATION PROTECTION

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The Effect of X Irradiation on the Macromolecular Organization of Escherichia coli

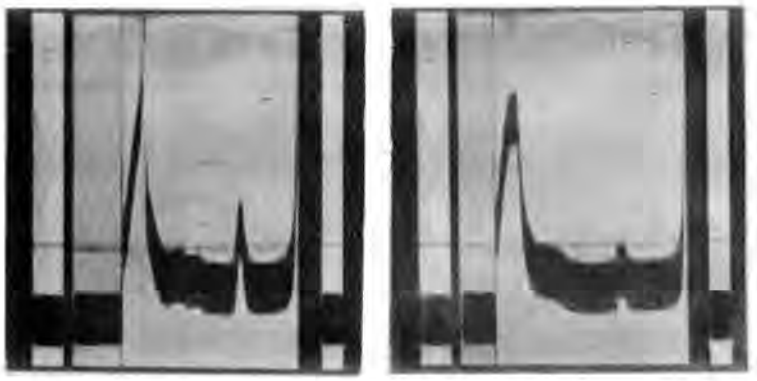
(Billen, Volkin of Biochemistry)

Studies on the macromolecular organization of Escherichia coli B/r are in progress as a continuation of our studies on X-ray-induced physiological changes in this organism. Other investigators have found that extracts of E. coli obtained by grinding with alumina contain three major boundaries as shown by ultracentrifugal analysis (Schackman et al., Arch. Biochem. Biophys., 38: 245-260, 1952). These major components possessed sedimentation constants of approximately 40, 29, and 5 S.[1] Studies of the 40-29 S component fraction separated from the whole extract revealed that this fraction consisted of particles 20-30 mμ in diameter, that it was composed mainly of ribonucleoprotein, and that it contained such enzymes as ribonuclease, catalase, and succinic-dehydrogenase. The 5 S component accounted for the major portion of the desoxyribonucleic acid (DNA) of the whole extract.

The data obtained in our investigation (using the same analytical procedure described by Schackman et al., ibid.) have shown that exposure of 10^{11} cells/ml of E. coli to 120,000 r of X rays[2] has no observable effect on the cellular components of this organism when analysis of the extracts, obtained by alumina grinding, is made immediately following exposure. However, if the cells are resuspended in M/15 phosphate buffer (pH 6.8) plus 1% glucose and incubated at 37°C for 90 minutes subsequent to X-ray exposure, marked changes are observed to have occurred in the component patterns as compared to no change in similarly treated control cells (Fig. 1). It can be seen in Fig. 1 that incubation of X-irradiated cells at 37°C for 90 minutes in buffer-glucose medium causes a marked decrease in the 40 S component. A loss of the sharp spike by the 5 S component is also observed. On the basis of these in vivo studies it may be concluded that there is no apparent immediate effect of X irradiation on either the ribo- or desoxyribonucleoprotein components as studied in the 40-29 S and 5 S

[1] Sedimentation constants are reported in Svedbergs: 1 S = 10^{-13} cm/sec/unit yield.

[2] A dose of 120,000 r caused a 99.5-99.9% decrease in the viable population of the cell suspension so exposed.



UNEXPOSED EXPOSED

FIG. 1. Ultracentrifugal patterns of E. coli extracts obtained as described in text. Each picture represents the pattern observed 24 minutes after reaching speed (42,000 rpm).

fractions respectively; however, upon postirradiation incubation of the cells in a medium containing glucose, there is a loss of ribonucleic acid (RNA) or protein or both from the 40-29 S fraction and most probably a depolymerization of the 5 S component. Confirmatory evidence on the loss of RNA from the 40 S particles was obtained by differentially centrifuging the extracts into a 25,000 rpm sediment, a 40,000 rpm sediment (29 S and 40 S particles), and a 40,000 rpm soluble fraction. Assay for phosphorus distribution in these fractions by a modified Schmidt-Thannhauser technique revealed, when compared with nonirradiated cells, that about 80% of the particle RNA was converted to material now found in the 40,000 rpm soluble fraction, as both acid-soluble and acid-insoluble fragments. The DNA in the 40,000 rpm soluble fraction, although ostensibly depolymerized as a result of irradiation and incubation, remain as acid-insoluble material. Experiments will be conducted to determine whether the changes here reported also occur in the absence of a metabolite such as glucose.

It has also been found that the 40-29 S components are responsible for some of the succinoxidase activity and all of the coupled oxidative-phosphorylation activity that has been found in the cell extracts. Thus the particles that constitute this fraction seem to be analogous to the mitochondria of animal tissues. Further studies of the effects of X irradiation on the components and the enzyme systems found in them have been initiated. It is hoped that these studies may lead to additional information about such X-ray-induced physiological changes as adenosinetriphosphate release from the bacterial cell and the inhibition of respiration which were reported earlier (ORNL-1244; ORNL-1393).

Formate Metabolism Studies

(Totter, Best)

The interpretation of effects of irradiation on metabolic patterns studied by means of tracers requires relatively complete knowledge of the normal distribution of activity following administration of an isotopically labeled compound. Such studies are being undertaken, employing rats as test animals. For the preliminary studies rats which had been injected with formate for other purposes were used.

Fractionation of various tissues from rats injected with isotopic formate at various intervals (0.5-10 days) before sacrifice is being carried out. The results of these experiments are being compared with similar results obtained by investigators who have used other compounds as sources of metabolic formate.

The results of the experiments in progress indicate that, unlike isotopic glycine, formate labels protein in at least two positions with different apparent turnover rates. Furthermore, the slower of the two components in liver protein has a "half life" of approximately 10 days as compared with the value of 100 hours given for glycine-labeled liver protein by Tyner et al. (Cancer Research, 12: 158, 1952).

Effect of Irradiation at Low Temperature on X-Ray Inactivation of *E. coli* B/r

(Stapleton, Edington)

In order to assess the relative importance of diffusible species produced by ionizing radiations within the bacterial cell we have begun an investigation of the sensitivity of *Escherichia coli* irradiated in liquid and in frozen suspensions. The range of temperature used will extend from liquid nitrogen temperature (80°-313°K) or just below that which will bring about heat inactivation of these bacteria. Preliminary results indicated that very little inactivation is brought about by rapid freezing of the suspension in liquid nitrogen and other freezing mixtures whose temperatures are below 240°K.

The sensitivity of oxygen-saturated and oxygen-free suspensions is being investigated at several temperatures over the range indicated.

Early results indicate that about an eightfold dose reduction is brought about by decreasing the temperature during irradiation of oxygen-saturated

suspension from 313° to 80°K, whereas oxygen-free suspensions show about a fourfold dose reduction under the same conditions.

Of utmost interest is the finding of an oxygen effect at all temperatures so far investigated. How this finding can be correlated with reduction in yield of oxidizing radiation-induced radicals by oxygen removal is not clear at present.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

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Radiation-Induced Mutations in the Mouse

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

The first objective in the major project undertaken by this group has been achieved. This was the obtaining of a reliable mutation rate at specific loci for mouse spermatogonia X-irradiated with 600 r. The second objective, to find whether the relation between dose and mutation rate is approximately linear or whether it departs significantly from linearity, is now occupying the major part of our effort. As stated in the latest Quarterly Progress Report (ORNL-1456), it will presumably take a long time to accumulate enough data to answer this important question even to the degree of accuracy needed for estimation of human hazards. The results obtained so far are reviewed briefly.

The first experiment attempted, after the original 600-r experiment, was one using a dose of 1000 r. It has already been reported (ORNL-1393, 1456) that the results obtained with this dose indicate a departure from linear increase in mutation rate with dose. As this indication strengthened, it seemed of vital importance to begin immediately an experiment at another dose level, rather than to wait for the final outcome of the 1000-r experiment. The 1000-r results showed a rate lower than that expected, on the hypothesis of linearity, from the 600-r rate. This suggested the possibility that the rates for doses lower than 600 r might be higher than expected from the 600-r rate. If this were true, then extrapolating from the 600-r rate to human hazards might underestimate the damage at the dose levels more frequently encountered by man. It seemed feasible to go as low as 300 r and still have an experiment that could be completed satisfactorily in a reasonable length of time. A new experiment, using this dose, was therefore started. This dose has the added importance of lying below the upper limit of doses that must be considered in evaluating genetic hazards in man. For example, 300 r can be accumulated in 20 years without exceeding the weekly permissible dose. Survivors of nuclear explosions or disasters could have received doses higher than this.

The rate obtained so far in the 300-r experiment is higher than expected from the 600-r results. However, many more mutations must be obtained before much confidence can be attached to the 300-r rate. This will probably take a considerable length of time with the present size of experiment. If the higher rate holds over the next few months it would be desirable to consider increasing the scope of this part of the project.

The 600-r experiment gave a rate about ten times higher than the rate found in similar Drosophila experiments. Since human hazards had been estimated from Drosophila rates, it was pointed out that the mouse results indicated that extra caution should be taken in drawing up permissible levels of exposure in man. The fact that the ratio of mouse to Drosophila mutation rates is not quite as high in the 1000-r as in the 600-r experiment cannot be taken as grounds for relaxing the caution imposed by the 600-r rate, because 600 r is closer to the dose levels likely to be encountered by man. The 300 r results, as far as they go, certainly show no justification for lowering estimates of hazard based on the 600-r data.

Extensive tests on the nature of the mutations produced in all experiments are continuing. More than one-half the mutations are proving lethal in homozygous condition.

Preliminary analysis of the data from the 600- and 1000-r experiments for effects on viability of the first generation offspring indicate that the genetic hazard to immediate descendants in man may not be negligible. If the 300-r experiment confirms this conclusion, it will be of considerable importance to test the over-all damage in an experiment designed specifically for this purpose.

The Effect of Prenatal Irradiation on Birth Weight in the Mouse

(L. B. Russell, Major)

The following data were obtained some time ago but have not previously been reported in Quarterly Reports.

Mean birth weights were obtained for groups of animals irradiated in stages differing by 24-hour intervals and ranging from day 1/2 to day 13-1/2 after fertilization (Table 3; Fig. 2). Several variables which are known to affect birth weight are controlled: (1) newborns are genetically uniform (F_1 hybrids between two inbred strains); (2) the maternal environment is genetically uniform; (3) parity is controlled, all animals coming from second litters. Other variables, especially litter size, could not be eliminated. Since some of the irradiated young had little or no milk, either because they were moribund or because they had to be obtained by caesarean

TABLE 3

AVERAGE WEIGHT OF NEWBORNS FOLLOWING IRRADIATION WITH DIFFERENT DOSES AT DIFFERENT STAGES OF DEVELOPMENT

	Dose (r)				
Stage treated	0	100	200	300	400
Control	1.42 (196)				
Days 1/2-4-1/2		1.43 (36)[a]	1.43 (33)		
Day 5-1/2		1.33 (8)	1.21 (22)		
Day 6-1/2			1.23 (31)		
Day 7-1/2			1.28 (26)		
Day 8-1/2			1.16 (32)		
Day 9-1/2			1.16 (33)	1.02 (8)	
Day 10-1/2			1.03 (31)	0.77 (22)	
Day 11-1/2		1.25 (54)	1.00 (54)	0.78 (52)	
Day 12-1/2			0.99 (17)	0.84 (24)	0.58 (11)
Day 13-1/2				1.00 (21)	0.69 (16)

[a]The figure in parentheses indicates the number of animals on which the average weight is based.

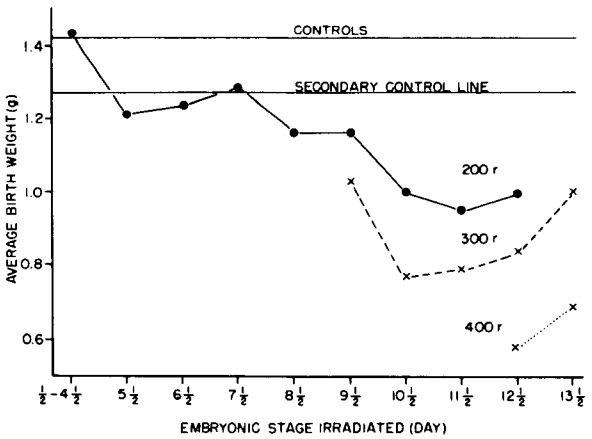

FIG. 2. Mean birth weights following irradiation with different doses of genetically homogenous mouse embryos at different stages in development (see also Table 3).

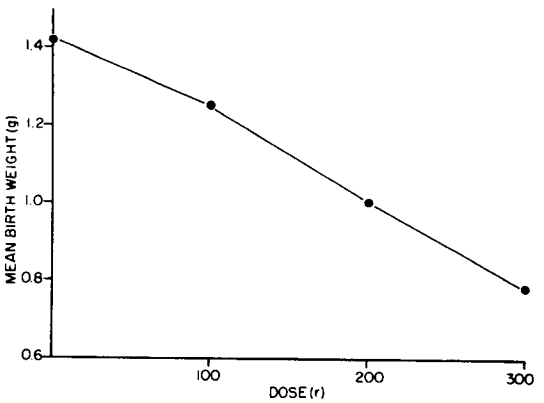

FIG. 3. Mean birth weights following irradiation with different doses of genetically homogenous mouse embryos 11-1/2 days postconception (see also Table 3).

section, a secondary control line was drawn in Fig. 2 to provide a liberal allowance for the amount of milk sucked by the control newborns between birth and weighing. It was found that mean birth weights for the 8-1/2- through 13-1/2-day stages of irradiation fell considerably below that control line. The 200- and 300-r curves were, in general, parallel with a mean difference of 0.2 g. Minima for both curves lay between the 10-1/2- and 11-1/2-day stages. The short portion of the 400-r curve available paralleled the 300-r curve in the rising portion between days 12-1/2 and 13-1/2.

Holding the time of irradiation constant, a more complete dose-weight series was recently obtained for day 11-1/2, which is close to the stage of maximum susceptibility to growth retardation. Points for different doses and for the control are shown in Fig. 3. Weight reduction per 100 r averages 0.22 g over the three available intervals.

PATHOLOGY AND PHYSIOLOGY

PATHOLOGY AND PHYSIOLOGY

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Uptake and Storage of Radioactive Iron by Mouse Liver

(Hampton**, Kahn†)

The method for separating and identifying ferritin and hemosiderin was worked out as follows:

The liver was fractionated into coagulable-insoluble iron compounds (hemosiderin-rich fraction, HRF) and incoagulable-soluble iron compounds (ferritin-rich fractions, FRF). The mice were sacrificed, and livers were removed, blotted, and homogenized with 2 ml of water in a Potter-Elvejhem glass homogenizer. Two 1-ml portions of water sufficed to wash the homogenate into a 15-ml centrifuge tube. The tubes were then suspended in a water bath at 75 °C for 15 minutes, with mild agitation. The coagulum resulting from this treatment was broken up, and the tubes centrifuged. The supernatant was decanted into a 10-ml volumetric flask: the coagulum was then washed twice with 2.5 ml of water, and its supernatants were added to the first. The flask containing the supernatant was filled to the mark and the sample was then ready for radioactivity determinations. This fraction is labeled the FRF. The twice-washed coagulum was digested with concentrated sulfuric acid, and the digest diluted to 10 ml. This fraction was labeled the HRF. Two ml each of the supernatant and the digest were transferred to counting vials for scintillation counting.

Using radioactive iron, the storage in liver of Fe^* injected intravenously into mice was studied. Following injection of 75 μg of iron the liver completed its uptake in about 3 hours. Most of the iron appeared first in the HRF. By the end of 15 hours this fraction had decreased, while a corresponding increase had occurred in the ferritin fraction. The values

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remained constant for the next 33 hours. In the tissue an equilibrium between ferritin and hemosiderin was established with the ferritin-hemosiderin ratio slightly above 1.0. Prior to the establishment of equilibrium this ratio was <1.0 (Table 4).

TABLE 4

RADIOIRON IN FRACTIONS OF MOUSE LIVER AT VARIOUS TIMES FOLLOWING INTRAVENOUS INJECTION OF 75 MICROGRAMS OF Fe*

Group	No. of mice	Mean weight (g)	Time killed after injection (hr)	Effective dose (±SD) (μg)	Mean Fe* in FRF(±SD)	FF(±SD)	HRF(±SD)	Mean FF/HRF (±SD)
A	5	28.2	1	69.21 (±1.47)	14.93 (±1.27)	9.07 (±1.53)	20.90 (±4.64)	0.46 (±0.18)
B	5	32.4	3	67.95 (±6.71)	17.28 (±2.55)	14.08 (±2.15)	22.85 (±4.05)	0.62 (±0.07)
C	5	30.2	7	68.56 (±7.77)	20.16 (±5.38)	15.42 (±4.21)	21.11 (±3.98)	0.73 (±0.07)
D	5	30.9	11	62.61 (±7.11)	22.55 (±4.59)	19.86 (±4.76)	14.79 (±2.17)	1.34 (±0.29)
E	5	32.5	15	64.88 (±3.31)	22.11 (±3.03)	20.59 (±3.03)	17.84 (±1.97)	1.15 (±0.07)
F	6	31.9	48	65.89 (±6.34)	22.01 (±2.66)	18.05 (±2.31)	15.35 (±2.56)	1.18 (±0.13)

All iron values are in terms of micrograms of radioiron. FF = Ferritin (antiserum precipitated). FRF = Ferritin-rich fraction. HRF = Hemosiderin-rich fraction.

SD = standard deviation

A study was made of the effects of varying the size of the iron dose on iron storage in the liver 15 hours after injection. No differences were noted in ferritin-hemosiderin ratios following injected doses which ranged from 12.5-125 μg of iron per mouse (Table 5).

It is suggested that as the tissue takes up iron, it does so in a form having characteristics of hemosiderin. This uptake is rapid. Iron may then be shifted from hemosiderin into a metabolic system which forms ferritin, the rate being such as to establish equilibrium before 15 hours after introduction of iron into the system.

TABLE 5

RADIOIRON IN FRACTIONS OF MOUSE LIVER 15 HOURS FOLLOWING INTRAVENOUS INJECTION OF VARIOUS DOSES OF Fe*

Group	No. of mice	Mean weight (g)	Injected dose (μg)	Mean effective dose(±SD)	Mean Fe* in Dose(±SD)	FF(±SD)	HRF(±SD)	Mean FF/HRF (±SD)
A	6	31.6	12.5	10.5 (±0.47)	2.25 (±0.60)	2.2 (±0.51)	2.06 (±0.41)	1.11 (±0.28)
B	7	28.7	25	23.6 (±1.37)	5.33 (±1.07)	4.62 (±1.10)	5.41 (±0.55)	0.85 (±0.16)
C	6	30.2	50	39.25 (±0.54)	8.94 (±1.78)	7.64 (±1.85)	10.05 (±1.31)	0.78 (±0.22)
D	5	32.5	75	64.88 (±0.24)	22.11 (±3.06)	20.59 (±2.98)	17.84 (±2.17)	1.15 (±0.07)
E	6	28.9	100	83.93 (±1.37)	25.66 (±3.92)	22.94 (±3.99)	21.17 (±4.58)	1.10 (±0.12)
F	7	31.8	125	104.84 (±0.78)	36.93 (±0.75)	36.08 (±2.86)	32.31 (±4.65)	1.13 (±0.20)

All iron values are in terms of micrograms of radioiron. FF = Ferritin (antiserum precipitated). FRF = Ferritin-rich fraction. HRF = Hemosiderin-rich fraction.

SD = standard deviation

Studies on the Incorporation of C^{14} Formate in the Blood Elements and on Platelet Life Span

(Odell, Gamble)

Recent observations made in this section have indicated that the control of erythrocyte loss into the lymph following intravenous administration of platelets into hosts with radiation-induced thrombocytopenia is exceedingly rapid. The meager knowledge of the life span of platelets now available is based on findings in thrombocytopenic or otherwise abnormal hosts and is therefore open to doubt. To fill this gap of knowledge, platelets of rats were labeled in vivo by the administration of C^{14}-labeled formate. For assay, blood was obtained from these rats at various intervals after a single administration of the formate, the blood elements were separated by fractional centrifugation, and the C^{14} activities of the platelets, plasma, and erythrocytes were determined. Plasma activity reached its peak within a day, indicating rapid formate incorporation; whereas platelet activity reached its peak on the third day, indicating utilization of the

plasma pool for platelet synthesis. A plateau of high activity in the platelets lasts for about 2 days and is followed by a rapid decline, suggesting a life span of 4-5 days. The erythrocyte activity of the same rats increases rapidly until the third day and then begins to level off. Fractional administration of formate during a 24-hour period resulted in a higher incorporation rate than single injection. The effects of total-body irradiation on the formation of these blood elements is being investigated.

Induction of Cataracts by Fast Neutrons

(Upton Christenberry, Hurst,* Farbstein, Ledford)

Experiments are in progress designed to determine the threshold cataract-inducing dose of fast neutrons for mice. The effects of neutrons of 14-mev energy (Cockcroft-Walton generator**), neutrons of approximately 2.5 mev (polonium-boron†), γ rays (Co^{60}‡), and 250-kvp X rays are being compared in an effort to establish their relative biological effectiveness (RBE) in cataract induction. Smaller numbers of rabbits and rats have been exposed similarly at carefully selected dose levels in order to compare responses in several species. Neutron- and γ-ray dosages have been fractionated at a single dose level to furnish information regarding additivity of injury, as available evidence points toward elevation of the RBE of neutrons as the duration of exposure is prolonged or irradiation repeated.

The following preliminary observations appear noteworthy: Grade I opacities of the lens appeared within 3 months in mice exposed to 3.3×10^9 n/cm^2 (2.5 mev) or to 333 r of γ or X radiation; and within 4 months after exposure to 100 r. While lesions have not yet been observed in mice exposed to lower dosages of radiation, on the basis of earlier studies their appearance is to be anticipated. In unpublished experiments LAF_1 mice exposed to 25 r developed Grade I opacities of the lens within 9 months, which progressed to Grade II 11 months after irradiation. Rf mice exposed to 16 r developed Grade I opacities later in life, but these failed to advance to Grade II even in old age.

In contrast to the observed sensitivity of mice, rats, and rabbits exposed to 3.3×10^9 n/cm^2 or to 333 r have failed to develop opacities in the first 8 months postirradiation. Data are still too few to permit precise calculation of the RBE of neutrons and γ rays in cataract induction, but it is evident from these experiments that the RBE of fast neutrons is high.

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* Member of the Health Physics Division, ORNL.

** Use of the Cockcroft-Walton generator was kindly made available by Dr. J. L. Meem and Mr. R. G. Cochran, Physics Division, ORNL.

† Source provided by Dr. K. Z. Morgan, Health Physics Division, ORNL.

‡ Source provided by Dr. C. W. Sheppard and Mr. E. B. Darden, Jr., Biology Division, ORNL.

It is also apparent that the marked radiosensitivity of the lens of the mouse's eye is a species characteristic. Evaluation of radiation damage in mice of the C57 black strain was complicated by the relatively high incidence of congenital cataracts encountered in these animals; accordingly, the experiments are being repeated with mice of the Rf strain, which have been found to be virtually free of congenital lenticular lesions. The exposures have been completed, and the animals are being examined periodically as unknowns with the slit lamp and ophthalmoscope.

Assay of Pituitary Tumors and Blood of Tumor-Bearing Hosts for TSH

(Burnett, Gadsden, Bonnie Anderson)

In order to understand the pathogenesis of the tumors induced by I^{131} and to define their character, it was found necessary to assay them quantitatively and qualitatively for hormone production.

The mouse has hitherto not been used by others for TSH assays. Earlier studies in this laboratory have indicated that TSH can be assayed in the mouse by total-body retention of I^{131} following a near tracer dose, as well as by the thyroidal retention of I^{131}. The total-body I^{131} retention (a counterpart of elimination) can be conveniently determined by lowering the entire animal in a high ionization γ chamber. Numerous experiments were performed to standardize this technique, determine its sensitivity, and attempt to refine it.

Figs. 4 and 5 give the response of mice to various quantities of standard TSH.* The results indicate that under the conditions of the experiment the threshold quantity giving a distinct response in mice is in the neighborhood of one Junkmann-Schoellor (J-S) unit. The degree of total-body retention and thyroidal retention are directly related to TSH concentration and can serve for its approximation within the range 1-7.5 units of TSH. The injections alone depress I^{131} retention probably by virtue of the stress reaction.

TSH in the blood of tumor-bearing hosts. —The results summarized in Table 6 indicate that 0.1 ml of plasma of mice bearing dependent tumors caused a marked stimulation of the thyroid of normal mice. In more recent tests, even 0.02 ml gave a distinct enhancement of iodine retention. The concentration of TSH in 0.1 ml of plasma of these mice based on the degree of I^{131} retention can be roughly estimated to be equivalent to 2-3 J-S units of TSH. Smaller quantities of plasma of the same type gave no consistent

* Obtained through courtesy of Parke-Davis & Company.

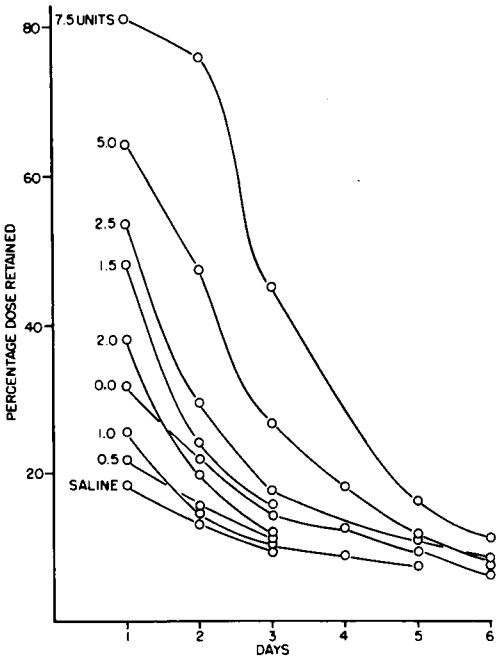

FIG. 4. The retention of a near-tracer dose of I^{131} in the whole body of mice following the injection of a standard TSH preparation.

TSH was given in three equal doses over a period of 3 days in volume of 0.2 ml per injection, the last dose being given 4 hours before the near-tracer dose of I^{131}.

The results of four independent assays are pooled. Each point represents the average value for 3-8 mice. The variability of values is now being reduced by more rigid standardization of the technique.

0.0 = no injection.

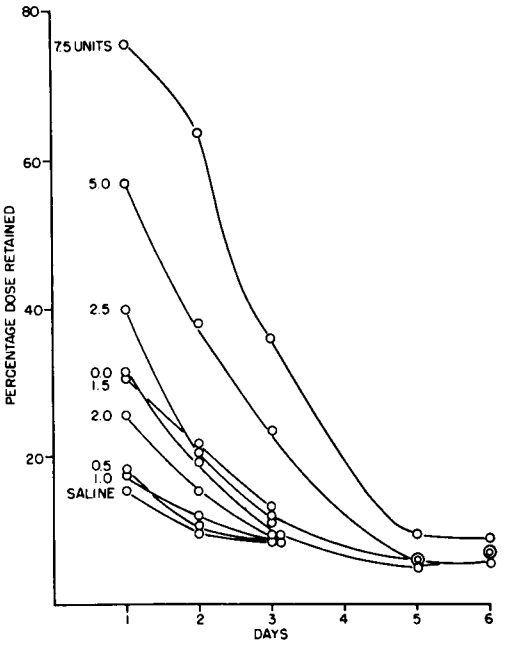

FIG. 5. Retention of a near-tracer dose of I^{131} in the thyroid region of mice following the injection of a standard TSH preparation (see legend to Fig. 4).

response detectable in mice by the isotopic technique used but gave an excellent morphological response in the chick in amounts of 0.02 ml of plasma. Plasma of normal mice bearing autonomous tumors tested in amounts of 0.3 ml gave a slight and questionable stimulative response. The plasma of radiothyroidectomized mice bearing autonomous tumors was inactive in these isotopic tests. Similarly, other experiments, not detailed here, indicate that the TSH content of the plasma of mice bearing autonomous tumors is much less than the plasma of mice bearing dependent tumors. It is the rule that intrinsic and extrinsic secretions of neoplasms diminish following the acquisition of autonomy.

TABLE 6

THE 24-HOUR RETENTION OF A NEAR-TRACER DOSE OF I^{131} IN MICE FOLLOWING INJECTION OF PLASMA FROM TUMOR-BEARING MICE

Assay no. →		22	20	18	16	6	20	16	6
	Volume	Percentage of dose retained in—							
Type of plasma	(ml)	Whole body				Thyroid region			
Saline[a] (control)	0.3	13.2[b]	30.3	28.6	-	14.0	23.7	-	10.0
Normal	0.1	17.1	21.9	-	-	-	19.0	-	-
Normal	0.3	-	-	-	27.7	-	-	12.3	-
Strain 3D[c]	0.02	-	17.8	35.0	-	-	12.7	-	-
Strain 3D	0.1	32.4	55.5	40.3	-	-	47.9	-	-
Strain 3D	0.3	-	-	-	47.6	61.7[d]	-	33.7	63.2[d]
Strain 3A[e]	0.1	-	28.4	-	-	-	21.9	-	-
Strain 3A[e]	0.3	-	-	-	29.7	-	-	16.7	-
Strain 3A[f]	0.3	-	-	-	24.2	-	-	12.4	-

[a]All injections were given in three equal doses over a period of 3 days, the last injection being given 4 hours before tracer dose of I^{131}.
[b]Each value given represents the average for four mice.
[c]D = dependent tumor in radiothyroidectomized host
[d]Serum
[e]Autonomous tumor grown in normal host
[f]Autonomous tumor grown in radiothyroidectomized host

The retention of radioiodine.—Retention of I^{131} of mice kept on a ration low in iodine was determined because it is desirable in future experiments to reduce the dose of I^{131} required for thyroid destruction and thereby minimize the radiation hazard, and to find out if the sensitivity of the host in TSH assays can be raised by reducing total-body iodine. Fig. 6 shows that 4 weeks of a diet low in iodine enhances the iodine-retaining ability of the body and of the thyroid of mice by about fivefold (24-hour values).

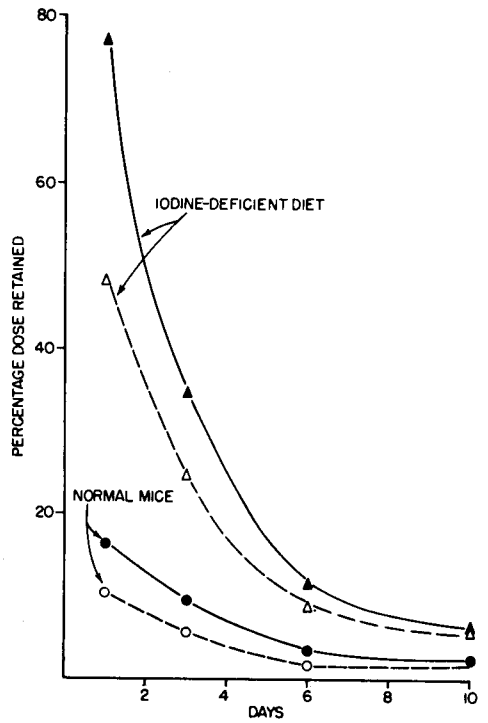

FIG. 6. The retention of a near-tracer dose of I^{131} in the whole body and thyroid region of normal and iodine-deficient mice following administration of 33 μc of I^{131}. Each point is an average of values in 10 mice.

——— = total-body retention

------- = thyroidal retention

Surgical Thyroidectomy

(Dent*)

In order to find out whether irradiation of the pituitary plays some role in the induction of pituitary tumors or whether stimulation given by lack of thyroid alone would result in the development of pituitary tumors, surgical thyroidectomies were performed in a strain of mice known to be highly sensitive to pituitary tumor induction by radiothyroidectomy. These animals are now about 7 months old and iodine uptake indicates that a large number of them are athyroid, the remaining hypothyroid.

In normal mice scintillation counter measurements after a near-tracer dose of I^{131} always gives much higher values in the thyroidal region than

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over the heart or mid-abdomen. In mice in which the thyroid was completely removed by surgery the prethyroidal values were lower than the other two (24-48 hours' determination). Incomplete thyroidectomy was indicated by increase of thyroid uptake values on repetition of the uptake study made a few months after the first test, when sufficient time had elapsed for regeneration of thyroidal remnants.

Conditioned and Autonomous Neoplasms

(Furth)

A study of the sequence of changes following destruction of the thyroid by I^{131}, which leads to the development of pituitary tumors, and that following total-body irradiation, which leads to the development of ovarian tumors, induced us to reconsider current concepts of the nature of cancer and of the essential change in the course of carcinogenesis. An analytic study of the available data supporting our concept on the evolution and nature of cancer has been submitted for publication.

Most current theories regard the basic alteration in cancer as residing not in the host but in the neoplastic cell, be this alteration a somatic mutation, or abnormal differentiation, or affliction of cells with viruses or self-perpetuating particles. In our article, the tumors which are surveyed find their origin in alteration of the host. Such tumors grow with little or no apparent restraint in altered hosts but do not grow in normal hosts until the cells gain autonomy. In such neoplasms, called dependent or conditioned, some cells ultimately become altered and give rise to autonomous growth. Evidence is presented to indicate that autonomy is a quantitative characteristic.

The best experimental material for the study of the principles involved in regulating numbers of cells is at present in the domain of endocrinology. Conditions can be created whereby uncontrolled proliferation of one cell type is obtained, resulting in a tumor-like growth. Creation of such conditions need not involve any intrinsic alteration in cells causing them to behave as cancer cells. Recent studies on the induction and transplantation of pituitary tumors have demonstrated that the change which initiates and maintains this neoplasm resides at first in the host and that, subsequently, modifications occur in both the hosts and neoplastic cells. A survey of the literature discloses that the same sequence of events holds for a large number of neoplasms. In contrast to the current trend to regard the basic change in cancer as a sudden alteration in cells, our analysis points to a sequence of gradual changes from normal through hyperplasia, to conditioned and autonomous growths.

More specifically, our analytic review deals with thyroid and pituitary interdependence in relation to tumor development in these two organs; gonadal-pituitary relationship leading to development of ovarian, testicular, and pituitary tumors; mammary glands and prostate tumors in relation to gonadal hormones; postulated conditioned neoplasm of the skin and other organs, including leukemia. It is concluded that alterations in either the host environment or in cells can give rise to neoplasms. Alteration in host environment may result in development of conditioned neoplasms; these are known to be readily preventable and reversible. Alteration in cells results in the development of neoplasms which are not reversible. Dependency and autonomy are recognized as relative and quantitative terms. Autonomy is never certain. Many autonomous growths pass through a phase of dependent growth, and the usual trend is toward gradual acquisition of greater autonomy. This sequence of events demands that greater attention be given to the study of normal growth-regulating mechanisms and of the very early changes in the evolution of neoplasms. The understanding of these events may ultimately lead to the prophylaxis of some cancers and to their control at their early phases of development.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson (Leader)
E. F. Phares Mary V. Long
M. I. Dolin Suzanne Berger
J. M. Adams*

Studies on the Reaction: Succinate $\rightleftharpoons$ Propionate + Carbon Dioxide

(Carson, Phares, Berger, Long)

Earlier studies (ORNL-1456) on enzyme preparations derived from Propionibacterium pentosaceum indicated that the reversible reaction—succinate $\rightleftharpoons$ propionate + CO_2— is the pathway through which propionic acid is oxidized. Coenzyme A dependence was demonstrated for the reversible reaction, and the presence of the following activated compounds was demonstrated: succinyl-, propionl-, and acetyl-CoA.

Present studies to be reported here show that the reaction is not stoichiometric for CO_2 and propionate, and indicate that a "one-carbon compound" is formed which is not CO_2, nor is at the oxidation-reduction level of either formate or formaldehyde.

Stoichiometry of the reaction: succinate $\rightleftharpoons$ propionate + CO_2 in the presence and in the absence of CoA (Expt. 1). —A cell-free, CoA-free (Dowex-1-treated) juice was used for tests on this reaction both in the presence and in the absence of CoA. The following reagents also were present: cysteine, tris buffer (pH 7.4), potassium adenosinetriphosphate, propionate-2-C^{14}, $C^{14}O_2$, Mg^{++}, and in an atmosphere composed of 90% helium and 10% CO_2. After a 2-hour incubation period, propionate and succinate were isolated, purified, and degraded; the results are presented in Table 7.

The data indicate that, in the presence of CoA, about thirty times more propionate than CO_2 is converted to succinate; and that the ratio of conversion of propionate and of CO_2 to succinate is eight times higher.

Non-stoichiometry of the propionate and CO_2 yield from labeled succinate (Expt. 2). —Totally labeled succinate (carbonyl to methylene ratio of C^{14} specific activity was 7:1) was acted upon by the same type of enzyme system and reagents as in Expt. 1, in the presence of CoA. The total C^{14} activity found in the propionate produced was fourteen times that of

* ORINS Predoctoral Fellow

TABLE 7

SUCCINATE-PROPIONATE EXCHANGE REACTION

Substrates, products, and degradation fragments	+CoA	-CoA
Average $C^{14}O_2$	13.6 *	10.5 *
Initial propionate-2-C^{14}	216 *	216 *
Final propionate-2-C^{14}	87.0 *	85.0 *
Average propionate-2-C^{14}	152 *	150 *
Final succinate-C^{14}	19.0 *	0.63 *
Methylene succinate carbons (I)	38.0 *	1.25 *
Carbonyl succinate carbons (O)	0.055 *	0.0125 *
Ratio I/O in succinate-C^{14} (A)	670	100
Ratio av. prop./av. CO_2 (B)	11.2	14.2
Ratio A/B	60	7.0

* c/s/μM

the CO_2, whereas the theoretical would be 1.3-fold. These data indicate that the one-carbon fragment (from the succinate carbonyl) is not readily converted to CO_2.

Accumulation of succinyl-CoA.—Analysis by spectrophotometric methods of succinyl-CoA accumulation were not possible, since CoA and ATP absorb too strongly in the wave-length region in which succinyl-CoA absorbs.

By the hydroxylamine method, about twice as much hydroxamic acid derivative was formed as in the controls, indicating a small formation of succinyl CoA.

Gamma-ketopimelic acid as an intermediate.—γ-Ketopimelic acid, a possible intermediate compound between succinate and propionate, was synthesized. The compound was added to the same ingredients described under Expt. 2. No C^{14} activity was found in the isolated γ-ketopimelic acid, and hence this compound is probably not an intermediate.

New Degradation Procedure for C^{14}-Labeled Succinic Acid

(Phares, Long)

Tracer experiments of the type described require many degradations of labeled succinic acid. The conventional methods involve many reactions and purification steps.

The Schmidt reaction (at atmospheric pressure) yields 60% of the carbonyl carbons, but only 10% of the methylene carbons. The low yield of the latter is due to the high volatility of the hydrazoic acid. In order to increase the yield of the methylene carbons, the reaction must be run at high pressure. The following compounds were heated at 70°C for 2 hours in a sealed 25-ml tube: 0.50 mM of C^{14}-labeled sodium succinate, 0.50 ml of 100% sulfuric acid, and 1.5 mM of sodium azide. The sulfuric acid residue was made alkaline, and the ethylenediamine (representing the methylene carbons) was recovered by partitioning between water and ethyl ether on a 1 x 5-cm Celite column. The C^{14}-labeled succinate of known specific activity gave 40% of the diamine having the theoretical specific activity.

BIOCHEMISTRY

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

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Elliott Volkin
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B. L. Strehler
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April B. Ottinger
M. Evelyn Purdy
M. J. Cormier

Nucleic Acid Structure

Nucleic acid-protein linkage (Volkin, Jones). —Preliminary experiments have been carried out in an attempt to determine the nature of the linkage between ribonucleic acid and protein in naturally occurring ribonucleoproteins. The sources of these latter materials have been (1) a purified rabbit liver microsome preparation, (2) a crude nucleoprotein from the soluble portion of rabbit liver cytoplasm, and (3) a ribonucleic acid-rich particulate fraction from *Escherichia coli*. It was first determined that these fractions were readily susceptible to hydrolysis by pancreatic ribonuclease. Subsequent to complete ribonuclease hydrolysis, the digests were exhaustively dialyzed, then the protein precipitated with acid. Hydrolysis of these residues with alkali revealed that about 10% of the original nucleotide material was further released by the action of base. Comparable results were obtained when snake venom diesterase was used in place of ribonuclease. Preliminary attempts to identify the protein-bound nucleotide(s) associated with the liver microsomal residue indicate that about 5-10% of the original cytidylic acid, and possibly some guanylic acid, remain attached to protein following the action of the nucleic acid-hydrolyzing enzymes.

Identification of the isomeric a and b adenylic acids (Khym, Doherty, Cohn, Volkin, Ottinger). —The degradative approach to this problem, outlined in the preceding report, has been followed up and is giving the data necessary finally to assign structures to the isomeric nucleotides discovered in this laboratory 4 years ago. Treatment of either one of the adenylic acids with Dowex-50-H^+ (strong-acid cation exchanger in the acid form) brings about the reactions shown in Fig. 7. At 100 °C, the half times of

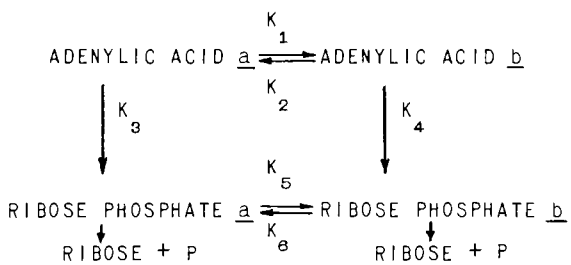

FIG. 7

the reactions shown are approximately 10 and 31 minutes for k_1 and k_2, the isomerizing reactions, and about 90 minutes for k_3 and k_4, the hydrolytic reactions. The half times of the k_5 and k_6 reactions are very long, under these conditions approximately 2000 minutes, due to the nonabsorption of the ribose phosphates on the resin catalyst, and the degradation of the ribose phosphates to free ribose is also very slow. Thus, the derived ribose phosphates come not from each other but from the amounts of their individual precursors present during the hydrolysis period. If this period is made sufficiently short (ca. 30 minutes), so that isomerization of the starting substance (e.g., adenylic acid a) is less than complete, the derived ribose phosphate should come predominantly from that form. Furthermore, the amounts of the two ribose phosphates should be proportional to the average concentrations of the two adenylic acids during the heating period, as calculated from the rate constants and the governing equations (derived for us by Dr. C. W. Sheppard):

$$a_t = a_\infty + b_\infty e^{-(k_1 + k_2)t}$$

$$b_t = b_\infty - b_\infty e^{-(k_1 + k_2)t}$$

$$a_t + b_t = a_\infty + b_\infty = a_0 + b_0$$

where a_t and b_t are the amounts of a and b nucleotide present at time t.

Starting with adenylic acid a, a 30-minute heating period (100°C) results in a 70/30 ratio of adenylic acid b to the a (equilibrium, or $b_\infty:a_\infty$, is about 75:25); the derived ribose phosphates are in the ratio of 57:43 (b:a) which is the ratio of the average amounts of adenylic acids a and b. Similarly, starting with the b isomer, the ribose phosphates are found in the proportion of 95:5, whereas the nucleotides have isomerized to an 80/20 b/a ratio.

From these and similar results, it appears that ribose phosphate a is derived from adenylic acid a. The next question is: what are the structures of the two ribose phosphates? To answer this, gram amounts of the two substances have been prepared on the large (33 cm^2) column and have been converted into various derivatives by nonisomerizing reactions. The reactions shown in Fig. 8 have been carried out. In these reactions, the a ribose phosphate, derived from adenylic acid a, has the properties of the 2-phosphate (optically active ribitol phosphate, oxidizable 1-methyl riboside phosphate) and the b phosphate the properties of the 3. Thus it appears that the original surmise, made 4 years ago, that the b adenylic acid is the 3', was correct and the classical formulation of Levene and Tipson, while derived prior to knowledge of the existence of isomeric forms, is also correct if one assumes, as is likely, that they were dealing with the less soluble b isomer.

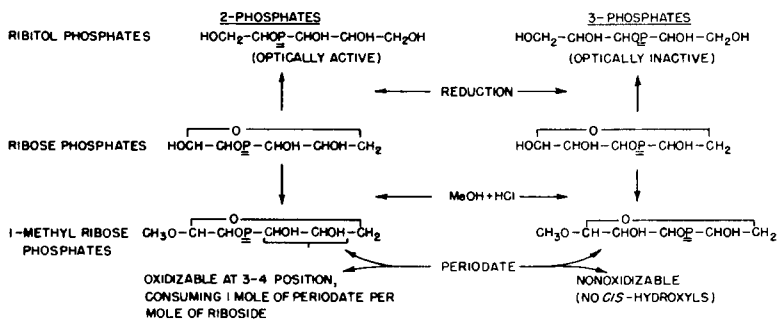

FIG. 8

Further work is planned to identify the acid-stable ribose phosphate which appears on long heating (perhaps the 4-phosphate derivative) and to expand the chemical evidence for the formulation of the a and b nucleotides as the 2' and 3' phosphoribosides.

Enzyme-Substrate Binding Studies

(Doherty, Purdy)

The ready hydrolysis of glyconyl peptide derivatives by enzymes suggested the synthesis of peptide derivatives of the simplest asymmetric aldonic acid, glyceric acid. Since it was desirable to obtain derivatives of both sterioisomers of glyceric acid, the resolution of DL-glyceric acid via the quinine salts was reinvestigated. Utilization of solvent systems different from those originally reported effected a good separation in reasonable yields of D- and L-glyceric acids. A literature survey revealed the lack of suitable glyceric acid intermediates for the synthesis of peptide derivatives. Therefore, the following series of reactions yielding crystalline derivatives in good yields at each step with DL-, D- and L-glyceric acids was developed. Glyceric acid was converted to the p-nitrobenzyl ester and benzoylated in pyridine to yield dibenzoyl-p-nitrobenzyl glycerate. This compound was reduced with Pd-H_2 to dibenzoyl glyceric acid and converted to dibenzoyl glyceryl chloride with phosphorus pentachloride. This acid chloride was coupled to the amino acid esters in the usual manner and the coupling product converted to the amide with methanolic ammonia. Crystalline amides were obtained with L-phenylalanine and L-tyrosine. The tyrosine amides were readily hydrolyzed by crystalline α-chymotrypsin.

Energy-Transfer Systems

(Strehler)

Bioluminescence: bacterial extract luminescence. —It has been found possible, for the first time, to obtain luminescence from cell- and particle-free extracts of luminous bacteria.

Some time ago, Strehler and Shoup* found that acetone-dried powders of the luminous bacterium, Achromobacter fischeri, would emit light for some minutes after suspending the powders in water. It has now become possible to prolong the luminescence for an indefinite period. The sensitive quantum counter apparatus available to us permitted a study to be made of physical and chemical factors influencing the luminescence of bacterial powders and, as a result, conditions for obtaining luminescence in cell-free systems were uncovered.

If the extracts were incubated at room temperature for an hour or so the luminescence was found to disappear. But subsequent addition of boiled extracts of A. fischeri effected the reappearance of light. Upon examining various biological intermediates it was found that DPN (diphosphopyridine-nucleotide) would replace the boiled bacterial extracts in this function. Reduced DPN gives a more immediate response, indicating that DPN gives rise to DPNH, the active intermediate, through the action of a DPN reductase in the extracts. The dinucleotide is apparently made rate limiting in the extracts through the action of a powerful DPNase.

While sufficient details of the process are at present unavailable to settle the function of DPN in bacterial extract luminescences, these preliminary findings are of interest for several reasons: first, aside from the previously published negative results, the activation of luminescence by an important biological electron-transfer agent such as DPN demonstrates again the comparative biochemical importance of this redox-system. Second, the sensitivity of the system to added DPN (0.1 μg gives a measurable response) should make available a rapid, sensitive, and specific test for this important intermediate. The application of techniques derived from this finding to certain problems in photosynthesis is projected as well as a study of certain aspects of oxidative phosphorylation.

Bioluminescence: firefly luminescence. —In cooperation with J. R. Totter of the Radiation Protection group, an attempt has been made to make use of the following reaction for DPN and pyrophosphate assay.

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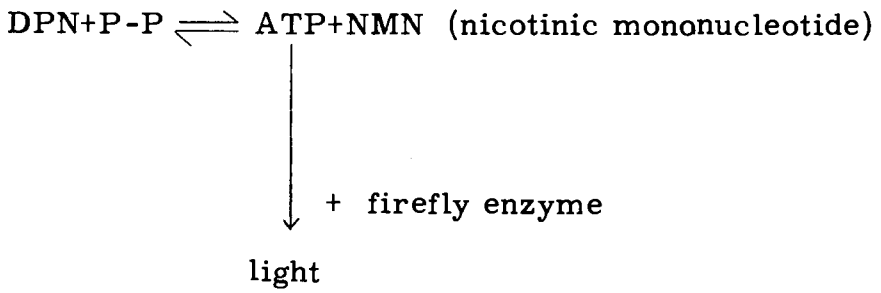

Preliminary results with a Kornberg enzyme prepared from yeast indicate the possible applicability of this method.

Oxidative phosphorylation (with Totter, Radiation Protection). —In order to test the possibility that oxidative phosphorylation involves the rapid turnover of dinucleotides important in oxidoreduction, radioactive adenylic acid was prepared using a yeast culture and radioactive formate. The radioactive adenylic acid was isolated by ion exchange and incubated with rabbit liver mitochondria in the presence and absence of substrate, the phenol extract of the mitochondria was paper-chromatographed and radioactive spots localized. Although the known oxidation-reduction dinucleotides were not appreciably affected, labeling did appear in other unknown fractions of the extract. It is hoped that these fractions can be isolated and identified.

PLANT BIOCHEMISTRY

N. E. Tolbert (Leader)
L. P. Zill Betty Ann Gwin

Biosynthesis of C^{14}-labeled sedoheptulose. —To isolate this seven-carbon sugar from plant saps it has been necessary to convert the free sedoheptulose to the dianhydride form (sedoheptulosan) by boiling in acid. This form will crystalize from a mixture of sugars but the sedoheptulosan has several disadvantages for biological work. By paper chromatography it can be shown to be contaminated with a small amount of glucose, and it requires repeated recrystallization to be obtained in a pure form, which is difficult when only small amounts of C^{14}-labeled material is available. More important, the sedoheptulosan represents an unphysiological form and its conversion back to the free sugar is only possible in 20% yield. The other 80% remains in the solution and gives rise to the possibility of competitive inhibition of enzyme systems to be studied.

In order to effect the separation of C^{14}-labeled free sedoheptulose from other sugars (chiefly fructose, glucose, and sucrose as shown by paper chromatography) found in the syrup from plant saps, an attempt was made to separate their borate complexes by ion-exchange chromatography. Although it was found that sucrose and glucose could be quantitatively removed, it was not possible to separate the sedoheptulose and fructose under the conditions used. The exchanger was a Dowex-1 column 0.85 sq cm x 2.5 cm, the borate form; eluting agents were potassium tetraborate solutions at a flow rate of 1.5 ml/minute. Since an exhaustive study of this problem was not made, it is possible that other conditions may be found to effect a separation of these two keto sugars.

The free sedoheptulose labeled with C^{14} has been isolated in milligram quantities by paper chromatography using thick paper chromatograms (Whatman 3MM) with a strip of Whatman 1 paper sewed along one edge. This strip dips into the solvent and acts as a wick and a valve to force the slow development of the thick paper chromatogram. The isolation of the sedoheptulose from sap of Sedum spectabile was carried out as previously described up to the conversion of the anhydride. The syrup (0.6 ml) containing roughly 100 mg of the sugar mixture was placed along the edge of the 3MM paper next to the Whatman 1 wick and developed in butanol-propionic acid water solvent for 48 hours. The sedoheptulose portion of the chromatogram was then cut out and run again in phenol and water, to separate it from a small amount of contaminating glucose.

Effects of near-infrared light on wheat and potatoes. —Thatcher wheat seeds moistened on cheesecloth, or planted in sand, were allowed to grow in total darkness, in sun light, and in the infrared light coming through a Corning #2600 filter which transmits between 7200 and 11,000 A with a maximum of about 80% transmission at 8000 A. A light source for the infrared was a 200-watt bulb with the far infrared removed by a water filter. The leaves grown in the dark etiolated with the outer colorless sheath elongating while the first leaves were stunted and remained far down inside the sheath. In the plants grown in the light the sheath ceased elongation upon coming through the soil and being exposed to light; the leaves then developed normally. In infrared light, the growth of the sheath and leaves appeared identical to the plants grown in the light except that they were without chlorophyll and appeared yellow from other pigments, such as the carotenoids. Thus the infrared light inhibited the growth of the sheath and stimulated growth of the leaves and development of yellow pigments.

Both the dark-grown and infrared-grown thatcher wheat plants contained little glycolic acid oxidase enzyme while the green plant contained a very large amount of this enzyme.

Sprouting of potatoes grown in the dark and in infrared light was compared. The potatoes were stored on moist cheesecloth. The potatoes in the dark developed numerous sprouts in a few days and the sprouts elongated rapidly. The potatoes under the near-infrared light did not sprout for a period several times longer and, when they did so, a smaller number of sprouts were formed and the elongation was much slower.

BIOPHYSICS

GENERAL PHYSIOLOGY

C. W. Sheppard (Acting Group Leader)
J. S. Kirby-Smith E. B. Darden, Jr.
N. G. Anderson Patty J. Mathias
Doris L. Craig

Radiological Physics

(Darden, Sheppard, Kirby-Smith)

Experimental results on the distribution of energies of β rays emitted by P^{32} bakelite discs have been compared with theoretical distributions established from such basic physical parameters as energy loss and collision cross section. Expressions for the energy distribution produced by emission of monoenergetic β rays from such sources are obtained by integration of the Boltzmann equation. For the high-energy end, the energies are obtained by the P_1 approximation of Weymouth, using the method of Laplace transforms but constant transport mean free path. At the lower end, the age method is applicable. The solution of the equations is mathematically difficult but can be obtained by the use of an electrical analog network. In the intermediate energy range, the two extreme approximations merge satisfactorily. Integration of these energy distributions over the P^{32} β-ray spectrum yields electron distributions which are in fair agreement with experimental results. Expressions are being developed for use in correcting for the backscatter of β rays from the anthracene crystal of the spectrometer. Also, theoretical corrections for absorption by the thin aluminum covering on the crystal are being derived.

Experimental determinations of the P^{32} spectrum from a phosphorus-bakelite disc in contact with the aluminum-covered scintillation crystal show a broad maximum in the region of 0.3-0.4 Mev. Experiments to observe the exact effect of the aluminum covering have been initiated. Preliminary results show that the foil produces a small attenuation but no significant change in the shape of the spectrum.

Neutron dosimetry studies are continuing in the ORNL 86-inch cyclotron. A larger lead-enclosed facility which can contain 20-30 mice has been installed; preliminary investigation indicates fluxes up to 3×10^8 neutrons/cm^2/sec, or about one-third the flux in the small facility. Some increase in relative γ-ray contamination is suggested. Small condenser type ion chambers with polyethylene electrodes coated with very thin conducting layers of aquadag have been successfully tested. The chambers are filled with ethylene gas. A Rossi type neutron dosimeter has been constructed and awaits filling with "tissue equivalent" gas.

Modification of the supply and control circuits of the Maxitron X-ray installation to permit operation of the X-ray tube at very low currents is in progress. These changes are being carried out to permit a study of the X-ray energy spectrum using the scintillation spectrometer.

Paramagnetic Resonance

(Kirby-Smith)

Equipment for the observation and study of paramagnetic resonance phenomena has been designed. Procurement of the required microwave apparatus and construction of a suitable magnet is under way.

Tradescantia Studies

(Kirby-Smith, Craig)

Dose-aberration frequency curves for *Tradescantia* microspores exposed to neutrons in the ORNL 86-inch cyclotron facility have been obtained for 4-day chromosomes. These preliminary results are in agreement with the curves of Conger and Giles for a fission neutron spectrum. The energy spectrum of the neutrons used in the present experiments appears to be lower than that for the fission neutrons, indicating energy independence of aberration frequency over the range of neutron energies.

Erythrocytes

(Mathias, Sheppard)

The use of the isolated ventricle of *Venus mercenaria* in the determination of acetylcholine content of tissue preparations has been initiated. A modification of a method successfully used in other laboratories has been found satisfactory. The method lacks as many confirmatory tests for the choline ester as provided by the frog rectus but is capable of considerably greater sensitivity. The new method has been used in preliminary experiments to determine the acetylcholine content of extracts of human blood, and to search for evidence of choline acetylase activity. As yet the results have been entirely negative.

State of Desoxyribonucleic Acid in Various Rat Tissues

(Anderson)

Investigation of the native state of desoxyribonucleic acid (DNA) in the cell has continued, using the procedure described in ORNL-1456. The considerable difference between the viscosity of these preparations and

purified preparations previously described has been accounted for by the finding that a number of procedures generally used in the purification of this substance depolymerize the native nucleic acid extensively.

The possibility that combination with protein or ribonucleic acid may be partially responsible for the high viscosity observed has been investigated by the use of enzymes and denaturants. Crystalline trypsin and chymostrypsin were shown to increase the viscosity, presumably because of the release of more DNA from protein combination. Urea and guanidine hydrochloride (4 M) which are strong protein denaturants also increased the viscosity. Ribonuclease has no effect. Treatment with desoxyribonuclease caused the viscosity to fall nearly to that of the salt solutions used as solvents. These observations support the view that the native substance is more highly polymerized than previously thought, and that the high viscosity observed in crude, rapidly prepared 1 M sodium chloride tissue suspensions is due almost entirely to the DNA present. Preparations containing only 0.045 mg of DNA/ml had a viscosity of 6 relative to that of a desoxyribonuclease-treated control. Previously available preparations generally required about 2 mg/ml to give comparable viscosities.

PHOTOSYNTHESIS

W. A. Arnold (Leader) M. Eleanor Schumacher

An infrared-sensitive photomultiplier was obtained from Dr. G. A. Morton of the RCA laboratories. With this tube we have been successful in getting a phosphorescent emission signal (with 1 mm^3/ml of Chlorella pyrenoidosa) that is fifty to one hundred times greater than the signal obtained with the combination infrared image tube and 5819 that was formerly used. However, at room temperature, the emission signal to the dark current fluctuation ratio is only twice that of the infrared image tube and 5819. This ratio must be at least ten times greater in order to distinguish chlorophyll A and chlorophyll B. At present we are working on a means of cooling the infrared-sensitive photomultiplier so as to increase the ratio.