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DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH
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Administration

Austin M. Brues, Director
 Leonidas D. Marinelli, Associate
 Director
 E. Lawrence Powers, Associate
 Director
 Rosson L. Cardwell, Assistant
 to the Director

A Conference on the Mineral Constituents of Bone was held under the auspices of the Division on January 14 and the morning of January 15 and was attended by one-hundred fifty-five investigators. The Division also sponsored the Carbon-14 Toxicity Conference that convened on the afternoon of January 15 and on January 16. Forty investigators participated in the carbon meeting.

PERSONNEL CHANGES

Dr. John W. Clark has joined the staff as an associate radiologist for a period of one year, under the Participating Institution plan. He graduated from the Harvard Medical School in 1943 and has been a staff radiologist at Presbyterian Hospital in Chicago and assistant professor at the University of Illinois Medical School. Dr. Clark will work with Dr. Howard H. Vogel, Jr., on problems of dosimetry associated with the neutron radiobiology program.

Dr. Daniel E. Atkinson has accepted a position on the faculty of the University of California at Los Angeles. He had been working with Dr. Solon Gordon on the mechanism of the plant growth hormone action.

BUILDING PROGRESS

Construction of the new building has progressed rapidly during this quarter. It seems likely that occupancy will take place during late spring or early summer. Most of the exterior work on the building has been completed, and many of the partitions and much of the electrical, ventilating, and plumbing work have been finished. Grading and landscaping will begin as soon as the weather permits.

Editorial

Antreen Pfau
Jane Williams
Helen Supert

An exhibit on radium toxicity, which was displayed at the 37th Annual Meeting of the Radiological Society of North America, Incorporated, December 2-7, 1951, Chicago, Illinois, was granted the Certificate of Merit award. The exhibit was based on work by L. D. Marinelli, W. B. Looney, R. J. Hasterlik, A. M. Brues, W. P. Norris, and A. F. Stehney. It was prepared by members of the Graphic Arts Department.

A Certificate of Merit for Research of Scientific Value award was granted by the American Academy of Orthopedic Surgeons, Incorporated, to W. B. Looney, R. J. Hasterlik, A. M. Brues, L. D. Marinelli, W. P. Norris, and A. F. Stehney, for their exhibit entitled, The Clinical Investigation of Chronic Radium Toxicity. The exhibit was executed by members of the Graphic Arts Department and Miss Frances Fee, staff artist. It was held at the Palmer House, Chicago, Illinois, January 26-31, 1952.

Publications and talks presented by staff members during the period covered by this report are given by title below.

PUBLICATIONS

Lorenz, E., D. Uphoff, T. R. Reid, and E. Shelton: Modification of Irradiation Injury in Mice and Guinea Pigs by Bone Marrow Injections. J. Natl. Cancer Inst., 12:197-201, August, 1951 (with National Cancer Institute).

Wassermann, F.: Electron Microscopic Study of the Submicroscopic Network of Fibrils as a Component of Connective Tissue. Anat. Record, 111:145-169, October, 1951.

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Powers, E. L. and D. Shefner: Tritium-induced Effects in Paramecium aurelia. Proc. Soc. Exptl. Biol. Med., 78:(2) 493-497, November, 1951.

Ehret, C. F.: The Effects of Visible, Ultraviolet and X irradiation on the Mating Reaction in Paramecium bursaria. Anat. Record, 111:528, November, 1951 (Abstract).

Tahmisian, T. N.: Mechanics of Cell Division. I. The Living Spindle. Proc. Soc. Exptl. Biol. Med., 78:(2) 444-447, November, 1951.

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Tahmisian, T. N., D. M. Adamson, and J. V. Passonneau: The Response of Melanoplus differentialis Cells to X irradiation Before and After Induction. Anat. Record, 111:(3) 158, November, 1951 (Abstract).

Vogel, H. H., Jr.: Cataract Formation and Retinal Damage Following X irradiation of the Heads of Newborn Mice. Anat. Record, 111:497, November, 1951 (Abstract).

Vogel, H. H., Jr.: Observations on the Social Behavior of a Group of Laboratory Mice Reared in a Multiple-escape Pen. Anat. Record., 111:490, November, 1951 (Abstract).

Barron, E. S. G.: Effects of Ionizing Radiations on Sulfhydryl Systems, in Biological Antioxidants, Transactions of the Fifth Conference, November 30 - December 1, 1951, New York, New York, Josiah Macy, Jr. Foundation, pp. 81-114.

Brues, A. M.: General Introduction to Radiation Effects on Higher Animals in Biological Antioxidants, Transactions of the Fifth Conference, November 30 - December 1, 1951, New York, New York, Josiah Macy, Jr. Foundation, pp. 41-80.

Arnold J. S.: Metabolism of Bone as Studied by Radioautographic Distribution of Calcium, Plutonium, and Radium. Am. J. Physiol., 167:765, December, 1951 (Abstract).

Patt, H. M., M. E. Blackford, R. L. Straube, and D. E. Smith: Effect of X rays on Thymocytes and its Modification by Chemical Means. Am. J. Physiol., 167:815, December, 1951 (Abstract).

Smith, D. E., E. B. Tyree, H. M. Patt, and E. Jackson: Effect of Total-body X irradiation on Metabolism of the Rat. Proc. Soc. Exptl. Biol. Med., 78:774-777, December, 1951.

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Allen, J. G.: Problems of Homologous Serum Jaundice after Plasma Transfusion. AMA Arch. Surg., 64:(1) 1-4, January, 1952 (Editorial).

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TALKS

Jacobson, L. O.: Factors Concerned in Recovery from Radiation Injury. Annual Meeting of the Hematology Club, Drake Hotel, Chicago, Illinois, November 1, 1951.

Allen, J. G. and C. E. Basinger: Effect of Intravenous Injection of Thromboplastin and Thrombin in Dogs. Central Society for Clinical Research, Chicago, Illinois, November 3, 1951.

Hasterlik, R. J.: Twenty-year Follow-up Study on Patients Injected with Radium Chloride. Central Society for Clinical Research, Chicago, Illinois, November 3, 1951.

Allen, J. G.: Radiation Injuries. American College of Surgeons, San Francisco, California, November 6, 1951.

Allen, J. G.: Effect of Intravenous Injection of Thromboplastin and Thrombin in Dogs. American College of Surgeons, San Francisco, California, November 7, 1951.

Patt, H. M.: Protective Mechanisms in Radiation Action. Memorial Center for Cancer and Allied Diseases, New York, New York, November 7, 1951.

Jacobson, L. O.: Spleen Shielding and Transplants on Recovery from Radiation Injury. Conference on Regeneration, American Institute of Biological Sciences, Bethesda, Maryland, November 9, 1951.

Patt, H. M.: Chemical Protection and Regeneration. Conference on Regeneration, American Institute of Biological Sciences, Bethesda, Maryland, November 9-10, 1951.

Patt, H. M.: Factors Influencing Recovery from the Effects of Radiation. Conference on Regeneration, American Institute of Biological Sciences, Bethesda, Maryland, November 9-10, 1951.

Hasterlik, R. J.: Treatment of Atomic Radiation Injuries. Northwestern University Medical School, Chicago, Illinois, November 14, 1951.

Barron, E. S. G.: The Mechanism of Ionizing Radiations. Sloan-Kettering Institute for Cancer Research, New York, New York, November 21, 1951.

Schubert, J.: Chemical Approach to the Treatment of Beryllium Poisoning. Chicago Section, American Chemical Society, Chicago, Illinois, November 23, 1951.

Hasterlik, R. J.: Implications of Atomic Energy for Industrial Health Programs. Industrial Health Class of the Department of Nursing Education, University of Chicago, Chicago, Illinois, November 29, 1951.

Jacobson, L. O.: Medical Efforts to Meet an Emergency. Nursing Services Department of the Chicago Chapter of the American Red Cross, Chicago, Illinois, November 29, 1951.

Hasterlik, R. J., W. B. Looney, and A. M. Brues: Late Skeletal Changes in Radium-treated Patients. Radiological Society of North America, Chicago, Illinois, December 2-7, 1951.

Marinelli, L. D., A. F. Stehney, and W. P. Norris: Determination of Radium Content in the Human Body. Radiological Society of North America, Chicago, Illinois, December 2-7, 1951.

Looney, W. B.: Biological Effects of External Irradiation. Illinois Dental Alumni Association, University of Illinois, Chicago, Illinois, December 5, 1951.

Allen, J. G., C. E. Basinger, J. J. Landy, and P. V. Moulder: Surgical Hemorrhage Caused by Blood Transfusion. Chicago Surgical Society, Chicago, Illinois, December 7, 1951.

Allen, J. G.: Discussion of a paper by Hugo Baum. The Possible Role of Hyperheperinemia in Human Sterility. Chicago Gynecological Society, Chicago, Illinois, December 21, 1951.

Stroud, C. P.: An Application of Factor Analysis to the Systematics of the Genus *Kalotermes*. Symposium on Statistical Problems in Experimentation. American Statistical Association, Boston, Massachusetts, December 26, 1951.

Ehret, C. F.: The Effects of Visible, Ultraviolet and X irradiation on the Mating Reaction in *Paramecium bursaria*. 48th Annual Meeting of the American Society of Zoologists, Philadelphia, Pennsylvania, December 27-30, 1951.

Tahmisian, T. N., D. M. Adamson, and J. V. Passonneau: Relative Sensitivity of Resting, Dividing and Differentiating Embryonic Cells to X irradiation. American Society of Zoologists, 118th Annual Meeting of the American Association for the Advancement of Science, Philadelphia, Pennsylvania, December 27-30, 1951.

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Vogel, H. H., Jr.: Cataract Formation and Retinal Damage Following X irradiation of the Heads of Newborn Mice. American Society of Zoologists, 118th Annual Meeting of the American Association for the Advancement of Science, Philadelphia, Pennsylvania, December 27-30, 1951.

Vogel, H. H., Jr.: Observations on the Social Behavior of a Group of Laboratory Mice Reared in a Multiple-escape Pen. American Society of Zoologists, 118th Annual Meeting of the American Association for the Advancement of Science, Philadelphia, Pennsylvania, December 27-30, 1951.

Jacobson, L. O.: Studies on Factors Influencing Recovery from Radiation Injury. United States Public Health Service, Institutes of Health, Bethesda, Maryland, January 7, 1952.

Lindenbaum, A.: Implications of New Knowledge about Proteins, Protein Enzymes, and Cells. Conference sponsored by the University Laboratory of Physical Chemistry, related to Medicine and Public Health, Harvard University, Boston, Massachusetts, January 7-8, 1952.

Allen, J. G.: Problems of Fluids and Electrolyte Balance in Surgery. American Academy of General Practice, Billings Hospital, Chicago, Illinois, January 9, 1952.

Looney, W. B.: The Clinical Investigation of Chronic Radium Toxicity. Chicago Roentgen Society, Chicago, Illinois, January 10, 1952.

Allen, J. G.: Anticoagulants in Surgery. American Academy of General Practice. Billings Hospital, Chicago, Illinois, January 12, 1952.

Buchanan, D. L.: Deposition and Turnover of C^{14} in Bone Carbonate. Conference on the Mineral Constituents of Bone, Museum of Science and Industry, Chicago, Illinois, January 14-15, 1952.

Looney, W. B.: Clinical and Pathological Studies on the Deposition of Radium in the Bones of Man. Conference on the Mineral Constituents of Bone, Museum of Science and Industry, Chicago, Illinois, January 14-15, 1952.

Norris, W. P.: Identification of Bone Constituents by Neutron Activation and Electromigration. Conference on the Mineral Constituents of Bone, Museum of Science and Industry, Chicago, Illinois, January 14-15, 1952.

Schubert, J.: Ion Exchange Physical Adsorption Concepts in Relation to Deposition of Radioactive Elements in Bone. Conference on the Mineral Constituents of Bone, Museum of Science and Industry, Chicago, Illinois, January 14-15, 1952.

Ehret, C. E.: Influence of Radiation upon the Sexual Response of P. bursaria. Botany Club Seminar, University of Chicago, Chicago, Illinois, January 21, 1952.

Schubert, J.: Ion Exchange Studies of Complex Ions. Physical Chemistry, Chemistry Department, University of Chicago, Chicago, Illinois, January 21, 1952.

Looney, W. B. and J. R. Blayney: Dental Roentgenographic Changes in Cases of Chronic Radium Absorption. Zoller Tuesday Seminar, University of Chicago, Chicago, Illinois, January 22, 1952.

Brues, A. M.: Medical Uses and Hazards of Radioactive Isotopes. Cancer Council of Cincinnati, Cincinnati, Ohio, January 23, 1952.

Jacobson, L. O.: Recovery from Radiation Injury. Joint Meeting of USAF Radiation Laboratory and USAF School of Aviation Medicine, Chicago, Illinois, January 28, 1952.

Scully, N. J.: Growth and Development of Red Kidney Bean and Opium Poppy in Relation to Photoperiod. Botany Club, University of Chicago, Chicago, Illinois, January 28, 1952.

Brues, A. M.: Basic Radiobiological Actions. University Comte, Harvard Medical School, Boston, Massachusetts, January 29-31, 1952.

Brues, A. M.: Quantitative Approaches to Understanding Cancer. Medical School Students and Faculty, Harvard Medical School, Boston, Massachusetts, January 29-31, 1952.

Brues, A. M.: Radiobiological Problems in General. Informal talk to Laboratory Staff, Massachusetts General Hospital, Boston, Massachusetts, January 29-31, 1952.

PROGRESS REPORT: MODIFICATION OF IRRADIATION INJURY
BY BONE MARROW INJECTIONS

E. Lorenz, D. Uphoff, and C. Congdon

Investigations are in progress to obtain as complete a histologic picture as possible after serial killings of bone marrow-treated mice. Since complete data are not available at the present time, a discussion of the findings will be reported later.

New data on survival under varying experimental conditions are given in Table 1. In this table a few data listed already in Table 1 of the previous report⁽¹⁾ are repeated for clearer presentation. The protection of homologous bone marrow injections was studied in a fourth strain of mice, strain L; experiments 2 and 3 show that both intravenous and intraperitoneal injections also afford good protection in this strain. In experiment 5 homologous bone marrow suspended in buffered saline was aged by letting it stand at room temperature for one hour before it was injected into irradiated mice. There is no significant difference in mortality of the two groups. In Figures 1 and 2 the picture of the circulating blood is plotted for the mice of the two experiments. A slight, but significant, delay in the recovery of leucocytes and reticulocytes in the two groups is observed on the seventh day, while the erythrocyte counts show no differences. This indicates that the factors in the bone marrow responsible for the recovery are little, if at all, affected by letting the bone marrow suspension stand for one hour. Since it has not been possible thus far to demonstrate the tissues in which intravenously injected bone marrow will be deposited, an experiment (9) was set up in which the bone marrow was injected into the spleen of C₃Hb mice irradiated with 900 r. The mortality in this group was 68 per cent. Incomplete histologic studies after serial killings of these mice indicate rapid spread of the injected cells, since within a few hours after injection the injected cells could not be identified. However, four to five days later the site of injection of the spleen showed large patches of hematopoietic cells, while the remainder of the hematopoietic tissue of the spleen was depleted. This hematopoietic activity in the spleen is slightly ahead of that observed after intravenous injection.

To obtain information on the dependence of the recovery upon the amount of bone marrow injected one group of strain A mice was given bone marrow intravenously obtained from the four long bones of a donor

Table I

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EFFECTS OF HOMOLOGOUS BONE MARROW INJECTIONS ON SURVIVAL OF MICE EXPOSED TO X RADIATION

Exp.	Dose (r)	Treatment	Strain	Animals (no.)	Number dying on indicated number of days																Mortality (%)	Remarks
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16-21		
1	700	None	L	39					5	1	9	4	4	4	3	3				2	90	
2	900	I.P.	L	20					2	2	2	2									40	
3	900	I.V.	L	20									2	1					1	2	30	
4	900	None	LAF ₁	28					2	4	4	9	4	3						2	100	
5	900	I.V.	LAF ₁	30				1		1		2		1							17	
6	900	I.V.	LAF ₁	10																1	10	Bone marrow injected 1 hr after collecting
7	900	None	C ₃ Hb	60				2	4	9	20	8	8	3	6						100	
8	900	I.V.	C ₃ Hb	56					2	6	6	2								2	32	
9	900	Spleen	C ₃ Hb	28			2	1	4	4	4					1				3	68	
10	900	None	A	22			2	12	6	1			1								100	
11	900	I.V.	A	20																	0	
12	900	I.V.	A	16			1							2							19	
13	900	I.V.	A	20								1	1								10	1/2 the bone marrow
14	900	I.V.	A	20																	0	Bone marrow 4 hr after irradiation
15	900	I.V.	A	16				1	1	6	2		1				1				75	Bone marrow 24 hr after irradiation
16	900	I.V.	A	20																	0	Bone marrow 32 hr after irradiation
17	900	I.V.	A	16						1			1								12	Bone marrow 48 hr after irradiation
18	900	I.V.	A	15						3	2	2	2	1			1				73	Bone marrow 72 hr after irradiation

Figure 1. Recovery of the erythrocyte count and per cent reticulocytes in LAF₁ mice following a total-body dose of 900 r after injection of fresh and aged bone marrow.

Figure 2. Recovery of the leucocyte count and per cent reticulocytes in LAF₁ mice following a total-body dose of 900 r after injection of fresh and aged bone marrow.

(approximately 1.5 mg) while another group received half this amount (experiments 12 and 13). Survival in these groups is approximately the same and the recovery of the picture of the circulating blood is not significantly different (Figures 3 to 6).

Experiments 14 to 18 were undertaken to study the time that can elapse between irradiation and bone marrow injection in order to obtain still significant recovery following a total-body dose of 900 r. These experiments indicate that excellent protection results when the time interval is increased from 4 to 48 hours and some protection is present for a time interval of 72 hours. Figures 5 and 6 give the picture of the circulating blood. The delay of the recovery in dependence upon time of bone marrow injection is apparent in these curves. At present failure to obtain excellent protection for a 24-hour interval cannot be explained.

Attempts have been made to study the cells of the hematopoietic tissues which are responsible for the recovery and whether or not tumor cells originating in hematopoietic tissues will also enhance recovery. A tissue brei of a lymphosarcoma which originated in the thymus of an irradiated strain A mouse was injected intravenously into mice of the same strain following a total-body dose of 900 r. All animals died within nine days after irradiation. Further experiments with other tumors are in progress. Improved survival was obtained, however, with intravenous injections of a brei of thymus tissue following a total-body dose of 900 r. The results are merely suggestive. Unfortunately 50 per cent of the injected animals died within a few minutes after injection because of pulmonary embolism, since the amount of tissue injected was too large. Three of the 6 animals surviving the injection survived the 21-day period.

Sufficient data have been accumulated to show that intravenous homologous bone marrow injections are also effective in chronic irradiation anemia. Sixteen guinea pigs of family 2 exposed to 8.8 r daily (8 hours) were removed from the exposure field when their erythrocyte count had reached approximately 2 million. They were injected intravenously with approximately 100 mg of bone marrow within one day after termination of the exposure. Fourteen are well on the way to recovery and only two died. In a previous experiment⁽²⁾ it was shown that guinea pigs of family 2 exposed under identical conditions invariably died after the exposure was terminated if their erythrocyte count was approximately 2 million.

Figure 3. Recovery of the erythrocyte count and per cent reticulocytes in strain A mice following a total-body dose of 900 r after injection of different amounts of bone marrow.

Figure 4. Recovery of the leucocyte count and per cent reticulocytes in strain A mice following a total-body dose of 900 r after injection of different amounts of bone marrow.

Figure 5. Recovery of the erythrocyte count and reticulocytes of strain A mice injected at 0, 24, 48, and 72 hours following irradiation with 900 r. (Arrows indicate time of injection.)

Figure 6. Recovery of the leucocyte count and reticulocytes of strain A mice injected at 0, 24, 48, and 72 hours following irradiation with 900 r. (Arrows indicate time of injection.)

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1. Lorenz, E., C. Congdon, and D. Uphoff. 1951. Progress report: Modification of acute irradiation injury by bone marrow injections. ANL-4713. Pp. 9-12.
2. Lorenz, E. 1951. Recovery pattern of the blood picture in guinea pigs following a limited total-body exposure to chronic gamma radiation. J. Chem. Phys., 48:264-274.

PROGRESS REPORT: EFFECTS OF IONIZING RADIATION ON
UNICELLULAR AGGREGATES. I. EFFECTS OF X IRRADIATION
ON TISSUE CULTURES

T. R. Reid* and M. P. Gifford*

The last progress report described the application of techniques for the quantitative determination of the number of viable cells in any given tissue culture to the study of survival patterns in tissue cultures following acute doses of X radiation.⁽¹⁾ This work has been continued and data are now accumulated for doses of X radiation of 250, 500, 750, 1000, and 2000 r (186 kv, 20 ma, 0.25 mm Cu and 0.55 mm Al, 380 r per minute). Every 48 hours following irradiation, four to six cultures from a control group and an equal number of irradiated cultures are chosen at random, fixed, and counted. The experiments are carried out over a period of from 10 to 13 days following irradiation.

Results to date show that at the 1000 r and 2000 r levels, growth is arrested for the first 24 hours following irradiation, after which there is a steady decline in the cell population to the point that, at 14 days, the number of cells per flask is less than that present at the time of irradiation (Figure 7). With 750 r (Figure 8) there is continued growth following irradiation for the first 6 to 8 days but at a much diminished rate compared with the controls. After the eighth day the cell population begins to fall off slowly. This picture is duplicated at the 500 r dose level except that the growth for the initial 6 to 8 days is more pronounced than that of the 750 r group. When 250 r is given there is a growth lag for the first 24 to 48 hours, then proliferation appears to continue at about the same rate as that in the control cultures. Work is still being done at this dose level in an effort to determine whether or not there is actually complete recovery. The constant variation throughout the experiment is not more than 20 per cent.

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Figure 7. Growth of control and experimental tissue cultures exposed to 2000 r X radiation. The broken line extending from the control curve represents the growth which would occur if the size of the flasks was such that normal proliferation continued without overcrowding.

Figure 8. Growth of control and experimental tissue cultures exposed to 500 750 r X radiation. The broken line extending from the control curve represents the growth which would occur if the size of the flasks was such that normal proliferation continued without overcrowding.

PROGRESS REPORT: EFFECTS OF SPLEEN PROTECTION ON
LYMPHOID TUMORS INDUCED IN C-57 BLACK MICE BY
TOTAL-BODY IRRADIATION

D. Uphoff and E. Lorenz

Kaplan⁽¹⁾ reported recently that local irradiation is ineffective in the induction of mouse lymphoid tumors and concluded, therefore, that an indirect systemic mechanism must be implicated in the induction of lymphoid tumors by irradiation. In local irradiation the spleen or bone marrow or both may be protected, and it is known that spleen protection or bone marrow injections are highly effective in restoring hematopoietic function. It occurred to us that prolonged depression of hematopoietic activity may be a factor in lymphoid tumor induction, especially since divided doses over several weeks are highly effective in producing such tumors.

Three groups of Kaplan's C-57 black mice were exposed to total-body X radiation in the following manner. The first group received four weekly doses of 225 r; the second group received the same treatment but with spleen protection; and the third group was given a single dose of 900 r with spleen protection. The treatment was completed when the animals were approximately three months old. Table 2 shows that the majority of the mice receiving the divided dose survived, while approximately 50 per cent of the mice of the third group died within a few days after treatment. Five and one-half months after completion of the exposures the incidence of lymphoid tumors of the first group is at present 36 per cent, 3 per cent in the second group, and 3 per cent in the third group. These results suggest that prolonged depression of hematopoietic activity may be a factor in eliciting lymphoid tumors.

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Table 2

LYMPHOID TUMOR INCIDENCE

Treatment	Treated (no.)	Survival (no.)	Dead (no.)	With lymphoid tumors (no.)
225 r, 4 weekly doses	60	56	22	20
225 r, 4 weekly doses spleen pro- tection	52	36	1	1
900 r, single dose, spleen protection	59	30	1	1

PROGRESS REPORT: METHODS OF CONTINUOUSLY RECORDING CONSTITUENTS OF RESPIRED AIR UNDER VARIOUS EXPERIMENTAL CONDITIONS

A. W. Pratt,* M. Eden,* and R. Koehn*

INTRODUCTION

It is generally agreed that respiratory metabolism is an accurate index of total-body metabolism. A knowledge of the changes in the concentration of the components of respired air is of considerable value in evaluating the effect of in vivo experimental procedures. In a previous paper⁽¹⁾ it was shown that in terms of general averages the oxygen consumption rate, the carbon dioxide production rate, and the respiratory quotient of experimental animals could be accurately measured by mass spectrometric analysis of the respired air. This analytical technique has the further advantage of being able to give rapid and accurate determinations of all volatile compounds of the respired air. The need for continuous analysis of oxygen and carbon dioxide concentrations in the respired air is apparent, but with the mass spectrometer this could be obtained only at the sacrifice of the high sensitivity of the mass spectrometric system and loss of the ability to analyze completely for all compounds of respired air samples.

The ideal would appear to be a system that would continuously analyze oxygen and carbon dioxide and from which samples of the respiratory exchanges could be obtained for mass spectrometric analysis for abnormal metabolites (e.g., acetone).

The construction of an instrument that will continuously record and analyze the carbon dioxide and oxygen concentrations in respired air is reported below.

DESCRIPTION OF APPARATUS

The oxygen analyzer as used in the system is a slightly modified form of a commercially available instrument commonly used in monitoring industrial combustion.** The principle of operation utilizes the unique

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**Hays Magnotherm Differential Oxygen Analyzer, Hays Corporation,
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paramagnetic property of molecular oxygen. The sensing unit consists of two chambers, a reference chamber and a measuring chamber, which have suspended in their center an electrically heated filament. Across the measuring chamber is a strong magnetic field.

In operation the oxygen-bearing gas is pulled into the strongest part of the magnetic field where the gas is heated by a hot filament. With heating, the oxygen loses practically all of its paramagnetic property. In this state, cooler, more magnetic gas forces it out of the field causing it to come in contact with the cool walls of the chamber where it loses its heat and regains its paramagnetism. This circulation cools the filament and causes changes in the electrical resistance. These changes in electrical resistance can be interpreted in terms of per cent change in concentration of oxygen.

The CO₂ analyzer is a commercially available thermal conductivity sensing element that has had no modification for this application.* Thermal conductivity may be simply described as a measure of the ability of a gas to dissipate heat. This property varies for different gases; air containing CO₂ being a significantly poorer conductor than air containing no CO₂. In this system the only gases present in significant amounts are N₂, O₂, and CO₂.

In operation there are two chambers, a measuring chamber and a reference chamber, arranged in an electrically balanced system. As the CO₂ concentration in the measuring cell increases a change in the electrical resistance is created. These changes can, by proper calibration, be interpreted as per cent change of carbon dioxide.

In Figure 9 a schematic diagram of the instrument is seen. Two parallel synthetic air trains have been constructed. One train forms the reference side of the system and the other train the measuring side. The reference gas passes first through the oxygen analyzer and then through the carbon dioxide analyzer and ultimately out into the atmosphere. The gas on the measuring side passes through a hydrator, an animal respiration chamber and a dryer, on through the measuring side of the oxygen analyzer and the carbon dioxide analyzer, and ultimately out into the atmosphere.

A fitting has been placed on the outlet of the test side for collection of samples for mass spectrometric analysis. Both air trains have a common source of compressed gas mixture containing 79 per cent N₂ and 21 per cent O₂. Each train has a separate flow regulator and flow-meter so that the minute volume of compressed gas in each train can be kept equal, constant, and continuously metered.

*Gow-Mac Thermal Conductivity Unit, Model 60-S, Gow-Mac Instrument Co., Newark 5, New Jersey.

Figure 9. Schematic diagram of an instrument for continuous recording and analysis of the carbon dioxide and oxygen concentrations in respired air.

There are two manometers in the system. One measures the pressure difference between the two trains at the input of the analyzing components. This difference in pressure is less than a millimeter of water. The other manometer measures the pressure of the atmosphere in the respiration chamber compared with ambient pressure. Recording is accomplished by the use of automatic recording strip chart potentiometers which are matched as to chart speed.

The hydrator consists of a wick suspended in a solution of potassium thiocyanate. This allows approximately 50 per cent hydration of the gas passing through the hydrator. The respiratory chamber is made of glass so that the experimental animal can be watched at all times. It consists of two chambers; the inner chamber houses the experimental animal and is enclosed by a water-tight jacket through which water from a thermostatically controlled reservoir can be pumped, thus controlling the temperature of ambient air of the inner chamber. Drying is accomplished by flowing the effluent air of the respiration chamber through a glass cylinder of drying agent.

PERFORMANCE

The preliminary data obtained thus far have demonstrated that there is excellent matching of the two analyzing components. The response time constant of the two analyzing components in our system is approximately 40 seconds, the delay being dependent upon the capacity of the air train system. The error of the analyzing components as checked by the mass spectrometer is less than ± 0.05 per cent. At present this instrument is being used to study continuously respiratory metabolism of experimental animals before and after irradiation. Insufficient data have been accumulated for inclusion in this report.

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Neutron Radiobiology

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THE GAMMA-RAY TURRET

H. H. Vogel, Jr., J. W. Clark, and D. L. Jordan

The radiation chamber being constructed for the Neutron Radiobiology program will have a source of fast neutrons at one end and a source of γ rays at the other. Experiments have been planned to test the radiobiological additivity of these two radiations in their acute lethal action on mice.

Eighteen sources of cobalt-60 were obtained from Oak Ridge. These sources were wafer-shaped, 1 cm in diameter x 2 mm thick. Each source was estimated to have an activity of approximately 3 to 3 1/2 curies. The turret was constructed so that all eighteen cobalt sources, each loaded on the end of an indexing rod, could be used to irradiate the animal chamber. Under certain experimental conditions half of the rods may be used for irradiation, while the other nine sources are protected by lead shielding. A third rotation places all eighteen sources behind lead shielding. In addition to this variation in the number of sources exposed for irradiation, the distance between the Co⁶⁰ wafers and the animal chamber can be varied. It is estimated that dose rates varying from 0.3 r per minute to 20 r per minute will be possible.

Loading the eighteen Co⁶⁰ sources was done by the Remote Control section by a method that was devised to meet our particular needs. It was necessary to apply the wafer-like sources to the tips of the indexing rods of the turret in such a manner as to assure a permanent, tight fit and at the same time to allow an easy method of removal if necessary. Also, the loading method had to be tailored to the use of master-slave manipulators.

The rods were fabricated with a groove around their circumference, about 1 cm from the end. Special aluminum caps were made to fit the rods. Each rod was then pushed into the cave through an access hole. The source was lifted from the pot, removed from its capsule and dropped into the Al cap. It was then measured at a distance of 25 cm by a Victoreen rate-meter. (Figure 10).

APPROX. $\frac{1}{4}$ SCALE

Figure 10. Readings in r per minute shown in relation to the proposed field of irradiation. Italicized figures indicate the nine sources that can be exposed while the others are shielded.

The cap, containing the source, was then pushed onto the rod. A special jig was placed over the cap, followed by a pneumatic dimpling device. The purpose of the jig was to guide the tips of the dimpler, which, when activated, placed two dimples into the Al cap at the exact plane of the underlying groove on the rod. This action effectively crimped the cap, containing the source, to a tight and permanent fit on the end of the indexing rod.

In one case it was necessary to remove the source from one of the rods. This was easily done by cutting the Al cap ahead of the crimp. The source was then recovered, and the remainder of the cap was peeled from the rod.

Before loading in the turret, each source was placed 25 cm from the thimble chamber of a Victoreen rate-meter. The values shown in Figure 10 are the readings in r per minute shown in relation to the proposed field of irradiation (40 x 40 cm). The italicized figures indicate the nine sources that can be exposed while the others are shielded.

EFFECT OF CLAMPING OFF SPLENIC CIRCULATION DURING
X IRRADIATION

L. O. Jacobson, E. K. Marks, and E. O. Gaston

ABSTRACT

The survival of mice in which the circulation to the shielded spleen is clamped off during exposure of the animal to 950 r and in which the clamp is released immediately after irradiation is approximately the same as survival in animals with spleen shielding without clamping.⁽¹⁾ In an experiment designed to determine whether clamping of the splenic pedicle during exposure to 1025 r without spleen protection would also enhance survival, the pedicles of the spleens of eight mice were tied before irradiation and released immediately after the exposure. None of these animals survived.

In a further experiment the pedicles of the spleens of 23 mice were tied off 10 minutes prior to irradiation and were released after the irradiation period (about 14 minutes). The circulation to the spleen was tied off, therefore, for a period of approximately 24 minutes. None of these mice survived beyond the eighth day.

In view of the observations of Jolly⁽²⁾ and Dowdy⁽³⁾ one might expect temporary clamping of the splenic pedicle during exposure of the mouse to 1025 r to enhance survival if the radiation injury sustained by the splenic tissue is sufficiently reduced by this procedure.

Further data are necessary before the reason for the ineffectiveness of this procedure can be resolved.

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PROGRESS REPORT: HEMATOLOGY

L. O. Jacobson, E. K. Marks, E. O. Gaston, and M. J. Robson

SPLEEN SHIELDING AND ALLIED STUDIES

The ability of cysteine to protect against the lethal effects of X radiation, as demonstrated by Patt⁽¹⁾, and the additive protection provided when animals are spleen-shielded during irradiation after the administration of cysteine 10 minutes before the irradiation period or following the administration of estradiol 10 days prior to irradiation were demonstrated by Simmons and Bethard.⁽²⁾ Mice were irradiated with 1025 r 10 minutes after the administration of cysteine, and four spleens from baby mice were implanted immediately after X radiation to demonstrate whether spleen shielding, spleen implantation, and cysteine also have an additive effect. In the small group of animals studied, pretreatment with cysteine followed by spleen implantation after irradiation were additive in enhancing survival. Cysteine failed to produce any beneficial effect on survival of irradiated mice when given 6 hours after 1100 r total-body irradiation with or without protection of the exteriorized spleen during irradiation.

THE EFFECT OF HOMOLOGOUS BONE MARROW INJECTIONS ON
THE SURVIVAL OF CF #1 MICE

Lorenz et al.^(3,4) reported that homologous bone marrow suspensions were effective in protecting LAF₁ and C₃Hb mice against the lethal effects of irradiation. Ten- to 12-week CF #1 female mice were exposed to 900 r total-body X radiation and injected intravenously with the marrow of 10-week CF #1 mice to determine the effect of homologous bone marrow injections. Preliminary studies indicate that the 21-day survival of this strain is approximately 50 per cent. The effect on the survival of CF #1 females given homologous injections of the marrow from young mice is currently under investigation.

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THE EFFECT OF AGE ON SURVIVAL OF CF#1 FEMALE MICE WITH SPLEEN PROTECTION

E. L. Simmons, L. O. Jacobson, E. K. Marks, and E. O. Gaston

Young adult, 10- to 12-week CF#1 female mice are used routinely by our group to investigate the mechanism of increased radioresistance following spleen shielding. The pooled results on some 170 mice exposed during the year 1950 to 1025 r total-body X radiation, with the exception of the shielded spleen, show a survival of 71.2 per cent as contrasted with a survival of only 2.15 per cent in 278 control animals in which the entire body was exposed to 1025 r.

Lorenz *et al.*⁽¹⁾ have reported that survival of C-57 black and strain A four-week mice, irradiated with spleen protection, was essentially no different from the survival of mice irradiated without spleen protection with the same dose. Irradiation of these strains at seven weeks, however, showed that spleen protection increased survival.

The experiments reported here were performed to determine the relative protective effects of spleen shielding in CF#1 female mice younger than 10 to 12 weeks of age. The CF#1 mice used in the laboratory arrive from Carworth Farms in New City, New York by air express when they are five to six weeks old. They are kept in the isolation rooms of the laboratory until they fall within the 10- to 12-week age span not only to insure complete recovery from travel and absence of disease but to allow them to adjust to the diet and conditions in the laboratory.

Four age groups of mice younger than 10 to 12 weeks were tested, the youngest being four to five weeks. This youngest group was especially ordered for delivery at this age and was tested during the week of arrival. Groups tested at five to six weeks of age were routine shipments that were used the week of arrival. Older groups were of similar stock but were allowed to mature in the isolation rooms for the required time intervals.

All mice were exposed to 1025 r X radiation. The LD₅₀ with or without spleen shielding for the younger groups studied has not been determined. Results are summarized in Table 3. Survival following spleen protection is higher in mice between the ages of six to twelve weeks than in mice between four to six weeks of age. The 138 mice tested at five to six weeks represent four separate experimental groups with consistently low survival results in each case, the pooled average survival being 9.4 per cent.

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Table 3

SURVIVAL OF MICE OF VARIOUS AGES FOLLOWING X IRRADIATION (1025 r)

Age (wks.)	Mice (no.)	Spleen shielding	Survivors (no.)	Survival (%)	Time of death after X irradiation (days)																											
					3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	26	30						
4-5	18	no	0	0		2		5	2	1	4		1	2											1							
4-5	34	yes	13	38.2		3		4		3		8		1																		
5-6	18	no	0	0					2		8	4	2		2																	
5-6	138	yes	13	9.4	1	6	16	44	11	16	15	4	8		2								1	1								
6-7	41	yes	26	63.4		3	2		1	2	2	2			1	1			1													
8-9	14	yes	9	64.3					2	1				1			1															
10-12	278	no	6	2.15	7	16	23	23	12	29	31	41	37	27	8	7	2	1	1	2	1	1				1	2					
10-12	170	yes	121	71.2	3	1	7	12	4	5		9	1	1	1	2			1		2											

Several possible explanations exist for the decreased survival observed in younger mice. Groups that showed the least protection from spleen shielding were, in each case, shipments of mice tested immediately upon arrival in the laboratory. This aspect would have to be ruled out by breeding our own mice and testing them between the ages of four to six weeks. Lorenz *et al.*⁽¹⁾ noted at autopsy that all young mice showed edematous intestines distended with fluid, which may indicate a greater sensitivity of the intestinal tract of young mice. Similarly, other organ systems in young mice may be less able to recover from radiation damage. The onset of sexual maturity is reported to be approximately six weeks⁽²⁾, so the possibility also exists that these findings may be related to some stage in endocrine-gonad development. Finally, it is possible that the protected spleens of younger mice are less effective in producing the "factors" that enhance survival in fully mature mice. This would seem doubtful, however, since even the spleens of baby mice, when transferred to the abdominal cavity of 10- to 12-week mice previously exposed to 1025 r, result in enhanced survival.

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Physiology

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PROGRESS REPORT: MECHANISMS OF RADIATION PROTECTION
IN MAMMALS

H. M. Patt, S. D. Mayer, and R. L. Straube

Earlier studies have revealed that the protection afforded rats by cysteine shows some proportionality to the dosages of cysteine and X radiation.⁽¹⁾ Our results with thymic cells irradiated *in vitro* suggest a similar relationship and indicate, moreover, that a given dosage of cysteine displaces the survival curve by a rather constant amount over a wide dosage range (50 to 1500 r).⁽²⁾ The maximal tolerated concentration of cysteine (0.02 M or approximately 2.3 mg per g of cells) appears to cancel 0.53 ± 0.019 r for each roentgen that is delivered to the cell suspension. We are carrying out additional experiments in the mouse in order to gain more quantitative information about the intact animal. The data obtained thus far indicate that the protection ratio for the maximal tolerated cysteine dose in mice (1.2 mg per g body weight) is 0.42 ± 0.043 .

In the more recent experiments, a standard dose of cysteine (1200 mg per kg I.V.) was given to mice (CF#1, females) after a fraction of the total radiation dose was delivered. The interval between successive radiation fractions did not exceed 5 minutes. The total dosages were either 700, 800, or 1000 r and the postcysteine fractions varied from 300 to 750 r (250 kv, 220 r per minute, 1.5 mm Cu hvl). Untreated controls were irradiated in an identical manner. In other experiments (to be reported later), the cysteine dose was also fractionated, a proportional amount preceding each radiation fraction. With the split-dose technique it is possible to evaluate the protection at sublethal dosages of radiation, and to investigate more fully the problem of reversibility, while still employing the simple criterion of lethality.

The effective roentgens received by the treated mice were read from a simultaneously determined dose-mortality curve for untreated animals (Figure 11). A protection ratio was then computed in the following manner, on the assumption that cysteine did not influence the radiation fraction preceding it.

Figure 11. Radiation dose-mortality curve for CF#1 female mice. (Weight, 19 to 25 g).

$$\text{Protection ratio} = \frac{(\text{postcysteine } r) - (\text{effective } r - \text{precysteine } r)}{\text{postcysteine } r}$$

Cysteine accounted for 125 to 335 r (208 ± 63 r) depending upon the magnitude of the postcysteine radiation fraction (Figure 12). On the other hand, the protection ratio ranged from 0.37 to 0.50 with a mean and standard deviation of 0.42 ± 0.043 . Thus, the data suggest that, in effect, cysteine neutralizes a fairly constant percentage of the radiation dose. These results may be explained equally well by postulating an interaction either with the radiation and its presumed toxic intermediates or with the biological system. In the former instance, the biological effectiveness of the radiation would be decreased, while in the latter case, the radiosensitivity of the biological system would be diminished. Further experiments along similar lines are in progress.

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Figure 12. Relationship between the dosage of radiation and the protection afforded by 1200 mg/kg of cysteine in the mouse. (Each point represents a group of 20 mice; the line is derived by multiplying postcysteine roentgens by 0.42).

Radium Toxicity

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PROGRESS REPORT: RATE OF DISAPPEARANCE OF RaSO_4
 DUST FROM THE HUMAN LUNG

L. D. Marinelli and P. F. Gustafson

Three of the five persons selected for study of RaSO_4 transfer from the human lung have been measured for total-body and lung content after a four- to five-month interval. From preliminary analysis it is possible to report the results shown in Table 4.

Table 4

PRELIMINARY ANALYSIS OF RATE OF DISAPPEARANCE OF RaSO_4

Remaining radium (%)				
Patient	Time interval (months)	Total*	Highest lung field**	Rn/total Ra
Ras	4	30-40	25	Approx. 0.5
Cha	5	60-70	50	Approx. 0.4
Kue	5	70-75	30	Approx. 0.6

*Absolute technique.

**Scanning method.

Despite their preliminary character, these data tend to show that the rate of elimination from the lung may vary significantly from patient to patient and that the patient showing the lowest lung elimination rate exhibits the lowest Rn to total Ra ratio.

It seems also clear that the rate of elimination from the lung is definitely faster than the rate of elimination from the whole body within the observational period reported. The data reported herein were taken at approximately six months after the accidental inhalation.

PROGRESS REPORT: THE INFLUENCE OF LOW, CONTINUOUS
DOSES OF AUREOMYCIN ON CF#1 FEMALE MICE

M. P. Finkel and G. M. Hirsch

For the past five years the rats and mice in this laboratory have been fed an exclusive diet of pellets that were developed by Dr. H. P. Morris at the National Cancer Institute and manufactured by Derwood Mills in Maryland. The Derwood diet has proved to be adequate for adult rats and mice, for breeding females, and for weanling animals. However, for a number of reasons it was decided to change the mouse food to "Lab-Blox for Mice," manufactured by Allied Mills, Inc., Illinois. Since a comparison of the lists of ingredients shows that the nutritional value of the Lab-Blox is at least equal to that of the Derwood pellets, it is assumed that this diet will be equally adequate. However, 1.4 mg of the antibiotic, aureomycin, is incorporated per pound of Lab-Blox, the rationale being the reported growth-stimulating properties of low doses of some of the antibiotics. There is the possibility that other incidental effects may follow the continuous feeding of small amounts of aureomycin, e.g., changes in the tolerance to radiation and to drugs and variation in the incidence of spontaneous tumors.

In an attempt to answer some of the questions arising from the use of a food containing aureomycin, we initiated a small experiment with CF#1 female mice. The conduct of the experiment includes daily observations of the animals, frequent weighings, sacrifice and autopsy of moribund animals, and roentgenography.

The mice arrived from Carworth Farms, where they had received a diet containing aureomycin, when they were 5 to 6 weeks old. They were immediately distributed (15 per cage) by a prearranged random system into 15 stainless-steel cages, five of which contained Derwood food (a total of 75 animals) and ten of which contained Lab-Blox (a total of 150 animals).

The average weight of each group of animals during the first five weeks of the experiment is plotted in Figure 13. Apparently 1.4 mg per pound of aureomycin in the food has no growth stimulating effect on CF#1 female mice between 5 and 11 weeks of age. Similarly, the sudden exclusion of aureomycin from the diet of animals that had received it from weaning to 5 to 6 weeks of age seems to have no detrimental effect.

Figure 13. Average weights of experimental and control groups during the first five weeks of the experiment.

Intestinal infection of mice is usually evidenced by diarrhea or by epilation and soreness around the anus. No such symptoms appeared among the 75 animals on the Derwood diet until the 35th day, when two animals in one cage had diarrhea. Of the 150 animals on the Lab-Blox, two showed these signs of infection on the 14th day, four on the 21st day, six on the 28th day, and four on the 35th day. Only three of the ten cages were involved. This is not a high incidence of infection for CF #1 mice in this laboratory. It does indicate, however, that the dose of aureomycin that the animals are receiving is not great enough to eliminate intestinal infections.

PRELIMINARY REPORT: TOXICITY OF CARBON 14

M. P. Finkel and G. M. Hirsch

A study of the long-term toxicity of carbon 14 was undertaken because of the possible hazard arising from its current widespread use in a vast number of fundamental investigations. It is a very weak β emitter (0.155 Mev) with a half-life of 5900 years. When carbon 14 is administered to animals as sodium bicarbonate, 97 to 98 per cent of the dose is rapidly eliminated through the lungs. Skipper, White, and Bryan⁽¹⁾ have reported that one week after the intraperitoneal injection of labeled sodium bicarbonate, retention in the mouse was 0.5 per cent. Only 0.1 per cent remained after 12 weeks and less than 0.05 per cent after a year.

In the present experiment, each of 50 young, adult CF#1 female mice (about 70 days old and weighing 25 g) and 50 young, adult Sprague-Dawley female rats (about 100 days old and weighing 200 g) received a single intraperitoneal injection of 500 μ g of $\text{NaHC}^{14}\text{O}_3$ (mouse dose, 20 μ c per g; rat dose, 2.5 μ c per g).^{*} The animals were permitted to live until moribund, at which time they were sacrificed with Nembutal[®] and examined for gross pathological changes. Roentgenograms were made after autopsy. Histological studies have not yet been done; consequently the data reported here are only tentative and will be revised and presented in greater detail at some later date.

No control animals were started specifically for this experiment. For final evaluation the rats will be compared with the hundreds of control rats and the mice with approximately a thousand control mice that have been maintained in the laboratory for other experiments. For purposes of comparison in this preliminary report, the pertinent data on 120 control mice that were received in the laboratory two months earlier are used.

The number of days to 50, 25, 10, and 0 per cent survival are given in Table 5. The rats lived as long as is usual under our laboratory conditions, and the carbon 14 injected mice fared as well as the control mice. Table 6 presents data on the morbidity of the animals based on gross pathological changes alone and study of the roentgenograms. It must be remembered that these are only tentative diagnoses. The incidence of disease among the injected rats was no different from that encountered regularly among control animals. There is the suggestion that among the treated mice the incidence of lymphomas was slightly elevated and the incidence of lung tumors was decreased. Statistical study of the final data will be necessary to settle these points.

^{*}We are indebted to Dr. Donald Buchanan for injecting the animals.

At present state of the analysis of the experiment, it appears that a total dose of 500 μc of carbon 14 given to rats and mice (2.5 μc per g and 20 μc per g, respectively) has little if any effect upon longevity and morbidity.

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Table 5

SURVIVAL (DAYS)

Survival (%)	C^{14}		Control Mice
	Rats	Mice	
50	300	500	450
25	400	600	550
10	545	625	660
0*	572	853	764

*Death of last survivor

Table 6

MORBIDITY (%)

Disease	C^{14}		Control mice
	Rats	Mice	
Pneumonia	100	10	21
Lymphoma	0-10	55	23-47
Mammary tumors	17	28	22
Lung tumors	0	55	71-82
Misc. tumors	0-5	21	18
Benign bone tumors	0	14	14
Malignant bone tumors	0	0	0

INVESTIGATION OF PLASMA PROTEINS LABELED WITH CARBON 14

K. A. Lathrop, P. V. Harper, and J. G. Allen

The data presented here were obtained in an experiment preliminary to study of the effect of ionizing radiations on the plasma proteins, particularly those involved in the clotting mechanism. Greenberg and Winnick⁽¹⁾ have demonstrated that C¹⁴-tagged glycine is incorporated in significant amounts into the blood plasma of the rat. Goldsworthy, Winnick, and Greenberg⁽²⁾ showed that C¹⁴-labeled glycine is converted to serine by the rat. Human plasma fibrin is known to be about 10 per cent serine on hydrolysis. Therefore, it was hoped that fibrin, together with the albumin and the globulins, would be tagged to give significant C¹⁴ counting levels following the administration of labeled glycine.

Ten mg of carboxyl-tagged glycine (0.5 μ c per mg) were injected into the tail vein of a 200-g albino Sprague-Dawley female rat. Specific activity measurements made on exhaled CO₂ samples taken during the 2 1/2-hour period following injection are shown in Figure 14. There was a rapid increase in the level of tagged CO₂ in the exhaled air during the first 30 minutes following injection. After 30 minutes the rapid formation of tagged CO₂ ceased abruptly. It is reasonable to suppose on this basis that the injected free glycine is no longer present as such in the animal after 30 minutes and that the formation of primary tagged products from it is limited largely to this period.

Blood samples were taken at intervals from one of the tail veins not used for injection of the glycine. Cells were removed and the plasma separated into fibrin, globulin, and albumin fractions by conventional precipitation methods. Specific activity values for these fractions plotted against time give the curves drawn in Figure 15. These curves may be interpreted to represent appearance of tagged protein in the plasma during the ascending portions, and the subsequent disappearance of these tagged molecules from the circulation during the descending portions.

Figure 14. Specific activity measurements of exhaled CO₂ samples taken during the two and one-half hour period after the injection of C¹⁴-labeled glycine.

55

Figure 15. Specific C¹⁴-activity values for fibrin, globulin, and albumin fractions plotted against time.

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AMINO ACID NITROGEN IN BLOOD PLASMA OF THE IRRADIATED DOG

K. A. Lathrop and J. G. Allen

After exposure of the dog to a lethal dose (450 r) of total-body X radiation, quantitative changes are observed in the nitrogen content of the blood plasma as determined by the Kjeldahl method.

1) By the fifth or sixth postirradiation day, total proteins decrease by about 20 per cent of the pre-irradiation level; subsequently returning to or exceeding this value before death about the tenth day.⁽¹⁾

2) Albumin decreases and the globulins increase.

3) Nonprotein nitrogen decreases about 15 per cent of the pre-irradiation level, reaching the minimum value on the sixth or seventh post-irradiation day.

The question arises as to what changes take place within the fraction comprising the nonprotein nitrogen of plasma, and as to their significance with regard to alterations in protein. Since, in the normal human being the NPN of the plasma is largely urea nitrogen with the amino acids making up the next largest fraction, it seemed of value to investigate the behavior of plasma amino acids in the irradiated dog.

Eight healthy mongrel dogs averaging about ten kg in weight were exposed to 450 roentgens of total-body X radiation. Two similar nonirradiated dogs were used as controls. In addition, each irradiated animal served as its own control for four to five days during the pre-irradiation period. The animals were maintained on a diet of Purina throughout the pre- and postirradiation periods. Blood samples were taken in the morning while the animals were in a postabsorptive state.

Plasma amino acid determinations were made by the method of Folin as modified by Frame, Russell, and Wilhelmi.⁽²⁾

Dividing each animal's postirradiation amino acid nitrogen values by its average pre-irradiation value gives ratios which indicate the amount of deviation from normal. These ratios were averaged for the eight irradiated animals to give the values plotted against time in Figure 16.

Figure 16. Average ratios of pre- and postirradiation amino acid nitrogen values in dogs

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PROGRESS REPORT: METABOLISM OF DEXTRAN

K. A. Lathrop and J. G. Allen

Since its introduction as a plasma substitute by Ingelman⁽¹⁾ dextran has been used extensively in some European countries. Because of the possibility of long time damage to the body, which has occurred with some other materials used as plasma expanders, it is desirable to study the metabolic fate of dextran. The preparation of the C^{14} -labeled dextran used for this purpose has been described earlier.^(2,3) For the animal experiments the dextran was tagged with 110 μ c of C^{14} per g. For man a six per cent isotonic solution of dextran (6 μ c per g) was used.

In all of our experiments, quantitative recoveries of urine, feces, and exhaled carbon dioxide have been made in order to determine the total C^{14} eliminated following the intravenous injection of tagged dextran. At death organ and muscle specimens are taken for determination of C^{14} retention.

For the purpose of quantitatively collecting exhaled carbon dioxide and separated excreta, the rat experiments were set up as shown in Figure 17. This arrangement immobilizes the lower part of the animal but allows the upper body free movement.

An early observation that specific activity measurements on CO_2 taken as spot checks before feeding were higher than those taken after feeding led us to divide the rat experiments into two groups. One group was allowed a normal food intake, while the other was maintained on a subsistence diet. A third group, given a normal diet and allowed unrestricted activity, is also under observation, at the present time.

Some of the data already collected are shown in Figures 18, 19, and 20. Cumulative CO_2 excretions are represented by Figure 18. Dextran metabolism in a rat allowed a normal food intake with restricted activity is shown in Figure 19; while Figure 20 represents an animal on a subsistence diet and restricted activity.

Although the data are not sufficiently complete to permit more than tentative conclusions concerning the metabolism of dextran, it appears that:

- 1) Dextran is metabolized similarly by the rat, dog, and man.
- 2) The rates of dextran excretion and metabolism depend on the metabolic needs of the animal.

A

B

Figure 17. Apparatus for collection of exhaled carbon dioxide and separated excreta.

Figure 18. Cumulative $C^{14}O_2$ excretion.

- (1) Rat-fed, unrestricted activity (2) Dog-fed, restricted activity
(3) Rat-starved, restricted activity (4) Rat-fed, restricted activity
(5) Man-fed, restricted activity

Figure 19. Dextran metabolism in a rat allowed a normal food intake with restricted activity.

Figure 20. Dextran metabolism in an animal on a subsistence diet and restricted activity.

3) About 50 per cent of injected dextran is excreted as such during the first three days, while a large part of that remaining in the animal is oxidized to CO_2 over a longer period of time.

4) After intravenous administration no dextran, nor any of its metabolic products, is excreted by way of the intestine.

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Clinical Investigation

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PRELIMINARY REPORT: EFFECT OF SALICYLATES ON
EXPERIMENTAL BERYLLIUM POISONING

A. J. Finkel and M. R. White

Acute experimental beryllium poisoning in mice, readily produced by the intravenous injection of soluble beryllium salts, usually terminates fatally in three days. Aurin tricarboxylic acid has been reported⁽¹⁾ as an immediate antidote for such poisoning, presumably because of its chelating and lake-forming properties as well as its relatively low toxicity. The present study was undertaken to ascertain if the salicylic acid portion of the aurin tricarboxylic acid molecule would be comparably effective in reversing experimental beryllium intoxication.

An LD₉₅ of BeSO₄ (0.7 mg per kg of Be) was injected intravenously into young adult CF #1 female mice, and the compounds tested for antidotal properties were typically administered intravenously one hour later. Sodium salicylate (*o*-hydroxy-benzoate) was studied at various dose levels. Results are given in Table 7, which shows this compound to be quite effective when given in adequate doses, either intravenously or intraperitoneally. Various analogues of salicylic acid were tried and those without the ortho-hydroxy-carboxylic acid grouping were uniformly ineffective. Sodium *p*-amino-salicylate gave only equivocal results. Sodium gentisate, which was of especial interest because gentisic acid is a product of salicylate metabolism, was unsuccessful as an antidote in the amount studied in spite of possessing the key chemical grouping.

Disodium catechol sulfonate, which possesses two hydroxyl groups in ortho position and which has been found to be effective in the treatment of experimental uranium poisoning⁽²⁾, was successful only in prolonging the time to 50 per cent death. The remaining miscellaneous compounds were entirely without antidotal value.

Sodium salicylate afforded protection when treatment with 400 mg per kg was delayed eight hours but not when treatment was postponed 24 hours. Pretreatment eight hours before beryllium was administered was ineffective.

Table 7

EFFECT OF SALICYLATES AND SALICYLATE ANALOGUES ON
EXPERIMENTAL BERYLLIUM POISONING IN MICE

Compound	Dose level mg/kg	No. mice	Survival at 14 days		Time to 50% death (days)
			No.	Per cent	
Sodium salicylate (Na <u>o</u> -hydroxy-benzoate)	600	20	20	100	---
	400	39	31	79	---
	200	10	6	60	---
	40	10	0	0	2.5
Sodium salicylate (intraperitoneal)	600	10	10	100	---
Sodium <u>p</u> -amino salicylate	660	10	2	20	2.5
	400	30	17	57	---
Sodium <u>m</u> -hydroxy-benzoate	600	10	4	40	2.4
Sodium <u>p</u> -hydroxy-benzoate	600	10	0	0	2.9
Sodium <u>o</u> -chloro-benzoate	600	10	0	0	2.8
Sodium gentisate (Na 2,5-dihydroxy-benzoate)	660	10	0	0	2.9
Disodium catechol disulfonate	800	10	5	50	8.4
	400	10	5	50	13.4
	200	9	3	33	4.3
	80	10	2	20	2.4
5-acetyl-4-oxohexanoic acid	800	10	0	0	1.9
Hexamethylenetetramine (intraperitoneal)	2080	10	0	0	1.9

Antidotal action is believed to result from the chelating action of the ortho-hydroxy-carboxylic acid structure, which reduces the availability of the toxic ionized beryllium by the formation of ionized complexes. Whether lake formation is required in addition to chelation in the treatment of chronic beryllium granulomatosis is a problem for future investigation.

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Radiochemistry

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COMPLEX PHOSPHORUS COMPOUNDS OF RAT BONE STUDIED BY
NEUTRON ACTIVATION AND ELECTROMIGRATION

W. P. Norris, T. R. Sato, and H. H. Strain

We have utilized electrical migration⁽¹⁾ for the examination of neutron irradiated bone. The samples of dried rat bone, of bone treated with ethylene diamine, and muffled rat bone (about 100 mg each), as well as small specimens of NaH_2PO_4 , CaHPO_4 , $\text{Na}_4\text{P}_2\text{O}_7$, NaPO_3 , and apatite were exposed to thermal neutrons for 45 days, dissolved in cold hydrochloric acid, and submitted to electromigration. They yielded several different zones of radioactive anions: four zones for dried bone, for bone treated with ethylene diamine, for CaHPO_4 , and for NaH_2PO_4 ; three zones for apatite, two zones for $\text{Na}_4\text{P}_2\text{O}_7$ and for muffled bone. On the basis of half lives and β energy, all of these anions were phosphorus compounds. As shown by a reference zone of radioactive orthophosphate (Oak Ridge), the principal zone from all the preparations of bone was orthophosphate. Pyrophosphate appeared only in muffled bone. The anions obtained in addition to orthophosphate from dried bone and from ethylene diamine-extracted bone have yet to be identified. At least two of these substances were obtained from apatite and from commercial preparations of phosphates. These constituents may have been present in bone or they may have been formed in the process of neutron irradiation. The results are summarized in Table 8.

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Table 8

ZONES DETECTED

70

Material	Treatment and source of P^{32}	?	$\begin{array}{c} \text{HPO}_4^- \\ \updownarrow \\ \text{H}_2\text{PO}_4^- \end{array}$	$\text{H}_2\text{P}_2\text{O}_7^-$	?	?	$(\text{PO}_3)_3^-$
CaHPO_4	Neutron activation	+	+		+	+	
NaH_2PO_4	Neutron activation	+	+		+	+	
$\text{Na}_4\text{P}_2\text{O}_7$	Neutron activation		+	+			
H_3PO_4	Oak Ridge - carrier free		+				
NaH_2PO_4	Added Oak Ridge H_3PO_4 and recrystallized 3 times		+				
$(\text{NaPO}_3)_3$	Heat above to 600°C						+
$(\text{NaPO}_3)_6$	Heat above to 900°C		+				
Rat bone	Muffled at 600°C - neutron activated		+	+			
Rat bone	Extracted with H_2O , alcohol, ether - neutron activated	+	+		+	+	
Mouse bone	Extracted with ethylene diamine - neutron activated	+	+		+	+	
Apatite	Apatite, Durango, Mexico - neutron activated		+		+	+	
Apatite	Apatite, Cumberland, England - neutron activated		+		+	+	
Apatite	Apatite, Renfrew, Ontario, Canada - neutron activated		+		+	+	

All ions were made to migrate in 0.1 M lactic acid.

ANALYSIS OF THE MESOTHORIUM SOLUTION USED FOR TOXICITY STUDIES

W. P. Norris and L. A. Woodruff

A portion of a mesothorium stock solution (Source No. 36) was obtained from Dr. M. S. Freedman of the ANL Chemistry Division and designated in our records as M-10. The records indicate that the MsTh was separated from Th in 1942 and therefore should now be in equilibrium with RdTh and its daughters. The solution contained a small amount of a fine white precipitate similar in appearance to BaSO_4 . The solution was shaken to suspend the precipitate, and a 10 λ sample was withdrawn and diluted to 100 ml with 0.1 N HCl.

A one-ml aliquot of this dilution (M-10.1) was put into a separatory funnel, and the RdTh was extracted at pH 1 with equal volumes of a solution of thenoyltrifluoroacetone in benzene. The phases were separated, and the extraction was repeated several times. The benzene phase was then rinsed with 0.1 N HCl, and a sample put on a Pt disc and counted. The RdTh (or MsTh) content was calculated as follows:

RdTh	5400	c/m total.
	$\times 2$	
	10,800	d/m/ml M-10.1 (dilution)
	1.08×10^8	d/m/ml M-10 (original)
	1.08×10^8	
	2.2×10^{12}	$= 5 \times 10^{-5} \text{ c/ml}$
		$= 50 \mu\text{c MsTh/ml M-10}$

BaSO_4 was precipitated from a sample of the aqueous phase and mounted on a Pt disc for α counting. This contained ThX and should have decayed with a half-life of 3.64 days. The sample contained 25,000 c/m, which is approximately 4 times the RdTh count and is what should be obtained from considerations of the decay scheme. However, the sample decayed too slowly, and the curve leveled off after seven days. This indicated the presence of Ra^{226} . Using the residual number of counts in the decay curve one can estimate the amount of Ra^{226} .

	2000	c/m
	$\times 2$	
	4000	d/m
	$\times 5$	(aliquot factor)
	20,000	d/m/ml M-10.1 Ra in equilibrium
	2×10^8	d/m/ml M-10
	5×10^7	d/m Ra/ml M-10
	5×10^7	
	2.2×10^{12}	$= 2.3 \times 10^{-5} = 23 \mu\text{c Ra}^{226}/\text{ml M-10}$

If Ra^{226} is present, it should be possible to plate out Po on silver. This was done with 1 ml of solution M-10.1, and the result was a disc with 330 α counts per minute. A pulse analysis showed that 70 per cent of this activity was due to Po^{210} . From this it was calculated that the solution contained Po equivalent to $10 \mu\text{c Ra}^{226}/\text{ml}$ if it was assumed that there had been complete retention of Rn since 1942 and no adsorption of Po on any of the surfaces with which it had been in contact. With these assumptions Po should have reached 23 per cent of its equilibrium value. Obviously the Ra^{226} value obtained by this method should be low. The Ra^{226} content was verified by using an ionization chamber and Lindemann-Ryerson electrometer. The ionization current decreased rapidly at first because of Th decay and then increased as the daughters of Rn grew in. These results also indicated that the Ra^{226} content was 23 μc per ml of M-10.

$$\begin{array}{rcl}
 332 & & \\
 \times .7 & & \\
 \hline
 232.4 & \text{c/m} & \\
 \\
 232 & & \\
 \times 2 & & \\
 \hline
 464 & \text{d/m} & \\
 4.64 \times 10^6 & \text{d/m} & \\
 \frac{4.64 \times 10^6}{.23} = 2.0 \times 10^7 \text{ d/m Ra} & & \\
 \\
 \frac{2.0 \times 10^7}{2.2 \times 10^{12}} = 1 \times 10^{-5} \text{ C Ra} & & \\
 10 \mu\text{c Ra/ml} & &
 \end{array}$$

It was concluded that the stock solution contained on the date of our analysis 50 $\mu\text{c MsTh}/\text{ml}$, 23 $\mu\text{c Ra}^{226}/\text{ml}$, and 2.1 $\mu\text{c Po}^{210}/\text{ml}$. These values are not in accord with the records, which indicate Source No. 36 to be substantially pure MsTh. However, there are no records to indicate that the laboratory previously had made a detailed analysis.

PROGRESS REPORT: EFFECTS OF X RAYS AND OF NITROGEN
MUSTARD ON GROWTH OF TETRAHYMENA GELEII

H. S. Ducoff

As part of a study on physiological factors influencing sensitivity to ionizing radiation and to radiomimetic substances, with particular reference to effects on cell division, a series of experiments have been performed on the ciliate, Tetrahymena geleii.* The cells in these experiments were grown in flasks of 2 per cent proteose-peptone at 25°; in most cases, they were centrifuged gently for 10 minutes and resuspended in an appropriate medium for treatment. Tetrahymena in broth, and in the dilute buffered medium⁽¹⁾ used for culturing chilomonads have thus been exposed to 250 kv X rays in doses up to 40,000 r; cells in broth and in distilled water have been exposed to nitrogen mustard (HN2) in doses ranging from 4×10^{-5} M to 1×10^{-3} M.

Figure 21 presents results of typical experiments with each agent. It can be seen that during the period of logarithmic growth, which ends at populations between 150,000 and 200,000 cells per cc, T. geleii has a generation time of just under 3 1/2 hours under our conditions. After centrifugation or transfer, the controls exhibit a lag of one to two hours; treated cells resume logarithmic growth after lag periods which are considerably longer than that of the controls. Thus, proteose-peptone-suspended Tetrahymena irradiated with 5000 r have a delay about 45 minutes more than the nonirradiated controls, compared with a five-hour longer delay following 40,000 r; 7.4×10^{-4} M HN2 in proteose-peptone cultures results in a delay about 2 1/2 hours longer than the controls. No killing was observed with the doses employed; this is not surprising in view of the recent report by Elliot⁽²⁾ that 850,000 r failed to kill Tetrahymena in proteose-peptone, and cells suspended in water were killed by 550,000 r.

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*Stock obtained from Dr. G. W. Kidder's laboratory.

Figure 21. Effects of X radiation and HN_2 on the growth of *T. geleii* in two per cent proteose-peptone.

CHANGES IN BLOOD PLASMA OF THE CHICK FOLLOWING X IRRADIATION

I. GLUCOSE

S. P. Stearner and E. J. Katz

Death in the initial period (24 to 48 hours) following X irradiation of young chicks is accompanied by evidence of alterations in liver function, e.g., multiple diffuse hemorrhages in the liver, decrease in the visible fat and glycogen content, and apparent change in the composition of the bile. Abnormal levels of some plasma constituents that are influenced by the activity of the liver (such as glucose, amino acids, proteins, and bile pigments) may be used as indicators of functional change. The effect of X irradiation on the plasma glucose level of newly-hatched chicks is reported here.

Plasma glucose levels of three-day chicks were determined at one hour, three hours, and five hours after X irradiation (1000 r, 43 r per minute). Blood was obtained by cardiac puncture. The micro-quantitative method of Nelson^(1,2), measuring no non-sugar reducing substances, was adapted to chick plasma of 0.1 cc and 0.2 cc aliquots by using zinc hydroxide filtrates. Loss of glucose through glycolysis was prevented by use of sodium fluoride in the collecting tubes. Determinations were also made in order to compare the effects of Nembutal[®] and ether anesthesia on plasma glucose concentration of control birds.

There was no significant difference in average plasma glucose levels between control and irradiated animals in either fasting or non-fasting chicks (Table 9). The relatively small decrease in plasma glucose concentration after a twenty-four-hour fasting period may be explained by the presence of a large amount of food in the yolk sac of three- to four-day chicks. Under the conditions of the experiment, the choice of anesthesia had no apparent effect. Although mean plasma glucose concentration remained unchanged following irradiation, values of 50 to 100 mg per cent above the control mean were observed in individual cases. This hyperglycemia was found at three and five hours following irradiation, usually in fairly sick birds and commonly associated with the presence of liver hemorrhages.

In contrast to the lack of a consistent change in the glucose concentration in irradiated chicks, a hyperglycemia in the initial postirradiation period has been reported in rabbits⁽³⁾, rats⁽⁴⁾, and guinea pigs⁽⁵⁾. In rabbits the change was found two to four hours after exposure; in rats an elevation was noted six hours after irradiation; in guinea pigs significant changes occurred within twenty-four hours.

Maintenance of the plasma glucose concentration in critically ill, irradiated birds indicates that glucose metabolism is being maintained. Apparently the plasma glucose level in the chick is not a sensitive indicator of any radiation-induced alteration in liver function. Preliminary investigation, however, has shown that in the initial period following irradiation there is a significant increase in both amino acid nitrogen and the concentrations of individual amino acids. This is being investigated further.

Table 9

PLASMA GLUCOSE (MG PER CENT) FOLLOWING X IRRADIATION
(1000 r, 43 r PER MINUTE) OF THREE-DAY CHICKS

	Controls			X irradiation		
	1 hr	3 hrs	5 hrs	1 hr	3 hrs	5 hrs
Fasting (ether)	253 (10)	250 (10)	248 (13)	255 (16)	270 (18)	249 (21)
Fasting (Nembutal®)	251 (6)	-	-	-	-	-
Food ad libitum (ether)	282 (23)	-	-	278 (21)	291 (23)	292 (21)

Number of animals in each group is indicated in parentheses.

SUMMARY

Plasma glucose determinations in newly-hatched chicks at one, three, and five hours after X irradiation showed no significant changes in mean value from control levels. Individual animals, however, showed a hyperglycemia of 50 to 100 mg per cent over control values three and five hours following irradiation. Such hyperglycemia was usually found in sick birds and was commonly associated with liver hemorrhages.

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X RADIATION TOXICITY IN THE CHICKEN

S. P. Stearner

The effect of X irradiation in the young chicken has been shown to be dependent on dose rate. Data now available permit a comparison of the radiation sensitivity of chickens of different ages up to maturity (six months after hatching). White leghorn cocks were either raised in the laboratory from newly-hatched chicks or were brought in one to two months before they were irradiated. The two groups of birds showed no apparent difference in radiation sensitivity. All birds were exposed to 1000 r, delivered at either 6 r or 43 r per minute. Conditions of irradiation were: 200 kv, 15 ma, 0.5-mm Cu and 3.0-mm Bakelite filters. A difference in rate was produced by varying the target-field distance. The birds were irradiated in a horizontal beam and were turned once during the exposure. Survival was observed for 30 days or more after treatment.

Survival of the different age groups following X irradiation is shown in Figures 22 and 23. All groups showed a large number of deaths in the initial period after a high rate exposure, while after a low rate exposure the period of highest mortality was between one and two weeks. After exposure at 43 r per minute, the initial mortality was similar in all age groups, but the small number of birds that survived this critical period does not permit an accurate comparison of sensitivity in the later period (Table 10). Radioresistance of the one-month chicks exposed at the lower dose rate was apparently greater than that of the older birds but resembled that reported in newly-hatched chicks.⁽¹⁾ There was no significant change in radiosensitivity between two and six months after hatching.

Ascites and hemorrhages in the proventriculus and duodenum were commonly seen at autopsy in birds that died in the initial period. Multiple diffuse liver hemorrhages were sometimes seen, and a few hemorrhages, urate deposits, and eschemia were occasionally observed in the kidneys. Animals that died later frequently showed hemorrhages in liver, duodenum, and kidneys; ascites; anemia; and jaundice. In a few instances multiple abscesses were seen in liver and spleen.

Sensitivity to X irradiation and the effect of variation in dose rate were not altered significantly during the maturation period in the chicken. In general, radiation death in the older birds was associated with less severe gross pathology than was usually seen in newly-hatched chicks. The physiological changes that follow irradiation of the mature bird have not been investigated.

Figure 22. Mortality of one- to six-month chickens after exposure to 1000 r at 43 r per minute. Number of animals in each group is indicated in parentheses.

Figure 23. Mortality of one- to six-month chickens after exposure to 1000 r at 6 r per minute. Number of animals in each group is indicated in parentheses.

Table 10

COMPARISON OF RADIATION TOXICITY IN CHICKENS OF DIFFERENT
AGES (X IRRADIATION - 1000 r)

Age (mo.)	Rate (r/min.)	Animals (no.)	Survival (%)	
			Initial (0-2 days)	Later* (3-30 days)
6	6	40	87.5	0
	43	43	34.8	53.3
4	6	34	94.1	12.5
	43	35	42.8	46.6
3	6	37	94.6	11.4
	43	36	36.1	30.8
2	6	36	91.7	3.0
	43	36	22.2	0
1	6	30	96.7	72.4
	43	32	15.6	60.0
2-4 (days)**	6	172	97.7	39.9
	43	180	17.2	16.1

*Per cent of three-day survivors at 30 days.

**Previously reported data.⁽¹⁾

SUMMARY

The mature chicken showed a response to variation X-radiation dose rate similar to that reported in newly-hatched birds. Survival following 1000 r at 6 r or 43 r per minute indicated that there was little or no change in radiosensitivity within the first six months.

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PROGRESS REPORT: THE MECHANICS OF CELL DIVISION

T. N. Tahmisian and G. Svihla

We have observed a very large nucleus in the premature ovum of Melanoplus differentialis. Prior to the meiotic division leading to the secondary oocyte the nucleus attains a diameter greater than $150\ \mu$. Nuclei in the primary oocyte stage vary in size from 80 to $150\ \mu$, depending on the degree of follicular maturation. The chromatin of these nuclei is visible with phase contrast microscopy.

In the more mature ovum the nucleus appears to move in an oscillatory rotational fashion. The moment of this movement varies from 10 to 45 seconds for each complete phase. This activity has been photographed by time lapse motion pictures.

These nuclei were dissected "free hand" out of the egg under a dissecting microscope. Chromatin and small particles were observed within the karyoplasm. The contents of the nuclei, except for the chromatin material, drop in viscosity when removed from the egg as judged by the increased Brownian movement.

We believe that this is the largest nucleus that has been observed in living material. It will be used in the near future as a test object in a study of the effects of radiation emanating from a point source.

PROGRESS REPORT: EFFECT OF X IRRADIATION ON ENZYMOGENESIS

T. N. Tahmisian and D. M. Adamson

The effect of X irradiation upon the acetyl choline content of the developing grasshopper embryo has been reported.⁽¹⁾ Attempts are being made to determine the choline acetylase content in irradiated and control postdiapause grasshopper embryos. Although our data are incomplete, there appears to be no immediate effect on the choline acetylase content of the irradiated embryo. However, as the irradiated embryo develops, the choline acetylase content is lower than in the control. This is consistent with reports in the literature. The acetylase content seems to depend on neural differentiation as well as on the functional activity of the differentiated tissues. There is no tissue differentiation in these irradiated embryos; hence, the acetylase content is low. The anabolic processes that are effected by 25,000 r X irradiation in turn affect the anabolism of the choline acetylase. That which is originally present in the embryo does not diminish in amount for 18 days after exposure.

LITERATURE CITED

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PROGRESS REPORT: REPURIFICATION OF LABELED IAA

R. E. Stutz, D. E. Atkinson, and S. A. Gordon

After the completion of the synthesis of labeled 3-indoleacetic acid-2- C^{14} (IAA)⁽¹⁾ the preparations were placed over P_2O_5 in an atmosphere of nitrogen for storage purposes. During the summer months the color of the preparations changed from a pale yellow to a dirty tan. This change was at first presumed to be caused by the presence of a volatile material in the P_2O_5 , although the internal radiation of the 15 per cent C^{14} -labeled preparation is approximately 500,000 rep per day.*

Since only the purest obtainable IAA should be used in the physiological experiments planned, repurification of the preparations is indicated. Column chromatography appeared to offer the best possibilities for a successful separation because, first, the original yellow impurity could not be removed by crystallization⁽¹⁾ and second, naturally occurring compounds having structures similar to IAA may be easily separated by paper chromatography.

If a column packed with washed paper pulp (Solka Floc - SW 200) and a one per cent NH_4OH butanol solution as the resolving solvent are used IAA may be obtained which melts at 169 to 170°C and has sharp ultraviolet adsorption peaks at 288, 279, and 220.5 $m\mu$ in water. IAA of this purity was secured from IAA which had been oxidized either by exposure to moist air or P_2O_5 .

Since there is now an appreciable difference in the appearance between the two preparations of labeled IAA, an estimation of the degree of decomposition caused by the internal radiation dose will be determined before any preparative separation is attempted. If this indicates that the storing of dry purified samples is impractical, small scale repurifications will have to be carried out as needed.

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*The possible extent of destruction caused by internal irradiation in dry organic compounds was called to our attention by Dr. B. M. Tolbert of the Radiation Laboratory, Berkeley, California.

PROGRESS REPORT: THE RADIOSENSITIVITY OF AUXIN BIOSYNTHESIS

R. P. Weber and S. A. Gordon

The conversion of tryptophan to indoleacetic acid (auxin) by the plant is an enzymatic process that shows immediate and prolonged inhibition following single low doses of X radiation.⁽¹⁾ In the series of reactions in which tryptophan is converted to indoleacetic acid, the immediate precursor is considered to be indoleacetaldehyde. The radiosensitivity of the reaction sequence leading to the aldehyde was therefore examined.

A study of indoleacetaldehyde levels in the mung bean (Phaseolus aureus, Roxbg.) immediately following low doses of X radiation (25 to 50 r) showed no decrease, but rather, a significant increase, or piling-up of the aldehyde, in contrast with nonirradiated controls. Elevated aldehyde levels were observed with all radiation doses studied up to 5000 r.

The simplest interpretation of the results described above is that a major locus of the radiosensitivity lies in the lability of the enzyme controlling the conversion of indoleacetaldehyde to indoleacetic acid.

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PHOTOPERIODIC RESPONSES IN PLANTS

INTRODUCTION

Photoperiodism refers to plant responses that are correlated with the duration and periodicity of the light and dark periods. The response is manifested in growth and development, and the effect is frequently, but not exclusively, on the reproductive phase. In most instances the effect of photoperiod on reproductive phases of growth has been simply to effect the initiation of floral primordia. After the initiation of these primordia, whether development of mature flowers and fruit occurs is dependent on a number of factors that may affect growth and development. Recent studies have indicated that, in the case of the red kidney bean, floral and fruit development is also governed by photoperiod.

A summary of the present results obtained from the experiments with red kidney bean and certain findings concerning floral initiation in Xanthium are presented.

XANTHIUM

Movement of the flowering stimulus. Photoperiodically, Xanthium is a short-day plant. While it will grow and remain vegetative indefinitely under a relatively long day, floral primordia are initiated promptly if the plant is subjected to but a single dark period of approximately 9 1/4 hours or longer. In accordance with experimental data available, the present belief is that the leaf is the site of synthesis of a substance or substances, and this synthesis is governed by the length of the dark period. The material formed in the leaf moves out of the leaf to the actively growing terminal meristems where it modifies cellular division to the extent that floral primordia are produced in place of the usual vegetative structures. These early floral structures can be seen under a dissecting microscope as early as four to five days after the plant has been subjected to a flower-inducing photoperiod. It has not been clear whether a continuous supply of the substance from the leaf is required during this four- to five-day growth period or whether a short initial stimulation is sufficient for floral initiation. Likewise, the actual time required for the material to move out of the leaf in required amounts has not been definitely known.

Experiments were designed to gain such information. Single-leaved Xanthium plants were prepared by removing all but the uppermost, fully-expanded leaf. These plants, which had been growing on an 18-hour vegetative photoperiod, were induced with a single 14-hour dark period, and the single leaf of plants of various lots in the subsequent light period was removed. Leaf removal was carried out at two-hour intervals starting at two hours after the close of the dark period. Ten days later all the plants were dissected to determine whether flowering had been initiated.

It was found that flowering had occurred in some of the plants that had their leaves removed six hours after the end of the dark period or 20 hours from the start of the dark period, but none of the plants that had the leaves removed earlier had flowered. Flower structures were present in all the plants that had their leaves removed eight hours after the end of the dark period and at all later periods.

These data indicate that if Xanthium is given a 14-hour dark induction, 20 to 22 hours are required for the production and translocation of the flowering substance in amounts sufficient for floral initiation. The substance apparently need not be supplied over a long period and it appears to move out of the leaf soon after it is formed. The data also indicate that the substance is not destroyed by light while it is still in the leaf, a point that had not been clarified to date, and in point of fact, the final effective amount leaves the leaf during the light period.

Light-dark relationships. Since the substance involved in floral initiation in Xanthium is produced in the leaf during the dark period, it would appear that perhaps a shorter time than 20 to 22 hours would be necessary for its production and translocation in amounts sufficient for floral initiation if the plant were subjected to a continuous dark period. This was tested by placing one-leaved Xanthium plants in a dark shed and removing lots at various intervals starting with 9 hours up to 30 hours of continuous dark, with a total of 10 periods. Two lots of plants were removed at each period: the leaves were removed at the end of the respective dark periods from one lot, and the plants of the second lot were transferred to a light period with leaves intact.

All the plants receiving dark periods from 9 to 30 hours followed by a post-dark light period produced flowers. In the case of those receiving no post-dark light period, only the plants on which the leaves were allowed to remain for at least 30 hours had flowered. Since the dark period is suitable for the formation of the flowering substance, these data suggest that with a given minimal but effective dark duration for synthesis, a post-dark light period has an enhancing effect in translocating the stimulus from the leaf to the meristem regions where floral initiation is accomplished.

Another experiment was carried out in which two lots of one-leaved Xanthium plants were induced and the leaves of all the plants were removed after 22 hours from the start of the dark period. One lot received a 16-hour dark period followed by a 6-hour light period and the second lot received a 22-hour dark period with no subsequent (post-dark) light period. In this case again all the plants receiving the post-dark light period flowered, while those receiving no post-dark light period failed to flower.

The post-dark light period is not essential for flowering, but apparently under the present experimental conditions light decreases the over-all time period required from time of formation of flowering stimulus in the leaf and its subsequent movement out of the leaf. Plants subjected to a continuous dark period apparently produce the flowering substance, but a longer period is required for its movement out of the leaf.

Since light is known to affect protoplasmic viscosity and permeability, it seems likely that these observed effects on apparent translocation are reasonable. In subsequent experiments it is planned to determine the minimal light intensity required to constitute a post-dark light period for this response and to determine the action spectrum for this phenomenon.

Effect of temperature on movement of the flowering substance.
An experiment was designed to determine the effect of low temperature during the post-dark light period on flowering, since the low temperature would tend to decrease the rate of translocation.

One-leaved Xanthium plants were induced with a single 14-hour dark period in a dark shed held at 75-80°F. At the end of the dark period one lot was transferred to a lighted room held at 50°F and a second lot was transferred to a lighted greenhouse bench at a temperature of about 80°F. The leaves of plants from both lots were removed at hourly intervals up to 10 hours after the end of the dark period.

If the leaves were left on the plants for at least 20 hours from the start of the dark period, the plants held at 80°F during the post-dark light period flowered. On the other hand, no flowering occurred in any of the plants held at 50°F during their post-dark light period.

These data again indicate that floral initiation in time is dependent both on the time required for movement of the flowering substance from the site of formation in the leaf to the stem and meristem regions and upon the time required for synthesis of an effective amount in the dark period.

RED KIDNEY BEAN

Effect of photoperiod on floral and fruit development in red kidney bean. In preliminary studies it was determined that red kidney bean (*Phaseolus vulgaris*) has been found to require a short photoperiod for both floral and fruit development. Subsequent experiments revealed that although essentially floral initiation occurs with any duration of photoperiod, including continuous light, as well as continuous darkness, the relative time of initiation of both first flower primordia and terminal inflorescences on the primary plant axis can be affected significantly by variation of photoperiod. The node position on the plant axis at which either first flower primordia or terminal inflorescences are initiated may be accepted as a reliable quantitative measure of the degree of flower stimulus attained as a result of differential plant treatments.

The earliest degree of flowering of kidney bean, as measured by the earliness of establishment of first flowers and terminal inflorescences, occurred with continuous darkness. For both of these flowering indices the greatest delay occurred with photoperiods of long duration, from 15 hours up to continuous light, while the short photoperiods of 13 hours or less the degree of flowering was intermediate to that expressed with continuous darkness as compared with long photoperiods. The position of the first flower node was found to vary from the fourth to the eighth node throughout the various photoperiod treatments. Under the same experimental conditions the location of the terminal inflorescence varied from the fifth to the ninth node.

Bean plants subjected continuously to photoperiods of 15 hours or longer failed to develop floral buds, with the result that they are also fruitless. With such photoperiods the flower primordia only develop to a small macroscopic bud stage. The fact that such buds are floral ones and have all of the normal floral structures present can usually be determined only with use of a dissection microscope. These buds frequently abscess if the long photoperiods are applied continuously for relatively long periods. Plants grown with 18-hour photoperiods have failed to develop floral buds beyond small macroscopic stages even with culture periods as long as 13 weeks. On the other hand, plants cultured from time of planting with photoperiods of 13 hours duration or less produce open normal flowers within 3 weeks from germination and bear mature sized pods and fruit within 5 weeks. As few as seven consecutive 15- to 18-hour photoperiods have been found to be effective in significantly inhibiting floral development of plants subsequently cultured with short photoperiods which normally induce early development of flowers and fruit (Figure 24).

The inhibiting effect of long photoperiods on floral and fruit development originates in the leaf, and as little as one primary leaf is effective in responding to photoperiod. Figure 24 indicates the nature of the

Figure 24. The growth and development of single-leaved, red kidney bean plants in response to duration of photoperiod: Planted November 20, 1951, on natural day (approximately 10 hours). The indicated photoperiod treatments were initiated when primary leaves were expanded on November 25, 1951. Photographed January 7, 1952. Floral buds and fruit developed only with continuous short, natural day photoperiods as shown by plant at extreme right.

inhibitory effect of long photoperiod upon floral and fruit development of single-leaved plants. If plants are maintained with but two primary leaves, one subjected to long photoperiod, the other to short, the long-day inhibitory effect on reproductive development prevails. The inhibitory effect of long day cannot be related to marked differences in photosynthate formed in leaves subjected to long day as against that formed in short-day leaves. This is true since photoperiods of different duration were given equal periods of high light intensity with the remaining hours of the respective photoperiods consisting of Mazda light of 30-50 fc. That the quantity of photosynthate was not involved was also substantiated by the fact that plants subjected to short photoperiods of high light intensity, which normally would flower and fruit, promptly failed to do so when 30 to 60 minutes of light of 30-50 fc was utilized to interrupt the middle of the dark or night period.

In addition to the inhibition of floral bud development in red kidney bean, a number of vegetative growth responses were also observed to be correlated with variable duration of photoperiod treatment. Generally photoperiods that inhibited floral bud development significantly promoted increased length of both the primary and lateral plant axis and resulted in a greater number of both lateral axes or shoots and number of expanded compound leaves. The observed correlation of increasing degree of vegetative growth with a decreasing or complete arrest of floral growth for kidney bean plants in response to increasing photoperiod duration suggests the possibility that a photoperiodic control of both the physiological level of auxin and the flowering stimulus may be operative in this plant species. Experiments designed to check this hypothesis are now being planned.

Microbiology

E. Lawrence Powers
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PROGRESS REPORT: MICROBIOLOGY

Re-examination of the data describing three biological effects (vegetative death, suppression of division, and death after autogamy) in Paramecium aurelia after increasing time of exposure to a nitrogen mustard compound has revealed that over the period of a year during which the experiments were performed there was a gradual shift in the quantitative relationships between two of these effects and dose, and that the direction of shift was different for each of the effects measured. At equivalent doses death after autogamy, a measure of genetic effect, increased and suppression of division decreased while death before cell division remained about the same.

Modification of these responses has been accomplished by varying temperature. At 30°C during exposure to the agent, death of cells before the subsequent first division is higher than that observed after a 25°C exposure temperature, but cell division is suppressed to a lesser degree. Postautogamous death is apparently not affected by this temperature difference. When the temperature is lowered to 18°C just after exposure to the agent at a temperature of 25°C, both vegetative death and suppression of fission are increased in magnitude.

It is not possible to explain these shifts in responses at the present time. However, it does appear that death after autogamy is not easily modified by environmental changes and that the shift in response noted over the year cannot be explained in these terms.

Biochemistry

E. S. Guzman Barron

Phyllis Johnson

Louise Seki

INACTIVATION OF YEAST ALCOHOL DEHYDROGENASE
BY X IRRADIATION

E. S. G. Barron and P. Johnson

ABSTRACT

Crystalline yeast alcohol dehydrogenase is a -SH enzyme that contains freely-reacting -SH groups and gives a strong nitro-prusside test. Titration by the amperometric method and by oxidation with iodosobenzoate revealed that the protein contained 10 -SH equivalents per mole. All of them seem necessary for enzyme activity. Inhibition of the enzyme with p-chloromercuribenzoate and with iodosobenzoate was only partially re-activated by the addition of glutathione or BAL.

Crystalline alcohol dehydrogenase was readily inhibited by X irradiation, the degree of inactivation being strictly proportional to X-ray dose. The ionic yield $\frac{M}{N}$, was 0.89, very close to that found for phosphoglycer-aldehyde dehydrogenase, which is 0.93. Complete protection of the enzyme against inactivation by X irradiation was achieved by the addition of 0.01 M glutathione or cysteine. Addition of glutathione after irradiation produced only partial reactivation.

The mechanism of inactivation seems to be due to oxidation of the -SH groups by the OH and O₂H radicals produced after irradiation of water. In this inactivation H₂O₂ plays no role, for 10⁻⁴ M had no effect at all. The enzyme was inhibited 23 per cent by 10⁻³ M H₂O₂.

STUDIES ON THE DISTRIBUTION OF FIXED CARBON

R. W. Swick, D. L. Buchanan, and A. Nakao

ABSTRACT

Rats and mice on stock diets were continuously exposed to air containing a constant low partial pressure of $C^{14}O_2$. This maintained an unchanging specific activity (s.a.) within the CO_2 -bicarbonate pool of the animal. Within two weeks the radioactivity of the total liver carbon reached a constant level. This constant s.a. was also found in the liver of mice born and raised in this labeled atmosphere. Such agreement was evidence that the total fixed carbon of the liver had become isotopically saturated. Therefore, a survey of the distribution of C^{14} in this tissue should quantitatively mirror the true distribution of CO_2 -derived carbon in the natural state. Approximately 3 per cent of the total liver carbon was derived from CO_2 . About 70 per cent of the fixed carbon was found in protein, 12 per cent in carbohydrate, 5 per cent in lipids, and 1 per cent in a crude nucleic acid fraction. Only 4 per cent of glycogen carbon and 1.4 per cent of glycerol carbon represented fixed carbon. About 1 per cent of the carbon in the unsaponifiable lipids and much less in the fatty acids was derived from CO_2 . Nearly 80 per cent of the fixed carbon in protein was in α -carboxyl groups of amino acids. While 73 per cent of the guanidine carbon of arginine was derived from CO_2 , the ornithine moiety was practically devoid of fixed carbon. The other essential amino acids that were examined and tyrosine contained essentially no fixed carbon. As measured by these experiments fixed carbon distribution differs considerably from that reported after isotope exposures of shorter duration.

STUDIES ON THE PHYSIOLOGICAL STATUS OF BONE CARBONATE

A. Nakao and D. L. Buchanan

ABSTRACT

Isotopic turnover studies have revealed that the specific activity (s.a.) of bone carbonate diminishes during manipulations preceding analyses; therefore a study of this phenomenon was undertaken. Rats were sacrificed after an exposure to $C^{14}O_2$ in air for two weeks, during which time only 35 per cent of the bone carbonate had exchanged with the labeled CO_2 -bicarbonate pool. In the bones of these animals the carbonate s.a. diminished to the indicated percentages (of untreated controls) in the following operations: extraction with ethylenediamine (1 hour), 54 per cent; boiling in 3 per cent KOH in glycol (90 minutes), 66 per cent; boiling in distilled water (1 hour), 91 per cent; and evacuation below 1 micron (3 days), 98 per cent. The CO_2 evolved during the 3 day evacuation had the same s.a. as the CO_2 -bicarbonate pool. Bones from animals exposed for different periods were ignited at 500° for 24 hours. The fractional diminution in s.a. with this treatment was proportional to the fractional replacement of carbonate by isotope during the exposure. These findings and others indicate that bone carbonate recently exchanged in vivo is more susceptible to removal by chemical and physical agents than older carbonate. To determine whether this time-dependent behavior results from diffusion through the bone matrix or from heterogeneity of carbonate within the bone salt, ground samples of this bone were fractionally acidified. The CO_2 evolved first had a high s.a. that diminished with each successively liberated fraction. This finding was independent of particle size down to 1 micron. We conclude that the carbonate heterogeneity lies in the bone salt.

PROGRESS REPORT: ORGANIC CHEMISTRY

The reaction of 3-aminophthalhydrazide (luminol) with hydrogen peroxide is accompanied by chemiluminescence⁽¹⁾, and an alkaline solution plus hemin, a catalyst, emits light when irradiated with X rays, γ rays, β rays, α particles, and neutrons, presumably through the intermediate formation of peroxide or oxidizing radicals.

An alkaline solution of luminol has been prepared for Dr. Isadore Berlman who will investigate the possibilities of using this as an aqueous system dosimeter.

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PROGRESS REPORT: IN VIVO CHEMICAL MECHANISMS OF
INTERACTION OF Be WITH ORGANIC COMPOUNDS

M. R. White and J. Schubert

Earlier work demonstrated that aurin tricarboxylic acid increases survival of mice acutely poisoned with beryllium ⁽¹⁾, and that the reactive groups (ortho-carboxyl-hydroxy groups) on the molecule are probably responsible for the reaction with Be. ⁽²⁾ Recently other compounds have been tested for their effect on the survival of mice given an intravenous LD₉₅ of beryllium sulfate (0.7 mg Be per kg). The following substances, given one hour after the Be injection, did not prevent death of the mice: anthranilic acid (12.4 and 40 mg per mouse), eriochrome cyanine (4 and 8 mg per mouse), mandelic acid (12.4 and 30 mg), aspartic acid (10.5 mg), and sodium malate (10.0). Sulfosalicylic acid in doses of 30 and 40 mg caused 80 to 100 per cent survival of the poisoned mice, while a dose of 20 mg gave partial protection.

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PROGRESS REPORT: RETENTION OF C¹⁴-METHYL-LABELED ATA
IN THE TISSUES OF RATS AND DOGS

A. Lindenbaum and M. H. Graves

Aurin tricarboxylic acid (ATA) has been shown to be effective in the treatment of experimentally induced acute berylliosis in mice and rats.⁽¹⁾ The effectiveness of ATA is believed to be due in part to prolonged retention of the dye around beryllium particles in the tissues. It was therefore considered important to determine the length of time a single, therapeutically effective dose would remain distributed among the body tissues in effective concentrations. Table 11 summarizes the data now at hand for rats and dogs given C¹⁴-methyl-labeled ATA intravenously.

Autoradiographs of rat liver, lung, and spleen removed 24 hours after intravenous ATA administration show a fairly uniform distribution of C¹⁴ activity throughout the tissues. In the kidney there is some cortical localization of activity. The periosteum and marrow of the femur and humerus appear to be uniformly active, while the inorganic bone matter is devoid of activity.

LITERATURE CITED

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Table 11

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C¹⁴ ACTIVITY IN RATS* AND DOGS[†] (%)

Tissue	1 hour rat	24 hours		1 week rat	4 1/2 mo. rat	6 mo. rat
		Rat	Dog			
Spleen	0.29 ± 0.00	0.42 ± 0.04	0.40 ± 0.04	0.46 ± 0.05	0.24 ± 0.01	0.20 ± 0.05
Kidneys	1.6 ± 0.00	3.9 ± 0.05	3.20 ± 0.05	4.32 ± 0.46	0.76 ± 0.03	0.78 ± 0.07
Liver	4.7 ± 0.30	9.1 ± 0.08	10.20 ± 0.55	7.36 ± 1.74	1.17 ± 0.40	0.88 ± 0.19
Lungs	1.0 ± 0.10	0.62 ± 0.04	0.58 ± 0.02	0.46 ± 0.18	0.15 ± 0.04	0.11 ± 0.02
Femurs	0.46 ± 0.05	0.53 ± 0.03		0.30 ± 0.04 [†]	0.19 ± 0.03 [†]	0.14 ± 0.00 [†]
Pelt		24 ± 4.0	30.7 ± 8.7	8.6 ± 0.80	3.2 ± 0.60	1.4 ± 0.00
Blood	21.0 ± 0.0	3.2 ± 0.3	7.58 ± 0.77			
Muscle		5.5 ± 0.3	10.49 ± 0.54			
G-I tract and feces		57 ± 2.0				
Urine		10.1 ± 1.9				

*Two or more rats in each time group received 80 mg ATA per kg.

[†]Two dogs received 40 mg ATA per kg.

[†]Value for right femur doubled.

PROGRESS REPORT: STUDIES ON POSTIRRADIATION HYPOTENSION

J. F. Thomson and E. T. Mikuta

INTRODUCTION

Since adenosine and 3-adenylic acid have been shown to decrease the blood pressure of rats to a greater extent than any other nucleic acid derivatives⁽¹⁾, it was felt that the fall in blood pressure observed shortly after X irradiation might be at least partially explained by the appearance in the circulating blood of adenosine derivatives as a result of destruction of nucleic acids or possibly of adenosine triphosphate.* The most convincing evidence of the role of such compounds in contributing to postirradiation hypotension would be the demonstration that there was an increased concentration of these compounds in the plasma of irradiated animals at a time when the blood pressure was diminished.

METHODS

Three-month, Sprague-Dawley female rats and four-month Tumblebrook guinea pigs were used in these studies; anesthesia and blood pressure recordings were carried out as previously described⁽¹⁾, with the exception that heparinization was omitted in certain experiments because of interference by heparin with the orcinol condensation. After measurement of blood pressure, rats were exsanguinated via the carotid artery, and the blood mixed with 1/5 volume of 3 per cent sodium citrate. Analyses were carried out on trichloroacetic acid extracts of the diluted plasma.

Although the analytical method for total pentose is not highly specific⁽⁴⁾,** a failure to demonstrate an increase in plasma pentose would be strong evidence against the hypothesis previously advanced.⁽¹⁾ Since glucose interferes to some extent in the pentose estimation, analyses for glucose were also carried out.⁽⁷⁾ In various other experiments, plasma or blood was analyzed for histamine⁽⁸⁾, nucleic acid phosphorus⁽⁹⁾, and non-protein nitrogen.⁽¹⁰⁾

*On the basis of work on larger animals, the desoxyribonucleotide and both ribonucleotides of adenine seem to be approximately equal in vasodilating action.^(2,3)

**More nearly specific methods employing aniline derivatives^(5,6) were unsatisfactory because only free pentose reacts.

RESULTS AND DISCUSSION

An average of 121 mm Hg ($\sigma = 16.4$) was recorded for the mean carotid blood pressure of 47 rats measured between one and two hours after exposure to 1000 r of 200-kv X rays. The average for 50 control rats was 140 mm Hg ($\sigma = 15.5$).

Although a slight increase in plasma pentose following exposure to X ray was observed in the first group of animals studied, subsequent experiments on more adequate numbers of animals failed to substantiate this preliminary finding, so that in consideration of the results as a whole, there was not a consistent difference between normal and irradiated rats in respect to total plasma pentose.

In one experiment in which whole blood rather than plasma was analyzed, the pentose content was unchanged one hour after irradiation but had decreased by 20 per cent at two hours. There was a hyperglycemia (28 per cent) at one hour, followed by a return to normal glucose levels at two hours. The blood NPN remained unaltered.

The possibility that vasodilatation was produced by more highly polymerized nucleic acid fractions, not extractable by cold trichloroacetic acid, was seemingly eliminated by the observation that no apparent nucleic acid phosphorus⁽⁹⁾ could be found in the plasma of either control or irradiated rats.* The possible role of protein moieties of nucleoproteins (e.g., histones) has not been assessed.

It was found that the blood pressure of guinea pigs was decreased from 62 ± 10 to 38 ± 1 within 50 minutes after exposure to 500 r, rising to 52 ± 5 90 minutes after exposure. Adenosine and adenylic acid, however, in doses which were strongly hypotensive in rats, were virtually inert when administered to guinea pigs.

Certain doubts about the concept that histamine is responsible for the fall in blood pressure after irradiation⁽¹²⁾ were raised by the finding that in a series of 21 exposed animals there was not a significant correlation between plasma histamine and blood pressure, measured on the same animal. Further, it was found that intravenously injected plasma from unexposed rats was generally as effective in lowering blood pressure as were plasma samples from exposed rats, despite the fact that normal plasma contained virtually no histamine while the exposed contained varying

*In this connection, it may be mentioned that a highly polymerized ribonucleic acid prepared⁽¹¹⁾ from cytoplasmic particulates of rat liver was as effective as a commercial sample of yeast ribonucleic acid, presumably appreciably degraded, in reducing rat blood pressure.

amounts up to 1.2 μg per ml. It is conceivable that the liberation of histamine is the most obvious manifestation of a shift in the metabolism of certain cells toward increased amino acid decarboxylation as an energy-yielding mechanism to supplement some radiation-induced impairment of the normal metabolic pathways, that the pharmacologic action of histamine would be offset by the concomitant liberation of amines with opposite action (e.g., tyramine), and that the initial fall in blood pressure is a central rather than a peripheral effect⁽¹³⁾, with histamine or other blood-borne agents contributing to the acute vasodepression only under certain circumstances.

The action of benzimidazole in competitively inhibiting the utilization of purines by certain microorganisms⁽¹³⁾ prompted the investigation of possible "anti-adenosine" activity. Benzimidazole in intraperitoneal doses of 100 mg per kg modified very slightly the response of the rat vascular system to adenosine or adenylic acid; however, this dose greatly attenuated the action of histamine. This antihistaminic action was confirmed in studies on the guinea pig ileum. Benzimidazole had only a slight effect on acute radiation toxicity, reducing from 19/20 to 15/20 the 30-day mortality of mice exposed to 800 r.

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TOPICS ON THE GROWTH OF CELLULAR AGGREGATES

G. A. Sacher

INTRODUCTION

The investigations summarized here have as their principal goal the development of a quantitative theory of the effects of ionizing radiations (and other noxa) on the growth and the steady states of tissues and organisms. An important requirement is that the equations for radiation injury should be deducible from the laws of normal growth and the known physiological actions of ionizing radiations. Existing theoretical treatments of growth were unsatisfactory for one or more of the following reasons:

1. The parameters have no obvious physiological meaning
2. Growth is described in terms of a single variable, usually the mass of the system
3. The equations are in some cases awkward, i.e., the labor necessary for an exact solution is not commensurate with the amount or quality of data at present available.

A method of attack free of the above difficulties has been developed which has been successfully used to fit curves of normal growth and which also can account for the effects of ionizing radiations on growth.

CONCEPTS AND METHOD

The growth of the various body organs and hence of the body as a whole is considered to be dependent on a complex substrate having a composition that is essentially that of the diffusible blood constituents. The substrate concentrations are in turn dependent on the ability of the organism to capture and transform energy from the environment, and this is (indirectly) a function of the dimensions of the various organs. Each tissue should be further differentiated into its vegetative and proliferative fractions. The organs and tissues can be considered to be in a secular steady state with respect to the substrate concentrations, at least for the slow growth rates and concentration changes which are characteristics of normal postnatal growth. The properties of growth which are thereby neglected,

e.g., lag period, inertia of growth, etc., have small time constants relative to the time constants of over-all growth. It may not be permissible to neglect these in considering sudden perturbations of growth. Growth of a tissue, averaged over volumes which are much greater than the volume of a single cell, can be considered in terms of a uniform average rate of synthesis of tissue mass. This can be equated to the steady state velocity of a chain of synthetic reactions. Theoretical and experimental knowledge of the behavior of steady-state chemical systems is sufficient to permit a reasonably accurate specification of the dependence of the rate of synthesis on substrate concentrations.

Nothing in the above schema is conceptually new, but on the other hand, the implicit set of equations could not be solved without tremendous simplification. The present contribution is the proposal of an approximate method that is simple enough to permit a solution while at the same time keeping the approximation error within tolerable bounds.

First it may be noted that while the total mass of the growing animal may increase by a factor of 50 from birth to adulthood, the ratios of the masses of the various organs to the body mass do not usually change by so much as a factor of two over this period.* Certain exceptions may be noted, of which the central nervous system and certain sense organs are the most conspicuous. The proposal is made that the growth of organs be represented by a dominating function that has the same form for all organs and essentially describes the growth of an average organ, plus a specific function that, as its name connotes, accounts for the departure of the growth trend of the specific organ from the general trend. Since the specific functions vary over a limited range, these functions can be represented by the linear term of an expansion in terms of some convenient function of the organ dimensions.

The end result of this approximation procedure is a system of simultaneous linear differential equations. Since the growth curves of laboratory animals are comparatively simple in form, it is probable that most organs contribute not as independent entities, but rather as constituents of larger organ systems, and these may be few in number. For the elucidation of these points it will be necessary to carry out multivariate analyses of growth curves and organ weights in normal and experimentally modified animals. There is no present application for the general theory, so further discussion will be restricted to simpler models.

*Extensive data on this point may be found in the literature on the allometric growth relations. See, for example, Donaldson, The Rat.

THE TWO-COMPONENT SYSTEM

For the purpose of growth analysis an organism may be thought of as consisting of two populations of cells, the proliferative and the vegetative. Let their numbers be, respectively, N_p and N_v . For this simplified model we introduce an empirical quantity, the growth-limitation function, $B(N)$, that comprises both the dependence of substrate concentrations on attained size and the dependence of rate of synthesis on substrate concentration. This is written in the form

$$B(N) = B_0 N_v^{-\alpha}$$

where B_0 is a constant and α is a small positive fraction.

A pair of nonlinear differential equations may be deduced:

$$\frac{dN_p}{dt} = N_p (B_0 N_v^{-\alpha} - d_p - m) \quad (1a)$$

$$\frac{dN_v}{dt} = m N_p - d_v N_v \quad (1b)$$

Here d_p and d_v are the death rates of proliferative and vegetative cells, respectively, and m is the migration (maturation) rate. Let N_p be the dominating function and define a new variable R as follows

$$N_p = R N_v$$

To simplify these equations two restrictions are imposed, both of which are admissible in application to postnatal growth

- 1) The exponent α is less than 0.3
- 2) The variable R changes during growth by less than a factor of 2.

With these restrictions the equations above can be approximated by the set:

$$\frac{d}{dt} \ln N_v + \frac{d}{dt} \ln R = h - j \ln N_v \quad (2a)$$

$$\frac{d}{dt} \ln N_v = p \ln R - q \quad (2b)$$

where the constants h, j, p, q are functions of the constants of Equations 1a, 1b.

The solutions are of the form

$$\ln N_v = P + Qe^{-\beta_1 t} + Re^{-\beta_2 t} \quad (3a)$$

$$\ln R = P' + Q'e^{-\beta_1 t} + R'e^{-\beta_2 t} \quad (3b)$$

The constants Q' , R' and Q , R are linearly related.

APPLICATIONS TO THE GROWTH OF NORMAL AND IRRADIATED RATS*

The mass growth curves of normal and irradiated rats have been fitted with expressions of the form

$$\ln M = P'' + Q''e^{-\beta_1 t} + R''e^{-\beta_2 t} \quad (4)$$

It has been found that two exponential terms are sufficient to describe the growth of rats from birth to maturity. A single exponential term is often sufficient to describe growth of rats from a weight of 25 g to maturity (Figure 25).

For application to the effects of radiations on growth, the most favorable case is that in which exposure to small daily doses is begun early in the course of growth and maintained until maturity (Figure 26). In this case, the effects of irradiation can be represented as a time-independent displacement of the values of the birth-rate and death-rate parameters (Equations 1a, 1b). The displacement of the irradiated from the control growth curve for very small daily doses is given by the sum of the linear terms of Taylor series expansions

$$\Delta \ln M(t) = \sum_{i=1}^n \left[\frac{\partial \ln M(t)}{\partial \alpha_i} \right]_{\alpha_i^0} \left[\frac{\partial \alpha_i}{\partial \rho} \right]_{\rho=0} \Delta \rho$$

where the α_i^0 ($i = 1 \dots n$) are the normal values of the n parameters of growth that appear in the expression for mass growth, and ρ is the dose rate (r per day). The resulting expression for the displacement of growth is of the form

$$\Delta \ln M = \left[C_1 + C_2 t \right] e^{-\beta_1 t} + \left[C_3 + C_4 t \right] e^{-\beta_2 t} + C_5$$

This expression can be made to fit radiation effect curves such as those of Figure 26. These weight effect curves are typical of the effects of continuous irradiation on a number of physiological systems, including

* The curve fitting reported here was carried out by Sylvanus A. Tyler, James E. Hall, and Joan Gurian.

Figure 25. The mass growth of male rats from age 25 days. The smooth line is a plot of the function:

$$\log_{10} M(t) = 2.570 - 0.864^{-0.030t} + 0.0476e^{-0.212t}$$

Figure 26. The effect of small daily doses of X rays on the growth of male Sprague-Dawley rats. Irradiation begun at age 53 days. Effect on mass growth, $E(t)$, expressed as

$$E(t) = \log_{10} M_c(t) - \log_{10} M_e(t) - C$$

where t is the time after beginning of irradiation, $M_c(t)$ and $M_e(t)$ are means of control and experimental groups respectively at time t , and C is a correction term which adjusts the mean difference between control and experimental animals to zero in the pre-irradiation period. The curves are graphical fits.

hematological variables, survival, number of cells in mitosis in a tissue, etc. It would appear, therefore, that the theoretical approach proposed here is potentially capable of giving a rational quantitative description of the effects of radiation on the growth and steady states of the whole organism and its tissues. One serious reservation is that it may be difficult to set up experimental conditions that will permit identification and quantitative evaluation of all the relevant parameters. This problem needs careful consideration. Another line of approach to the general problem of the effects of radiations on cell populations is considered in the following section.

LABORATORY MODELS OF TISSUE-LIKE SYSTEMS

Present evidence indicates that the regulatory mechanisms which govern the growth and the steady states of animal tissues are highly complex. Numerous neural, humoral, and vascular reflexes have been implicated, but exact knowledge of their mode of action is still lacking. In these circumstances a direct mathematical attack on the problem has little hope of attaining fruitful results. It was concluded that answers to definite questions about the behavior of cell populations could be better obtained by setting up laboratory models of tissue-like systems and comparing their actual behavior with the behavior predicted by the mathematical model. The experimental setup would consist basically of a thermostatted culture vessel in which a free-living bacterium or protozoan would be cultured continually, with provisions for exchange of medium and for the removal of cells at rates which are under the control of the investigator. The kinetics of such a system should be primarily governed by the supply of substrate and the theoretically expected behavior could be calculated by solving the equations for such a system.

Mathematical analysis of simple models has been undertaken. The simplest situation leads to nonlinear simultaneous differential equations for which a general solution is not yet known. It has been possible however to discuss the steady state solution, its stability and the nature of the return to the steady state from infinitesimal displacements. When the normal population dynamics become sufficiently well known by a combination of theoretical and experimental analysis it will be possible to study the dynamics of an irradiated system and to compare the observed results with the results expected on various hypotheses about the effects of radiations on the population structure.

Development of simple continuous-culture systems has been under way and it is hoped that a satisfactory mechanism will soon be in operation.

Biophysics

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INITIAL STUDIES ON A FAST NEUTRON DETECTOR - PART II

I. B. Berlman

In a previous report,⁽¹⁾ some initial tests on the layer counter were described. This report describes a continuation of that work.

Mr. Marinelli has developed a formula relating the maximum pulse heights of the layer counter to the energy of the incident neutrons and to the thickness of the scintillating layers. The formula indicates that the maximum pulse height should be proportional to $E^{1/3} \times X^{2/3}$, where E is the energy of the incident neutrons, and X is the thickness of the scintillating layers. This formula is based on the following assumptions: 1) that the maximum range of the recoil protons is greater than X , and 2) that the light output of the scintillator increases linearly with proton energy.

The variation of pulse height for monoenergetic neutrons but for different X 's has been tested. In each case the total amount of scintillator and the total amount of lucite were the same. D-T neutrons of approximately 14-Mev energy were used. The results, as shown in Figure 27, indicate that the maximum pulse height is proportional to $X^{2/3}$ to within 5 per cent. In each case the end point was determined by a final count that was too small to be indicated on this type of graph.

Variations of maximum pulse height with a variation of neutron energy have as yet not been performed because there is a lack of other good monoenergetic neutron sources.

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Figure 27. Variation of maximum pulse height with layer thickness.

Neutron energy = 14 Mev

1/8" lucite spacers

1 mil = 40μ

ABSORPTION CURVES OF SCINTILLATING SOLUTIONS

I. B. Berlman

Kallmann and Furst⁽¹⁾ have published the emission bands of various hydrocarbon scintillating solutions as well as curves for total light output versus concentration. They have also shown that in the various solutions tried the total light output increased with increasing scintillator concentration up to a certain concentration, beyond which the light output either stayed constant or decreased with increasing concentration.⁽²⁾ This report deals with some preliminary absorption curves made on some of the scintillating solutions in order that the extent to which these findings depend on the self-absorption in the solution can be investigated. It is also of interest to determine from these curves whether the absorption maximum shifts with increasing concentration of the scintillating solute and to note whether there is an effect on the absorption by the addition of a second nonquenching solvent.

A Beckman spectrophotometer, Model DU, was used for these measurements. Typical results are those shown below. Curve I of Figure 28 is a plot of density, D , versus the wave length, λ in Angstrom units, $\text{\AA}$, of the absorption of one-half g of terphenyl per liter of xylene as measured against a standard of one-tenth g per liter of terphenyl-xylene solution. It is noted that the peak is below 3250 $\text{\AA}$. Curve II of Figure 28 is a plot of the absorption versus wave length for a seven g per liter terphenyl-xylene solution, when a one and one-half g per liter terphenyl-xylene solution is used as a standard. This curve indicates that the absorption, D , increases as one goes to higher concentrations in a terphenyl-xylene solution. It is noted that the maximum of the absorption peak now is above 3250 $\text{\AA}$. Since the emission band is given⁽¹⁾ as 3250 to 4000 $\text{\AA}$ there is self-absorption at the larger terphenyl concentrations. Similar curves are obtained for anthracene in xylene but here the absorption peak is at 3900 $\text{\AA}$ while the emission band is given as 3900 to 4400 $\text{\AA}$.⁽¹⁾

Curve III of Figure 28 is an absorption plot of a solution of one liter of mineral oil in a liter of one g per liter of terphenyl-xylene solution. The standard was one-tenth g per liter terphenyl-xylene solution. Thus, the total concentration of terphenyl was one-half g per liter of solvent. It is to be noted that Curve III coincides essentially with Curve I. The absorption characteristics in the mixture appear to be related only to the terphenyl concentration.

Curve I of Figure 29 is the same as Curve I of Figure 28. Curve II of Figure 29 is the same as Curve III of Figure 28, except that now the

Figure 28. Absorption curves.

- Curve I. One-half gram per liter terphenyl-xylene solution versus 0.1 gram per liter terphenyl-xylene solution as a standard.
- Curve II. Seven gram per liter terphenyl-xylene solution versus 1.5 gram per liter terphenyl-xylene solution as a standard.
- Curve III. One liter per one liter paraffin oil-terphenyl-xylene solution (one gram per liter) versus 0.1 gram per liter terphenyl-xylene solution as a standard.

Figure 29. Absorption curves using 0.1 gram per liter terphenyl-xylene solution as a standard.

Curve I. One-half gram per liter terphenyl-xylene solution.

Curve II. One liter per one liter dioxane-terphenyl-xylene solution (one gram per liter).

solvent is dioxane instead of mineral oil. Here too, the only absorption change seems to be a function of terphenyl dilution.

SUMMARY

From these preliminary investigations the following seem to be true for the solutions tested: 1) the absorption, D , increases with concentration; 2) the absorption peak shifts toward longer wave lengths as one goes to higher concentrations; and 3) the absorption decreases when certain nonquenching solvents are added to the scintillating terphenyl-xylene solution, by an amount corresponding to the dilution of the terphenyl. On the basis of points 1 and 2 it may be stated that for the terphenyl-xylene solution self-absorption increases with increasing concentration.

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moving likewise. A further improvement, as shown in Figure 30D, resulted from readjusting the thermal gradient from a very unstable condition (5°C colder at the top than at the bottom) to a more reasonable condition (3° warmer at the top than at the bottom).

It should be pointed out that these difficulties did not result from mechanical effects of the air movements. The phenomenon is rather one of ions moving with the air instead of moving with the collecting fields. This can result either from ordinary ions well away from the chamber where the electric field is small or from hypothetical "heavy ions" moving within or in the vicinity of the chamber itself. Such ions would have to have mobilities of the order of 100 to 1000 times less than ordinary air ions whose mobilities are of the order 1 to 20 cm per second per volt per cm.⁽³⁾ Such particles might be very fine dust. Ordinary dust and lint can hardly be the cause, because there was no improvement when air which had been filtered through the commercially available Filter Queen vacuum cleaner filters was blown into the shield.

It is hoped that sufficient stability in ionization current can be attained without too much more effort. At present a more detailed study of the temperature distribution is being made as well as a re-examination of other possible difficulties, which, now that the principal problems have been lessened, may be of some consequence.

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PROPOSED CALORIMETRIC DOSIMETRY OF FAST NEUTRONS

H. E. Kubitschek

The problem of the dosimetry of prompt neutrons has arisen in connection with the neutron toxicity program.⁽¹⁾ It is desirable to state the dosage in terms of the energy dissipation per gram of tissue, rather than a relative number such as the number of "n" units of exposure, since it is then possible to specify accurately the average relative biological effect for the fast neutrons of the prompt spectrum as compared with the effect of γ radiation from Co⁶⁰.

Little, if any, absolute fast neutron dosimetry has been done, primarily because of the low efficiencies of detection of fast neutrons, which must be separated from other radiation components, such as γ rays and slow neutrons, and because of the rapid variation with energy in the sensitivities of most detectors. Furthermore, the calculation of the fast neutron energy loss in hydrogenous media is made difficult by both the rapid slowing down and scattering which quickly degrades the neutron beam, and by the highly irregular shapes of biological objects. The method of choice would seem to use a hydrogenous detector nearly equivalent to the biological object both in atomic constitution and in shape, and practically insensitive to γ radiation. The energy dissipated in this tissue equivalent detector will then be the same as that lost to the biological target.

Tissues obviously vary in atomic constitution even in the same animal, but for chemical and radiobiological work the following values are often accepted⁽²⁾ and termed standard here:

oxygen	65 per cent by weight
carbon	18 per cent by weight
hydrogen	10 per cent by weight
nitrogen	3 per cent by weight

Alternatively, tissue composition is sometimes assumed as $C_5H_{40}O_{18}N$.⁽³⁾ Two outstanding tissue equivalent substances have been used by Rossi and Failla, a plastic* and a gel.⁽³⁾ In thin slabs these will absorb energy from the prompt neutron spectrum within 3 per cent and 1 per cent greater, respectively, than that which would be absorbed by standard tissue.

*Markite Standard No. 5, manufactured by the Markite Company,
155 Waverly Place, New York City.

Of the numerous methods available for detecting neutrons, pulse or track counting methods are not easily adaptable to this task since they are designed primarily to measure the energetics and spectra of neutron reactions. It should be noted, however, that a rather useful proportional counter has been built by Hurst, Ritchie, and Wilson⁽⁴⁾, the energy response of which simulates the first collision dosage in collimated beams with an accuracy of about 10 per cent.

Nevertheless, the most appropriate detectors should determine directly, after any necessary calibrations, the total energy given to the target. Three such detectors are proton recoil ionization chambers, nuclear track plates, and calorimeters. Of these, the ion chambers are most sensitive.

Proton recoil ion chambers for neutron detection are described extensively by Rossi and Staub.⁽⁵⁾ Since they measure the number of ion pairs produced per unit time in a known gaseous volume, the energy released in that volume may be computed once the mean energy lost per ion pair formed by the recoil protons, is known. Though highly selective for neutrons, these chambers are usually larger than many experimental animals. Moreover, difficulties with this method arise through "wall effects" and through inaccuracy in the knowledge of W , such as:

1) First, in the absence of reference calibrations the chamber must be tissue equivalent not only in atomic but also in molecular constitution, since W depends strongly upon molecular arrangement. For example, CH_4O has a mean relative total ionization for alphas of 1.22 (compared with air) while CO and H_2 are 1.01 and 0.99.* Thus, there would be serious error in assuming that the mean relative ionization of the simple gas mixtures that can be used were the same as that for tissue. It would be, in fact, a very difficult task to approximate closely a molecular equivalence to tissue, both theoretically, since a knowledge of tissue structure is incomplete, and experimentally, since tissue made gaseous would decompose and equilibrate only partially as vapor. Thus tissue equivalence, which is necessary to obviate changes in neutron scattering, is not obtainable for the gas, and deviations in W from the true value may well be of the order of 30 per cent.

In practice, the gas in the ion chamber must be a simple mixture, making it necessary to calibrate the ion chamber. The simplest calibration is the exposure of the tissue equivalent chamber to a γ -ray source that in turn has been calibrated with an air-equivalent chamber. The assumption is then made that the ratio of W for electrons and protons is the same in the tissue equivalent chamber as it is in air. This assumption is untested. A correction of probably less than the order of 10 per cent could be involved⁽⁷⁾ for fast protons and recoil electrons.

*Values of Kovarick and McKeehan listed by Rutherford, Chadwick, and Ellis, p. 82.⁽⁶⁾

2) Again, W is not a constant with variation in proton velocities at low energies. Gray estimates that at velocities corresponding to 0.25 Mev the value of W will be about 8.5 per cent greater than at 2 Mev. Hence, if one accepts the prevailing values of W , obtained at energies higher than 2 Mev, the energy absorbed from the beam will be overestimated, especially since low energy recoils are present for all incoming neutron energies in equal probabilities with other energies. For the spectrum of prompt neutrons this error would not be more than a few per cent. However, in the event that the ion chamber is calibrated against electromagnetic radiation, it should be kept in mind that W increases here also as electron velocities are reduced, with a change of the order of 20 per cent for very low γ -ray energies. Eisl's measurements, discussed by Gray, show that W is increased in air by about 10 per cent over the value for very fast electrons when the recoil energy is reduced to 10 kev.

3) Stopping powers for protons may be slightly different for the gaseous state of a substance as compared to the liquid or solid. If this is the case, those protons generated in the walls of the ion chamber will not lose energy in the wall at the same rate as in the gas, and ion production would not then be constant throughout the chamber. However, this effect is quite small, not over 2 per cent.⁽⁷⁾

More important is the fact that a large fraction of the energy lost by the neutron beam appears in the production of recoil ions of carbon, oxygen, and nitrogen. In standard tissue this amounts to 10 to 20 per cent of the energy loss. Here again the value of W will be different from that for fast protons, since these ions will be moving quite slowly. Such ions would be quite rare in a γ -ray calibration, and therefore cannot be compensated for by that technique. In view of the above, it is doubtful that an ion chamber can measure dose rates of prompt neutrons within an error of about 10 per cent. A precision determination of dosage could be made by calibration of the ion chamber with monoenergetic protons throughout most of the recoil energy range intended for study, and especially in the low energy range.

Proton sensitive nuclear track plates inserted into a tissue equivalent target may also be used for dosimetry. Here again, exposure would not be measured by counting tracks, since such a method is tedious and becomes inaccurate for proton energies below 1 Mev. Instead, the procedure would be to measure the emulsion blackening densitometrically. Unfortunately, this technique is fully dependent upon a calibration of grain density as a function of proton and other nuclear recoil energies, perturbed by the silver bromide in the emulsion. The method, furthermore, would not discriminate against γ radiation.

The calorimetric approach, on the other hand, avoids all sources of error so far indicated except that of discrimination against γ radiation.

PROGRESS REPORT: IONIZATION IN AIR FROM BETA-RAY POINT SOURCES

S. S. Brar and R. K. Clark

A grid wall ionization chamber has been built and its operating conditions determined.^(1,2) A wire mesh with plastic cover was necessary to reduce the effects of electro-static pick-up and dust to the point that consistent ionization readings could be obtained. In order to establish all the necessary conditions for a precise β -ray measurement, we found a larger shield essential. The principal condition to be met by the large shield is that its presence should not influence the ionization in the chamber by scattering within its volume β rays from the source. This requirement is met by making the shield large enough that the sum of the distances from the source to any point in the shield, plus the distance from this point to the chamber, is less than the maximum range of the β particles. Such a shield is certainly adequate, although it may be somewhat larger than is essential. In addition to these requirements it was thought that the shield should serve to keep dust away from the chamber. Since there was some uncertainty as to the ultimate effectiveness of such a shield, it was thought best to make it somewhat smaller than optimum in order to avoid expense and gain time.

The resulting shield is an 18-foot cube, with the ion chamber at its center. The wall of the shield consists of Copperskin, a copper foil bonded to a tar paper backing. This material was draped over a frame of 1 1/4-inch aluminum tubing, and the seams closed by stapling to 1/2-inch x 2-inch wood boards. The only apparent disadvantage of this shield was that it is not as large as might be required for P^{32} . This 1.7-Mev emitter calls for a 23-foot shield. Since this one was 18 feet, it would be large enough for emitters of energy up to about 1.3 Mev only. (This is assuming that elastic scattering is the only scattering occurring at the shield.)

It was soon discovered that this shield was in some way inadequate. A typical trace from the Brown Strip-Chart Recorder taken at the time the operating conditions were determined is shown in Figure 30A. This was with the small shield in place, and the source of ionization was a 100-mg Ra capsule at about 4 meters. Figure 30B shows a similar trace taken with the same source at about the same distance, but with the large shield instead of the small one. Study and elimination of many other possibilities revealed the source of difficulty to be the movements of the air within the shield. Figure 30C shows the improvement resulting from the stiffening of the loosely draped walls. These walls had been moving with every minor draft entering the room; hence, the air inside the shield had been

Figure 30. A. Typical ionization current observed with small wire mesh shield and plastic dust cover. (Full scale = 100 mv).

B. Typical ionization current observed under poorest conditions of air movement. The walls of the shield were loosely draped and the temperature was 5.5°C lower at the top than at the bottom. (Full scale = 100 mv above and = 1000 mv below).

C. Typical ionization current observed with the walls of the shield stiffened but with the thermal gradient still the wrong direction. (Full scale = 1000 mv).

D. Typical ionization current observed with the walls stiffened and the thermal gradient corrected. (Full scale = 100 mv).

In each case the ionization was produced by γ rays emanating from a radium source of about 100 mg at a distance of roughly four meters from the ionization chamber.

Furthermore, it is absolute, measuring directly the total fast neutron energy loss into heat production. It does not measure, however, that energy lost to chemical reactions. Energy loss to reactions in tissue is extremely difficult to predict since chemical decomposition depends upon a large number of factors in as yet indeterminable chemical complexes. For an estimate, however, for organic liquids, specifically covalent materials, Allen⁽⁸⁾ indicates that about four molecules react per 100 electron volts of energy absorbed. These have highest probability of occurring through single bond breaks. Since single bond energy values for the hydrocarbons are the order of 5 ev or less, the amount of energy lost to radiation decomposition and oxidation reactions should be about 10 to 20 per cent. Some of this energy, however, may subsequently be converted to heat, or possibly exothermic reactions may also occur. Thus, the calorimetric method without calibration may be almost as accurate as the tissue equivalent ion chamber calibrated with γ rays.

In addition, the calorimetric method is appealing, precisely because it may allow the determination of the energy lost to chemical reaction. This may be accomplished in two quite different ways: 1) The fast neutron density incident upon the calorimeter can be calculated in the neutron density incident upon the calorimeter can be calculated in the neutron toxicity program. If the calorimeter is built to approximate a planar sheet, then energy absorption from the beam will occur predominantly through neutron single collisions, for which case the energy loss may then be computed, and compared with that measured. 2) A more accurate procedure would be to take a calibrated source of α rays and distribute it within the calorimeter in such a way that no α radiation could escape. The difference between the known source energy and that measured would give the reaction energy. This procedure may be more conveniently performed with X rays of suitable energy, but in this case, of course, the energy lost to chemical reaction would be expected to deviate further from that lost by recoil protons.

Calorimetry has been applied quite successfully by Laughlin and Beattie to the dosage determination for a 22.5-Mev betatron,⁽⁹⁾ where the flux involved was quite large. The X-radiation dissipation was 5.1 milliwatts, which gave a temperature rate of rise in their calorimeter of about 10^{-4}C° per second. With the energies available in the neutron toxicity program, energy absorption rates in the target will be of the order of a hundred-fold less, giving temperature changes of about 10^{-6}C° per second. Although it does not appear possible to build a thermistor bridge circuit of the type used by Laughlin and Beattie to measure much less than 10^{-5}C° , the equilibrium temperature rise expected is much greater, about 10^{-3}C° , so that the calorimetry appears feasible.

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PROGRESS REPORT: THE IONIZATION TYPE HALIDE DETECTOR

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Two models of the ionization type halide detector have been set up in the Radiological Physics section of the new permanent physics building and are now being operated.* It has become increasingly apparent that the response of this type of detector depends very strongly on surface effects. Electrode surfaces have been varied in a number of ways, e.g., by applying solid materials, by electrical discharges, by washing with water or acids, by flaming, or by adding gaseous materials continuously. The results have been evaluated in terms of stability, speed of response, and sensitivity.

The sensitization of electrodes by the introduction of alkali salts into a gaseous discharge inside the detector appears to have considerable promise. For example, the absolute sensitivity of an experimental detector (i.e., microamperes per part per million of Freon 12) was increased by a factor of over 500 as the result of a gaseous discharge, operated for ten minutes in argon that had been passed over heated KCl before entering the space between the red-hot platinum electrodes. The electrode that was negative in the discharge was made positive in the detector. The detector prepared in this manner gave adequate sensitivity, quick response, and good stability and reproducibility for very low Freon concentrations, but the signal decreased with increasing time for concentrations of only a few parts per million (referred to as signal decay).

These results appear as if K^+ ions were driven into the surface of the platinum and subsequently released, either at a low rate in the presence of an atmosphere of air or at a greater rate in the presence of Freon or its decomposition products. Since the ionization potential of potassium is only about 4.3 electron volts, the thermionic work function of platinum in the presence of air would not have to be as large as that of clean platinum (6.3 e.v.) to assure that at least part of the potassium evaporating off a platinum surface would leave as K^+ ions. On the basis of this hypothesis, signal decay is associated with a condition in which potassium is leaving the surface at a greater rate than it is being replaced.

In some sets of experiments, it has been found that the tendency of the Freon signal to decay with increasing time has been reduced by adding

*Setting up the equipment and initiating operations in the new laboratory was not quite as straightforward as had been expected. The electrical circuitry and heating system of the new building caused unexpected variations which have since been minimized.

a few per cent of hydrogen to the air stream. This, too, appears to be a surface phenomenon. At least some, and possibly most, of the hydrogen is burned into water in the reaction zone, but the introduction of water vapor into the detector has been found to have an entirely different effect. Small concentrations of a number of other gases have been tried and several show a stabilizing influence, but the experiments have not yet been extensive enough to draw rigid conclusions. Sorption seems to be involved here, since the effects of certain changes in gas concentrations appear very slowly and depend upon the order in which the changes are made.

A more extensive literature survey has been made, with special attention devoted to the pyrolysis of chlorinated hydrocarbons and to the design and construction of positive ion sources.

Of the positive ion sources that have been constructed in the laboratory, not all have been Freon sensitive. This, too, may be a surface effect, or it may be an indication that a catalyst must be available to decompose the Freon before the reaction products can release ions.

Ion currents varying from a fraction of an ion per molecule of CCl_2F_2 , to more than eight ions per molecule have been observed. All of these cannot possibly be fragments of CCl_2F_2 , unless they make several trips, crossing back as neutral fragments.

The application of alternating, collecting potentials has been found to give results that are very informative and useful, particularly in the case of the General Electric type of detector. In a unit of this type, the background (no Freon) ion current does not depend very much on the polarity of the collecting potential, while the Freon signal, on the other hand, is much greater when the hotter inner electrode is made positive than when it is made negative. If one uses an A.C. collecting potential, then, the D.C. component of the ion current exhibits a more favorable signal to background ratio and any leakage current tends to be balanced out. In addition, it has been found that the decay of signal that attends higher Freon concentrations is sometimes less with A.C. than with D.C.

If the amplifier is replaced by an oscilloscope and an A.C. collecting potential is used, one obtains a graphic picture of the ion current as a function of time. It was found that new General Electric units vary considerably from one to the other in this respect and from the detector built in this laboratory. The wave form is changed greatly when Freon is added. It is also affected by the presence of other gases such as ammonia, nitrous oxide, hydrogen, butane, and methane.

The results of field tests can only be as accurate as the calibration of the equipment. A leak for the metering of Freon into an air stream at very low rates has been set up in the laboratory and carefully calibrated.

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