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Editors:

Austin M. Brues, Director, Biology Division
Hermann Lisco, Director, Medical Division

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Administration

Dr. Austin M. Brues, Director
Biology Division
Dr. Hermann Lisco, Director
Medical Division
Dr. Raymond E. Zirkle
Mrs. Josephine Graff
Mrs. Jean Gilbert

During the period covered by this Quarterly Report several staff changes occurred.

New Staff Members: Dr. Janet V. Passonneau has joined the staff as associate biologist. She has been a teaching fellow in physiology at Harvard University since 1945.

Robert S. Stutz, formerly research assistant in biochemistry at the University of Wisconsin, has joined Dr. Gordon in phytoradiological research.

Visiting Investigators: Dr. Irvin E. Liener, instructor in agricultural biochemistry at the University of Minnesota, came for a four-month period to investigate problems in carbon¹⁴ metabolism with Dr. Buchanan.

Lt. Com. Robert A. Conard, M.D., U. S. N., who is associated with the Naval Radiological Defense Laboratory, San Francisco, has joined the staff for a period of one year and is investigating acute radiation physiology with Dr. Patt.

Dr. B. Vincent Hall, associate professor of zoology at the University of Illinois, is investigating some problems in cancer biology for the coming year.

Dr. Arthur M. Cloudman: We have suffered a great loss in the unexpected sudden death of Dr. Cloudman August 7, 1949. Dr. Cloudman received his Ph.D. at the University of Michigan in 1931 and served on the faculties of the Universities of Maine, Vermont, and Michigan. He was a Lucius M. Littauer Fellow and pathologist at the Huntington Memorial Hospital, Boston, in 1932 and 1933. Since then he was research pathologist, director and treasurer of the Roscoe B. Jackson Memorial Laboratory until 1948 when he joined our staff as senior biologist. He was an authority in the genetics and pathology of tumors, and was in charge of an active program in radiation carcinogenesis.

In addition to his scientific work, Dr. Cloudman's contribution to the Laboratory must be measured in greater terms. His practical, quiet

humor and joy in living did much to pull the staff through many difficult periods, and he was a friend always ready to employ his intellectual ingenuity, meticulous thoughtfulness, and common sense, alike to greater and smaller problems in scientific work and administration.

Promotions: Dr. Norbert J. Scully was promoted to senior physiologist and Dr. R. Flynn to associate veterinarian.

Editorial

Mrs. Antreen Pfau

A cumulative index of ANL Biological and Medical Divisions Quarterly Reports is in preparation. The index will cover the period of November, 1946 to May, 1949, and will be issued as an ANL report.

Directions for submitting manuscript to pertinent journals have been collected by Miss Helen Supert, who will add to this compilation from time to time at the request of staff members.

PUBLICATIONS

Schubert, Jack: Application of Ion Exchange to the Separation of Inorganic Cations in Ion Exchange, Academic Press, New York (1949), pp. 167-222. Ed., F. C. Nachod.

Barron, E. S. Guzman, Sherman Dickman, J. A. Muntz, and T. P. Singer: Studies on the Mechanism of Action of Ionizing Radiations. I. Inhibition of Enzymes by X rays. J. Gen. Physiol., 32: No. 4, pp. 537-551, March, 1949.

Barron, E. S. Guzman and Sherman Dickman: Studies on the Mechanism of Action of Ionizing Radiations. II. Inhibition of Sulfhydryl Enzymes by Alpha, Beta, and Gamma Rays. J. Gen. Physiol., 32: No. 5, pp. 595-605, May, 1949.

Watson, C. J., S. Schwartz, W. Schulze, L. O. Jacobson, and R. Zagaria: Studies of Coproporphyrin. III. Idiopathic Coproporphyrinuria; A Hitherto Unrecognized Form Characterized by Lack of Symptoms in Spite of the Excretion of Large Amounts of Coproporphyrin. J. Clin. Investigation, 28: No. 3, pp. 465-468, May, 1949.

Hasterlik, R. J.: Beryllium Poisoning. Physics Today, 2: No. 6, pp. 14-17, June, 1949

Schubert, Jack and E. E. Conn: The Radiocolloidal Behavior of Some Fission Products. Nucleonics, 4: No. 6, pp. 2-11, June, 1949.

Zirkle, R. E.: Relationships between Chemical and Biological Effects of Ionizing Radiations. Radiology, 52: pp. 846-855, June, 1949.

Jacobson, L. O., E. K. Marks, E. Gaston, and M. H. Block: The Effects of Nitrogen Mustard on Induced Erythroblastic Hyperplasia in Rabbits. J. Lab. Clin. Med., 34: No. 7, pp. 902-924, July, 1949.

TALKS

Jacobson, L. O.: The Effect of the Nitrogen Mustards on the Coagulation of Whole Blood. Meeting of the Blood Club, Atlantic City, New Jersey, May 1, 1949.

Barron, E. S. G.: Plant Respiration - The Last Steps to Reaction with Atmospheric Oxygen. Botany Club, University of Chicago, May 9, 1949.

Norris, W. P.: Radiotracer Techniques and Examples of their Use in Biological Research. Food Industries Advisory Committee, Sky Top, Pennsylvania, May 10, 1949.

Lisco, H.: Public Health Aspects of Nuclear Energy. School of Hygiene and Public Health. Harvard University, Boston, Massachusetts, May 11, 1949.

Brues, A. M.: Radioactive Isotopes in Medicine. Lake County Medical Society, Hammond, Indiana, May 12, 1949.

Lisco, H.: The Toxicology of Radioactive Isotopes. Department of Pharmacology, Harvard Medical School, Boston, Massachusetts, May 12, 1949.

Brues, A. M.: Atomic Energy and Agriculture. Radio Station KRFD, Columbus, Ohio, In conjunction with the 5-day Convocation at Denison University. June 1, 1949.

Brues, A. M.: Some Peacetime Developments in Atomic Energy Research. Denison University, Granville, Ohio, June 1, 1949.

Marks, E. K.: The Influence of Spleen Protection on Whole-body Radiation Injury. Annual Convention of the American Society of Medical Technologists, Roanoke, Virginia, June 20, 1949.

Robson, M.: Effects of Radiation on Immune Reactions. Annual Convention of the American Society of Medical Technologists, Roanoke, Virginia, June 20, 1949.

Sanderson, M.: Observations on a Clotting Defect Following X irradiation: Detection by Protamine Titration. Annual Convention of the American Society of Medical Technologists, Roanoke, Virginia, June 20, 1949.

Skirmont, E.: Radiation Hazards and Control. Annual Convention of the American Society of Medical Technologists, Roanoke, Virginia, June 20, 1949.

Brues, A. M.: The Principles of Measuring Cell Growth. Tissue Culture Association Course. Cooperstown, New York, July 8, 1949.

PRELIMINARY REPORT

by
Vladimir T. Clemens and Sarmukh S. Brar

In this preliminary report the microdensitometer described below has been designed to answer the need for an instrument that will measure quantitatively with reasonable accuracy the relative amount of light transmitted by the radioautograph. Thus it can be used for measurement and calibration of blackening of radioautographs.

The microdensitometer consists of a collimated point light source, focussed from the bottom on the radioautograph; a phototube and amplifier to collect the portion of the light transmitted through the film; a Brown strip chart instrument for continuous recording of any variation in transmitted light intensity due to various degrees of blackening of the radioautographs; and a special scanning device so that all points of the radioautograph can be scanned.

The experimental details involved in the design of a satisfactory light system have been completed. A collecting and collimating light system for the Pointolite lamp, which was selected after trials with several sources, has been worked out and is shown in Figure 1. The Pointolite lamp circuit (100 C. P.) is shown in Figure 2.

A collimated light beam of approximately 0.008 inches in diameter is available now to illuminate the radiogram while scanning.

Preliminary measurements indicate that an elemental area of 0.012 inches in diameter can be scanned for blackening with an adequate response to differentiate clearly between density over a range of an Eastman step tablet. Figure 3 shows the detail of microdensitometer.

The top view of the scanning carriage, light source photocell mounting, and plate holder are shown in Figure 4.

Figure 5 shows mounting of the phototube, with the micrometers for proper adjustment, mounting for the optical system is shown in Figure 6, and the carriage control, knobs, and register are shown in Figure 7. Figure 8 illustrates the motor for automatic scanning of radioautographs.

Figure 1. Collecting and collimating light system for the Pointolite lamp.

Figure 2. Pointolite lamp circuit (100 C. P.).

Figure 3. Photograph showing detail of the microdensitometer.

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Figure 4. Photograph showing top view of the scanning carriage, the light source photocell mounting, and the plate holder.

Figure 5. Photograph showing the mounting of the phototube and the micrometers.

Figure 6. Photograph showing mounting for the optical system.

Figure 7. Photograph showing carriage control, knobs, and register.

Figure 8. Photograph showing motor that is used for automatic scanning of radioautographs.

In the past few weeks the microdensitometer has been used successfully for recording the pattern in a section of liver tissue which had been stained for phosphatase study by Gomori's method (Mrs. Rietz and Dr. Brues); determination from radioautographs of the deposition of C^{14} in bones of mice treated with this isotope (Mrs. Stroud and Dr. Brues); and to analyze the diffraction pattern taken with electron microscope (Mr. Sayre).

ACKNOWLEDGMENT

The authors wish to acknowledge the cooperation of Dr. Robert J. Moon, who initiated this work.

ELECTRON MICROSCOPY

by
Joseph Sayre

Electron diffraction studies: Considerable electron diffraction work was done during the past quarter in order to work out effective techniques for using the electron microscope as a diffraction camera. All of the particular samples studied were of evaporated metals upon collodion films which are the most readily prepared and have the simplest molecular structure.

Whenever a crystalline structure is subjected to a beam of mono-energetic electrons, a diffraction pattern consisting of a concentric series of rings of different intensities is formed. The diameter of the brightest ring in the pattern is dependent upon the spacing of the individual atoms or molecules in the unit cell. In the case of a metal such as gold the unit cell is cubical and face-centered, there being an atom of gold at the center of each face of the unit cell. The spacing between these atoms is known as the lattice constant, d . Before the electron microscope can be used for qualitative diffraction work it is necessary to measure the diameter of the brightest ring, D for some known structure, and calculate the instrumental K from the formula;

$$K = d \times D \quad (1)$$

The lattice spacing for some 1300 crystalline substances has already been determined by X-ray diffraction and is compiled in the Handbook of Chemistry and Physics. Once K has been determined from the pattern of a known substance, the lattice constant for an unknown crystalline substance can be calculated by measurement of the ring diameter from the pattern of the unknown and by substitution into the formula:

$$d = \frac{K}{D} \quad (2)$$

which is merely equation (1) solved for d .

The ring diameters from diffraction patterns cannot be measured accurately from the photographic plate since the diameter is only about 1 cm. Measurement from enlargements of the plate are not feasible either because the photographic paper shrinks nonuniformly during drying.

The recording microdensitometer was thus adapted to measure the ring diameters. This unit consists of a mechanism for scanning a very small area of a photographic plate and a recording device for automatic

plotting of the transmission of the plate at the point being scanned. Both the scanning mechanism and the recorder chart drive are operated by synchronous motors. The ratio of the chart feed to the scanning speed is approximately 50 to 1 so that the ring diameter is expanded on the recorder chart to about 50 cm.

Close examination of a diffraction pattern shows that the density of a single ring is not uniform but varies through a maximum within the limits of the ring width. Figures 9 and 10, which are reproductions of the microdensitometer chart, illustrate this point diagrammatically in the rounded dips in the transmission curve at each side of the ring. The correct ring diameter is measured from these 2 points of minimum ring transmission, or maximum ring density as indicated on the chart by distance $\underline{D}$. The slight increase in the transmission curve at the center of the plot is due to the pin point scratched into the emulsion of the plate at the center of the pattern. This point is used in aligning the scanning path through the center of the pattern. Figure 11 A and B are enlargements of the diffraction patterns of copper and aluminum.

The lattice spacing for aluminum and copper as determined by X-ray diffraction is 4.04145 and 3.608 Å, respectively. If $\underline{D}$ is measured and K calculated for these two metals the result will be a check upon the validity of the electron diffraction technique. The computation is thus:

$$K_{Al} = 49.2 \times 4.04145$$

$$K_{Al} = 198.8$$

$$K_{Cu} = 55.2 \times 3.608$$

$$K_{Cu} = 199.2$$

where $\underline{D}$ is measured in centimeters. The reproducibility of K is dependent primarily upon two factors; the ability to measure the ring diameter accurately, and the ability to reproduce exactly the same settings of the electron microscope controls, particularly the diffraction control.

Electron diffraction studies of more complex structures are, of course, more difficult, but the method is wholly feasible and would appear to be facilitated on use of the recording microdensitometer for measurement of the ring diameter.

Magnetic shield insert for the projector pole piece: Normally, as the magnification is increased in the electron microscope the image of the specimen becomes larger and rotates. The axis of this rotation does not and cannot be made to coincide with the center of the viewing screen while still maintaining optimum magnetic alignment. At the higher steps of magnification the image may shift completely out of the field of view. It

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Figure 9. Microdensitometer chart used to measure ring diameter of copper.

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Figure 10. Microdensitometer chart used to measure ring diameter of aluminum.

Figure 11 A. Enlargement of diffraction pattern of copper.

11 B. Enlargement of diffraction pattern of aluminum.

is then necessary to shift the specimen stage controls for each change in magnification. If a particularly small specimen is being studied it may be necessary to reduce the magnification and carefully examine the entire grid opening before the particular detail in question is found again.

This effect may be explained as being due to the leakage of magnetic flux about the top of the projector pole piece into the area of the beam. This leakage exists because of the difficulty of machining a projector pole piece that fits perfectly into the projector coil, and yet is easily removable. The possibility of getting a leakage field that would be symmetrical is even more remote. Since the projector coil current is varied to obtain the different steps of magnification, the leakage flux changes also, and the image spirals out of the viewing area instead of about the center of the viewing area.

Following the suggestion of Dr. James Hillier of RCA, who gave the above interpretation of this matter, an insert of Armco metal and brass was made in the shops for the projector pole piece of the electron microscope. The insert is shown in Figure 12 A, and in place in the projector pole piece in Figure 12 B. The Armco cylinder, positioned by the brass disc at each end, is of high magnetic permeability (conductivity) and forms a path for the leakage flux outside of the path of the beam. Figure 13 shows two series of micrographs made with and without the insert at increasing magnifications. Figure 13 A is without the insert, and shows that the field of view changes drastically at the higher steps. Figure 13 B is illustrative of results when the insert is used.

A

Figure 12 A. Armco metal and brass insert for the projector pole piece of the electron microscope.

Figure 12 B. Insert in place in the projector pole piece.

B

A

7000

10,000

14,000

17,000

23,000

B

Figure 13 A. Photomicrograph made without the Armco metal and brass insert.

Figure 13 B. Photomicrograph made with insert in place.

Hematology

Dr. Leon O. Jacobson
Dr. Matthew H. Block
Mrs. Edna K. Marks
Dr. Eric Simmons

THE EFFECT OF X RADIATION ON ANTIBODY FORMATION*

by

Leon O. Jacobson, Melba E. Robson, Edna K. Marks,
and Marvin C. Goldman

INTRODUCTION

Hektoen⁽¹⁾ in 1915 first conclusively demonstrated that total-body exposure of experimental animals to ionizing radiation suppressed the usual antibody response to antigens injected shortly before or shortly after irradiation. Hektoen's observations led him to ascribe this suppression to the destructive effect of irradiation on lymphatic tissue and bone marrow. These findings have been corroborated by a number of workers and have recently been reviewed by Taliaferro.⁽²⁾ Craddock and Lawrence⁽³⁾ reported in a recent publication that total-body exposure of rabbits to 250 r X radiation effectively suppressed the formation of antibodies to antigens (typhoid vaccine and washed red cells from sheep) administered 8 hr after irradiation.

This communication relates an attempt to study the capacity of the rabbit to form antibodies during a period in which all lymphatic tissue of the body with the exception of the spleen or appendix is essentially destroyed.

MATERIALS AND METHODS

Young adult rabbits (Swift's snuffle-free), weighing approximately 2 kg, were used in this study. The general setup of the experiments is shown in Tables 1 and 2.

*From the Argonne National Laboratory and the Department of Medicine of the University of Chicago. Supported in part by a grant from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

Table 1

The effect of lead protection of the surgically-mobilized spleen
on antibody response

Group	Treatment	Antigen*	Rabbit no.	0 or con- trol†	Hemolysin titer - interval in days after immunization					
					7	10	14	21	28	35
I	None	1 cc 2% sheep cell I.V.	P-902	0	40	5120	5120	640	--	--
			Q-45	10	40	1280	160	80	20	--
			Q-39	0	0	1280	1280	320	320	--
			Q-203	0	640	640	640	320	80	80
II	None	None	P-895	0	0	0	20	0	--	--
			Q-41	0	10	0	0	0	10	--
			Q-93	0	80	20	10	20	10	--
			Q-98	0	20	20	20	10	0	--
			Q-201	0	0	0	10	0	--	--
III	Spleen mobili- zation only	1 cc 2% sheep cell I.V.	P-669	0	0	80	40	0	0	--
			Q-00	0	1280	2560	5120	5120	2560	--
			Q-197	0	2560	5120	640	320	320	160
IV	Splenectomy only	1 cc 2% sheep cell I.V.	P-921	0	40	5120	5120	320	--	--
			Q-103	0	2560	2560	5120	5120	2560	--
			Q-93	0	10	20	40	40	40	10
V	800 r total- body irradiation	1 cc 2% sheep cell I.V.	P-890	0	0	0	0	0	--	--
			Q-50	0	0	0	0	20	40	--
			Q-31	0	0	0	0	80	--	--
			Q-204	0	10	--	10	40	40	80
			Q-192	-	0	0	0	10	0	0
VI	800 r total- body irradiation	None	P-674	20	0	--	--	--	--	--
			Q-46	0	0	0	0	0	0	0
VII	800 r total- body irradiation with lead shielding of sur- gically mobilized spleen	1 cc 2% sheep cell I.V.	P-896	0	0	320	--	--	--	--
			P-495	0	0	40	80	5120	20	--
			Q-40	0	40	160	640	0	0	--
			Q-47	0	80	160	320	20	80	--
			Q-105	0	0	80	1280	5120	80	--
			Q-202	0	40	80	40	0	0	0
			Q-199	0	0	160	160	20	20	20
			Q-194	0	0	20	40	--	80	10
VIII	800 r total- body irradiation with no shielding of mobilized spleen	1 cc 2% sheep cell I.V.	P-892	0	0	20	--	--	--	--
			Q-48	0	0	0	0	--	--	--
			Q-43	10	10	0	--	--	--	--
			Q-99	0	0	0	0	0	0	--
			Q-18	0	0	0	0	0	0	--
			Q-189	0	0	0	0	80	80	40
			Q-190	0	0	0	10	10	10	10

*Antigen given 24 hr after treatment.

†Blood drawn prior to immunization.

Table 2

The effect of lead protection of the surgically-mobilized appendix
on antibody response

Group	Treatment	Antigen*	Rabbit no.	0 or con- trol†	Hemolysin titer - interval in days after immunization					
					7	10	14	21	28	35
I	None	1 cc 2% sheep cell I.V.	P-808	0	40	640	640	640	40	--
			Q-133	0	1280	640	640	640	320	--
			Q-251	10	2560	5120	5120	5120	1280	320
II	None	None	P-730	0	0	0	0	0	--	--
			Q-79	0	0	-	0	0	0	--
			Q-256	0	0	0	0	0	0	0
III	Appendix mobil- ization only	1 cc 2% sheep cell I.V.	Q-141	10	2560	5120	5120	1280	640	--
			Q-222	0	1280	5120	2560	640	320	160
IV	800 r total- body irradi- tion only	1 cc 2% sheep cell I.V.	Q-373	0	0	0	0	0	0	0
			Q-231	0	0	0	0	0	--	--
V	800 r total- body irradi- tion only	None	Q-51	0	0	0	0	0	0	0
VI	800 r total- body irradi- tion with lead shield protect- ing appendix	1 cc 2% sheep cell I.V.	P-810	0	0	0	0	640	--	--
			Q-137	0	0	40	320	80	80	--
			Q-236	0	40	320	320	320	160	80
			Q-265	20	80	2560	5120	640	320	640
VII	800 r total- body irradi- tion with sham shield protect- ing appendix	1 cc 2% sheep cell I.V.	P-809	0	0	0	--	--	--	--
			Q-140	0	0	0	0	10	40	--
			Q-132	0	--	0	0	0	10	--
			Q-224	0	0	0	0	0	0	0
			Q-267	0	0	0	0	0	0	10
			Q-273	0	0	0	0	0	0	0

*Antigen given 24 hr after treatment.

†Blood drawn prior to immunization.

Experiments involving the spleen: Animals were divided into 8 groups as follows:

Group I consisted of normal untreated rabbits immunized simultaneously with the others of the series.

Group II consisted of normal untreated rabbits with no immunization.

Group III consisted of rabbits which were subjected to surgical mobilization of the spleen followed 24 hr later by immunization.

Group IV consisted of rabbits which were splenectomized, followed 24 hr later by immunization.

Group V consisted of rabbits exposed to 800 r total-body X radiation followed 24 hr later by immunization.

Group VI consisted of rabbits exposed to 800 r total-body X radiation with no immunization.

Group VII consisted of rabbits exposed to 800 r total-body X radiation except that during irradiation the spleens were protected by lead shields. Immunization was carried out 24 hr later.

Group VIII consisted of rabbits exposed to 800 r total-body X radiation with spleen mobilization but with no protection of the spleens during irradiation. Immunization of these animals was carried out 24 hr after irradiation.

The rabbits of all groups, including the control animals, were anesthetized with Nembutal (38 mg per kg total body weight) administered intravenously. Deaths from Nembutal rarely occurred when the drug was injected slowly, over a period of 5 to 10 min., depending upon the amount given. If injected more rapidly, death from respiratory failure was common.

The spleen of each animal of groups III, VII, and VIII was mobilized and gently withdrawn from the abdominal cavity through a left upper quadrant incision (Figure 14). In the animals in group III, which were not irradiated, the spleen was exteriorized, wrapped in moist gauze, and left for 1 hr (the approximate time required to deliver the radiation to the exposure groups). In group VII the spleens were placed in lead shields during irradiation. Particular care was taken to avoid rupture of the blood vessels of the pedicles. The lead shields* (Figure 15 C and D) consisted of two parts that, when put together, formed a cylindrical tube with a slit measuring $3\frac{1}{4} \times \frac{3}{16}$ inches along the long axis. This slit prevented compression of the splenic pedicle. The walls of the cylinder were $\frac{1}{4}$ of an inch thick. The procedures for the animals in group VIII were the same as those for group III with the addition of irradiation.

*The lead shields have recently been redesigned and will be described in a future Quarterly Report.

A. Mobilized spleen.

B. Mobilized spleen in lead shield.

C. Approximate position of rabbit and shield during irradiation.

Figure 14. Photographs showing technique of shielding spleen with lead during irradiation.

Figure 15. Lead shields for protecting the rabbit appendix (A and B) or spleen (C and D) during the administration of total-body X radiation.

Twenty-four hours after operation and X irradiation, each animal in groups I, III, IV, V, VII, and VIII received 1 cc of a 2 per cent suspension of washed sheep cells intravenously. Blood specimens were obtained on all rabbits before treatment (base line) and at 7, 10, 14, 21, and 28 days after immunization.

The hemolysin titers were determined by making serial dilutions of the rabbit sera (1:10, 1:20, 1:40, etc., to 1:5120) in 0.5 cc amounts, adding 0.5 cc of lyophil guinea pig complement and 0.5 cc of a 2 per cent suspension of washed sheep cells, incubating 1 hr at 37°C, and refrigerating overnight. The hemolysin titrations on each series (those animals treated and immunized on the same day) were done at the same time to eliminate differences in complement or sheep cell suspension. The titer of each particular serum was that dilution that gave complete hemolysis.

Experiments involving the appendix: The experiments involving lead protection of the surgically mobilized appendix (Table 2) were carried out in an identical manner to the experiments involving spleen protection except that the surgical incision was made in the right lower quadrant of the rabbit's abdomen (Figure 16). The appendix was placed in a cylindrical lead container (1/4 inch thick throughout) with an opening at one end for the proximal end of the appendix and a narrow slit, 3 1/2 X 3/16 inches long, along one side with several sharp prongs through which the mesentery was hooked (Figure 15 A and B). This allowed the blood supply of the appendix to remain intact and prevented the appendix from slipping out of the shield during the irradiation period.

Dosimetry: The X rays were generated in a 250-kv machine operating at 15 ma. A 1.0-mm Cu filter and a 3.0-mm Bakelite filter were used. The half-value layer in copper of the filtered beam was 2.0 mm. The exposures were measured with a Victoreen condenser r-meter equipped with a 100-r chamber. Measurements were made in air at the position occupied by the center of the animal's body. The dose rate averaged 15.5 r per min. at 33 inches; a total dose of 800 r was delivered.

The amount of X radiation that the lead-protected spleens of group VIII received has not been accurately measured as yet but the dosage varied depending on the position of the slit relative to the direct beam. In experiments now in progress these shields have been modified to reduce the scatter radiation entering the slit. This has been accomplished by using a baffle type opening for the appendix, mesentery, and spleen pedicle.

A. Mobilized appendix.

B. Mobilized appendix in shield.

C. Approximate position of rabbit and shield during irradiation.

Figure 16. Photographs showing technique of shielding appendix with lead during irradiation.

RESULTS

Experiments involving lead protection of the spleen: As is indicated in Table 1, animals given 800 r total-body X radiation and immunized one day later (group V) either developed no demonstrable hemolysin titer or developed titers of 1:80 or 1:40, which were demonstrated on the 21st day and the 28th day, respectively, after immunization. In the normal animals (group I) and in animals that were subjected to surgical mobilization of the spleen (group III) or splenectomy (group IV) hemolysin titers of 1:5120 were produced by the 14th or 21st day; the titers had diminished by the 28th day after immunization. Rabbits given 800 r total-body X radiation exclusive of the surgically-mobilized, lead-protected spleen (group VII) produced antibodies to sheep cell antigen comparable to normal non-irradiated control animals except that the maximum titer was usually reached on the 21st rather than on the 14th day.

Experiments involving lead protection of the appendix: As is indicated in Table 2, rabbits given 800 r total-body X radiation exclusive of the surgically-mobilized appendix and immunized 24 hr later produced antibodies reaching titers essentially comparable to the control groups. In rabbits given total-body X radiation including the appendix, the formation of antibodies was suppressed.

DISCUSSION

In a previous communication⁽⁴⁾ it was reported that the 28-day LD₅₀ for mice exposed to total-body X radiation exclusive of the surgically-mobilized, lead-protected spleen is nearly twice as great (975 r) as the LD₅₀ for mice exposed to total-body X radiation including the spleen (550 r). It has also been demonstrated⁽⁵⁾ that lead protection of the surgically-mobilized spleen of mice during the delivery of 600 r total-body X radiation obviated the development of anemia and significantly lessened the severity and duration of the leucopenia and thrombocytopenia that regularly follow total-body exposure at this level. In fact, preliminary data⁽⁶⁾ show that no anemia of significance and only a transient leucopenia and thrombocytopenia occur after exposure of mice to 975 r total-body X radiation if the surgically-mobilized spleen is lead-protected during delivery of the radiation. It is apparent that the spleen, under these circumstances, assumes functions that are of signal importance in increasing survival following radiation exposure. The capacity of the spleens of these mice to compensate so quickly for the destruction of hematopoietic tissue elsewhere in the body may be the significant factor. On the other hand, the retention of the capacity to produce antibodies, as is demonstrated in this paper, may play a role in increasing survival as well may other factors yet unknown. Further studies on antibody formation are in progress as well as hematologic and histologic investigations of rabbits, mice, rats, and guinea pigs using the general techniques described in this paper.

CONCLUSIONS

These experiments corroborate Hektoen's original classic findings that antibody formation is suppressed by total-body X radiation. It has been demonstrated, in addition, that if the spleen or the appendix of the rabbit is protected by lead shielding during total-body irradiation the capacity to produce antibodies to an injected particulate antigen is retained, even though lymphatic tissue elsewhere in the body is largely destroyed.

LITERATURE CITED

1. Hektoen, L.: J. Infectious Diseases, 17:415 (1915).
2. Taliaferro, W. H. and L. G. Taliaferro: The Effect of X rays on Immunity. NNES (mpts) Div. IV, Vol. 22B (CH-3891).
3. Craddock, C. G. and J. S. Lawrence: The Effect of Roentgen Irradiation on Antibody Formation in Rabbits. M-2011 (1948).
4. Jacobson, L. O., E. K. Marks, M. J. Robson, E. Gaston, and R. E. Zirkle: The Effect of Spleen Protection on Mortality Following X irradiation. J. Lab. Clin. Med., (in press).
5. Jacobson, L. O., E. K. Marks, E. O. Gaston, M. Robson, and R. E. Zirkle: Proc. Soc. Exp. Biol. Med., 70: No. 4, p. 740 (1949).
6. Jacobson, L. O., E. K. Marks, E. O. Gaston, and M. Robson: Unpublished data.

BLOOD PICTURE OF CHRONICALLY IRRADIATED LAF₁ MICE

by

Egon Lorenz and Leon O. Jacobson

After the original experiments dealing with the exposure of LAF₁ mice to 8.8 r, 4.4 r, 2.2 r, 1.1 r, and 0.11 r, given in 8 hr per day, were finished,⁽¹⁾ another experiment was set up in which LAF₁ mice, 2 months of age, were exposed to 8.8 r, 4.4 r, and 1.1 r given in 8 hr per day in order to make extensive histologic studies of progressive changes. Thirty-four male and 34 female mice were exposed on each dose level. A corresponding number of mice served as nonirradiated controls. Four male and 4 female mice were sacrificed and autopsied at intervals of 2 months. The histologic studies will be reported by Dr. William Bloom.

Blood data were taken on all animals. Blood counts were made 1 week prior to exposure, the day the exposure was started, at the end of the first week of exposure, and at intervals of 4 weeks thereafter. In addition weight records were kept of all animals. These data are presented here because more frequent counts were taken in the early part of this experiment than in the original experiment and furthermore more animals were available during the first 6 months. The data (Figures 17 to 28) confirm the findings of the original experiments: that the total leucocyte and absolute lymphocyte values of the peripheral blood are significantly depressed only in the 4.4 r and 8.8 r groups, this depression is established early and maintained throughout the experiment. Erythrocyte and hemoglobin values are reduced significantly only in the animals exposed to 8.8 r.

It was found in the original experiment that the weight of the irradiated animals, especially that of the male animals exposed to 1.1 r, exceeded that of the control animals significantly reaching a maximum weight at approximately 70 weeks after the exposure was begun. The weight values found in this experiment (Figures 29 and 30) confirm this observation.

LITERATURE CITED

1. Lorenz, Egon et al.: Biologic Effects of Long-continued Whole Body Irradiation with Gamma rays on Mice, Guinea pigs, and Rabbits. Part III NNS (mpts) Div. IV, Vol. 22B (CH-3698).

Figure 17. Leucocyte values of chronically irradiated LAF₁ female mice.

Figure 18. Leucocyte values of chronically irradiated LAF₁ male mice.

Figure 19. Lymphocyte values of chronically irradiated LAF₁ female mice.

Figure 20. Lymphocyte values of chronically irradiated LAF₁ male mice.

Figure 21. Heterophil values of chronically irradiated LAF₁ female mice.

Figure 22. Heterophil values of chronically irradiated LAF₁ male mice.

Figure 23. Erythrocyte values of chronically irradiated LAF₁ female mice.

Figure 24. Erythrocyte values of chronically irradiated LAF₁ male mice.

Figure 25. Platelet values of chronically irradiated LAF₁ female mice.

Figure 26. Platelet values of chronically irradiated LAF₁ male mice.

Figure 27. Hemoglobin values of chronically irradiated LAF₁ female mice.

Figure 28. Hemoglobin values of chronically irradiated LAF₁ male mice.

Figure 29. Weight values of chronically irradiated LAF₁ female mice.

Figure 30. Weight values of chronically irradiated LAF₁ male mice.

EFFECT OF IRRADIATION ON A TRANSPLANTABLE MOUSE LYMPHOMA

by

Joanne Weikel Hollcroft and Egon Lorenz

----- A considerable amount of data has been collected on the effect of intravenously-administered radon and its decay products and of external total-body X irradiation on a transplantable mouse lymphoma. These data can be summarized as follows: In 144 radon-treated and 229 X-irradiated animals the tumor did not recur in 3.5 per cent of the animals of each group (Table 3). The animals were irradiated when the tumor had reached a size of 500 to 1000 mm³. Following exposure the tumor regressed in size often until it was not palpable any more but the tumor resumed growth several days later with the exception of the 3.5 per cent mentioned above. Life span of the animals in which the tumor recurred was increased over that of control animals which died 30 to 40 days after transplantation.

It was noted that the regression of the tumor was more pronounced in the radon-treated mice, also more animals died within 2 weeks after the radon treatment than within 2 weeks after X irradiation (Table 3). To compare the doses received, we compared the experiment in which 36 mice were given 0.015 mc per g radon intravenously with that in which 20 mice were given 400 r X radiation. In 4 of the radon-treated mice and in 3 of the mice exposed to X radiation the tumor failed to recur. The energy absorbed by an animal receiving 0.015 mc per g of radon is 39,160 ergs per g,⁽¹⁾ which is equivalent to 420 r if 93 ergs per g are taken as equivalent to 1 r. The energy absorbed from 400 r of X radiation is 37,200 ergs per g (1 tissue r = 93 ergs per g). When using the data of Lea⁽²⁾ for the conversion of absorbed α energy to roentgens (1 r α radiation from Rn + Ra A + Ra C is equivalent to 109.26 ergs per g) the 0.015 mc per g radon treatment is equivalent to 375 r. This α -ray roentgen is obtained by an extension of the definition of the roentgen to α radiation. Thus the doses given in these 2 experiments are roughly comparable as is the response of the blood picture and the destruction of a number of tumors.

The tumor in all the animals of Table 3 was transplanted subcutaneously. While in the X-ray-treated mice the tissue dose can be assumed to be uniform, this does not hold true in the radon-treated mice. As the radon is given intravenously and as the α particles are mainly responsible for radiation injury, it is conceivable that a subcutaneous tumor may receive a smaller tissue dose than other parts of the body where the tissue is more vascularized. For this reason the tumor was transplanted intramuscularly. When 10 of these animals were treated with 0.014 mc per g of radon, the tumor disappeared in all animals and did not recur in 6,

Table 3

Effects of irradiation with X rays or intravenously administered radon and its decay products on a transplanted, nongeneralizing mouse lymphoma

Treatment	Number of mice	Number surviving 15 days after treatment	Number cures
0.015 mc/g Rn	36	24	4
470 r X radiation	20	20	0
100 r X radiation	20	19	0
200 r X radiation	69	69	0
300 r X radiation	60	60	0
400 r X radiation	20	19	3
0.0045 mc/g Rn	10	10	0
0.0081 mc/g Rn	10	10	0
0.012 mc/g Rn	30	30	1
0.016 mc/g Rn	29	7	0
300 r + 300 r X radiation 6 days apart	20	18	1
0.012 mc/g + 0.012 mc/g Rn 6 days apart	13	11	0
300 r + 300 r X radiation 4 days apart	20	16	5
0.012 mc/g + 0.022 mc/g Rn 4 days apart	16	4	0

while 4 mice died from the treatment. When 10 of the mice with intramuscular transplant were X-irradiated with 400 r, the tumor disappeared in all animals but recurred in all but one. These experiments are being continued.

A preliminary study of the effect of X rays and the effect of α and β rays from radon and its decay products on another transplantable lymphoma was made. This lymphoma generalizes to other lymphoid structures and also affects the blood picture. The tumor appears about 6 days after subcutaneous transplant; blast forms appear in the differential 10 days after transplant, and the white count begins to rise approximately 12 days after the transplantation, as shown in Figure 31.

When treated 7 days after transplanation with either 400 r X radiation or 0.014 mc per g. radon, the tumor regresses but recurs (Figure 32).

Although the leucocytes decrease after treatment they increase in the terminal stage as do those of the controls (Figure 31). The course of the leukemia appears to be independent of tumor size. These experiments are also being continued.

LITERATURE CITED

1. Hollcroft, J. Weikel and E. Lorenz: Chronic External Irradiation, Quarterly Report, Biological and Medical Divisions, Argonne National Laboratory, November 1948 to February 1949, Eds., A. M. Brues and H. Lisco (ANL-4253) p. 35.
2. Lea, D. E.: Actions of Radiations on Living Cells. MacMillan and Co., Inc., New York (1947), p. 8.

Figure 31. Leucocyte and lymphocyte counts of animals with transplanted generalizing lymphoma (controls and irradiated animals).

Figure 32. Tumor growth following treatment with X radiation or radon

THE 30-DAY LD₅₀ AND ACCOMPANYING BLOOD PICTURE OF HYBRID GUINEA PIGS

Preliminary Report

by

Egon Lorenz, Delta Uphoff, and Harriet Sutton

References in the literature to the LD₅₀ for guinea pigs are scant. Bloom⁽¹⁾ gives this dose as 175 r while Jacobson et al.⁽²⁾ state that guinea pigs and dogs show approximately the same sensitivity of blood and blood-forming tissues, the 30-day LD₅₀ for dogs being about 315 r. Therefore a determination of the 30-day LD₅₀ for hybrid guinea pigs of the colony that was used in the chronic experiments was undertaken to compare this dose with the mean lethal dose in chronic exposures and to compare the effects on blood and blood-forming organs for acute and chronic exposures.

To obtain as uniform a distribution of energy within the animal as possible in comparison with that obtained in γ irradiation, a double tube setup was used. One tube irradiated the animal from above, the other from below. The distance from the middle of the animal to each tube was 54 cm. The irradiation conditions were as follows: 186 kv, 20 ma, and 0.5 mm-Cu and 0.55-mm Al filters plus inherent filtration were used. The dosage rate was measured in air at the place of the middle of the animal to 63 r per min. The animals were irradiated singly in thin cardboard containers covered with cheese cloth. The containers were such that the animal could crouch comfortably but could not turn or lie down.

Thirty male and 30 female guinea pigs of approximately 3 months of age were used in each experiment. The animals were received at approximately 1 month of age and were kept for 2 months in the laboratory under observation to make sure that only healthy animals were used in the experiments. During the 2-month interval animals died from coccidiosis and unknown causes. Complete blood counts were taken on 10 animals. One group received a total dose of 220 r and the other, 260 r. The error of each dose is estimated as ± 5 per cent.

Doses were measured on a dead 640-g guinea pig with a 100 r Victoreen condensor chamber, and the results are given in Table 4.

Table 4

Tissue doses of X rays

Location of chamber	Dose (r per min.)
air middle of animal	63
within center of animal	64
on top of middle of animal	61
underneath middle of animal	67
in rectum of animal	66
in mouth of animal	52

These data show that the tissue dose is fairly uniform and comparable to the air dose with the exception of the head which is to be expected. The exposure to 220 r killed 27 per cent of the animals within 30 days and the exposure to 260 r killed 38 per cent.

The confidence limits of the experiments are given by:

$$T = \frac{P - P^1}{\sqrt{\frac{PQ}{N}}}$$

where P is the per cent expected, P^1 is the per cent observed, Q is the per cent not expected ($1 - P$), and N is the number of animals. The 95 per cent confidence limits obtained for the 30-day LD_{50} for 60 animals are 63 per cent and 37 per cent killed; in other words, a dose that kills such a number of animals that the percentage lies within these limits has a 95 per cent chance of being the LD_{50} . A third experiment will be performed soon in which the same number of animals will be exposed to 320 r to establish the 30-day LD_{50} more accurately. It can be stated however from the data gathered from the 2 experiments that this dose will lie between 250 r and 300 r for the hybrid guinea pigs that were used.

Mortality in these 2 groups reached a peak between the 10th and 12th days following irradiation at a time when blood counts had reached their lowest values. In the 260 r group only a second smaller peak was observed on the 20th day after the blood picture had almost returned to normal. It is probable that this second peak of death may have been caused by an infection with failure of immune mechanisms. It was not caused by a hyperheparinemia as described by Jacobson *et al.* (3) as the clotting time did not change materially after the exposure (Figures 33 and 34). Blood picture data are given in Figures 33, 34, 35, and 36.

A decrease in all elements of the peripheral blood started shortly after the exposure and reached its maximum on approximately the 12th day. This was followed by a rapid recovery within the following weeks. At autopsy the animals usually showed extensive ecchymoses, mainly of the small intestine, stomach, heart, and lungs. These ecchymoses were more pronounced than those observed in chronically exposed animals. Effects on the weight of the animals were not very pronounced as shown in the curves of Figure 37. The weight minimum occurred approximately at the same time at which the minimum values of the blood picture were found. Comparing the effect on the blood picture in acute and chronic exposures, it may be seen that the effects are strikingly similar as far as the abrupt decrease of the blood elements is concerned. In chronically exposed guinea pigs the blood picture shows little change until the dosage is almost lethal, then an abrupt decrease of all blood elements sets in (the exceptions are the platelets which decrease gradually shortly after the exposure is begun) leading to death of the animal from pancytopenia which frequently occurs within a week. Recovery is, however, quite different in chronic exposures. If the chronic exposure of guinea pigs is stopped after an anemia as low as 2.5 to 2.8 millions erythrocytes has developed, the blood picture of those animals which survived comes back to normal very slowly. It takes 1 month and longer to reach values that are near normal. Thus damage to the blood-forming organs is considerably more severe than in acute exposures.

It is of interest to compare the acute 30-day LD_{50} with the mean survival time and mean accumulated dose which caused death from pancytopenia in chronic exposures (Table 5). While as far as comparable pictures of the peripheral blood are concerned, the chronic dose (8.8 r level) is approximately 7 times that of the acute dose, as far as blood-forming organs are concerned this ratio is probably considerably less as judged from the slow return of the picture of the peripheral blood to normal.

Figure 33. Erythrocyte, hematocrit, hemoglobin, and platelet values and clotting times of hybrid guinea pigs given a single total-body exposure of 220 r X radiation.

Figure 34. Erythrocyte, hematocrit, hemoglobin, and platelet values and clotting times of hybrid guinea pigs given a single total-body exposure of 260 r X radiation.

Figure 35. Leucocyte, lymphocyte, and heterophil values of hybrid guinea pigs given a single total-body exposure of 220 r.

Figure 36. Leucocyte, lymphocyte, and heterophil values of hybrid guinea pigs given a single total-body exposure of 260 r.

Figure 37 A. Effect of a single total-body exposure of 220 r X radiation on the weight of hybrid guinea pigs.
B. Effect of 260 r.

Table 5

Mean survival and mean accumulated dose administered to
chronically exposed hybrid guinea pigs

Dose level (r)	Mean survival time (days)	Mean accumulated dose (r)
8.8	187	1700
4.4	653	2900
2.2	987	2200

LITERATURE CITED

1. Bloom, W.: Summary in Histopathology of Irradiation from External and Internal Sources, McGraw-Hill Book Co., Inc., New York (1948), NNES (mpts) Div. IV, Vol. 22I, p. 750, First edition. Ed., W. Bloom.
2. Jacobson, L. O., E. K. Marks, and E. Lorenz: Radiology, 52:371 (1949).
3. Jacobson, L. O., E. K. Marks, E. O. Gaston, J. G. Allen, and M. H. Block: J. Lab. Clin. Med., 33:1566 (1948).

COMPARISON OF THE BIOLOGIC EFFECT OF 186 KV AND 11.4 MEV X RAYS

Preliminary Report

by

Allen B. Eschenbrenner and Theodore Wang*

In an earlier report⁽¹⁾ it was shown that the pattern of injury and recovery of the mouse testis following total-body exposure to 186 kv X radiation, as reflected in testes weights, is correlated with the dose that is administered. The minimum weights of testes are dependent upon dose and the time required to effect the minimum weights as well as the time required for the weights to return to normal is a function of dose.

This dose response relationship suggested that a similar study using the 10 mev betatron of the Naval Ordnance Laboratory as a source of X rays might make it possible to establish a relationship between biologic effect and physical dose.

Accordingly, a preliminary investigation was initiated with the following experimental setup. Isodose curves were established by means of densitometer readings of exposed photographic film. From this data a special holder was designed and constructed to confine the mice (Figures 38 and 39) during irradiation.

The annular chambers consist of celluloid walls with Lucite top and bottom plates in which there are vent holes. Six mice were irradiated at a time and dosage was measured by a Victoreen thimble chamber r-meter. The mice were held in a horizontal position approximately 30 inches from the X-ray tube. The sound level at this position was 95 decibels with a fundamental frequency of 1920 cycles per second (due to vibration of the betatron laminations).

A Textolite cap of 1-inch wall thickness was required to provide electronic equilibrium in the thimble chamber. With a 1-inch cap the ionization was 1.9 times that obtained in air with no cap. The space available in the supporting mouse frame did not permit the attachment of a cap to the r-meter chamber, so that the actual dose to the mice was the indicated value multiplied by a factor between 1 and 1.9 depending upon the depth within the mice. Irradiations given were 165 r indicated (33 r per min. for 5 min.) and 300 r indicated (37 r per min. for 8 min.).

* Formerly, U. S. Bureau of Standards; present address: National Cancer Institute, National Institute of Health, U. S. Public Health Service, Bethesda, Maryland.

Figure 38. Lucite holder used to confine mice during irradiation.

Figure 39. Mice in place in Lucite holder.

The spectrum of the "10" mev Naval Ordnance Laboratory betatron has been determined by M. Weiner and P. Wang at the National Bureau of Standards, and has been found to be 11.4 ± 0.2 mev peak with a distribution in essential agreement with the theory of Heitler.

One control group of 6 mice was exposed to conditions similar to those of the experimental group (including 7 min. of sound) with the exception of X irradiation.

A limited number of mice was irradiated so that the full range of damage and recovery pattern established for 186 kv X ray could not be studied. Instead, the most critical period of time was studied. Groups of 4 mice were sacrificed at intervals of 1 week from 2 to 7 weeks after irradiation. The testes were dissected free of fat and epididymides and carefully weighed on a torsion balance. Average values obtained are shown in Figure 40 as per cent of weight of the testes of the control mice. For purposes of comparison data previously obtained using 186 kv X radiation are shown in the same figure. It is seen that the pattern of loss and recovery of testes weight is the same for 186 kv and for 11.4 mev X rays strongly suggesting that we are dealing with the same biologic response in both instances. It is concluded that the response of the mouse testis to irradiation will prove to be a valuable tool for subsequent studies on the relation between biologic effect and physical dose in the multimillion volt range.

LITERATURE CITED

1. Eschenbrenner, Allen B.: Chronic External Irradiation, Quarterly Report, Biological and Medical Divisions, Argonne National Laboratory, August to November 1948, Eds., A. M. Brues and H. Lisco (ANL-4227) p. 28.

Figure 40. Average values of testes weights of mice following X irradiation.

COMPARISON OF THE 30-DAY LD_{50} FOR MICE FOLLOWING TOTAL-BODY IRRADIATION WITH X RAY AND INTRAVENOUSLY-ADMINISTERED RADON

Preliminary Report

by

Joanne Weikel Hollcroft and Egon Lorenz

A comparison is being made on the 30-day LD_{50} for mice following total-body irradiation with X ray and intravenously-injected radon in equilibrium with its short-lived decay products. Two sublines of strain A males, one of Heston designated in the following as subline I and the other of Bittner designated as subline II, were used in these experiments since the first subline will not be available again for several months.

To simulate irradiation conditions for both radon and X radiation, the X-ray dose was given over a period of 2 hr, divided into 3 or 4 intervals. In addition, the dose rate was staggered so that the highest dose rate was used at the beginning and then gradually lowered. This was accomplished by added filtration. The irradiation conditions were 186 kv, 20 ma, and focus-middle-mouse distance, 162 cm.

The experimental data shown in Figure 41 indicate that these 2 sublines of strain A mice are markedly different. The 30-day LD_{50} for subline I for radon is approximately 0.017 mc per g of radon or 44,375 ergs per g of tissue, or, taking Lea's conversion value, 425 r. The 30-day LD_{50} for X ray for the same subline is estimated from the curve of Figure 41 to be 580 r. This dose corresponds to 53,940 ergs per g of tissue.

The 30-day LD_{50} for subline II appears to be greater than that of subline I for X ray but less for radon. The range of the values of the radon 30-day LD_{50} for subline II is greater than that of subline I. Estimating the 30-day LD_{50} from the curves in Figure 41 for subline II we obtain a value of approximately 0.014 mc per g radon. This corresponds to 36,540 ergs or 350 r taking Lea's conversion value. In the X-irradiation experiment 3 groups of mice of subline II have been used so far. All 3 values lie within the confidence limits of the 30-day LD_{50} . Assuming 600 r to be the LD_{50} we obtain 55,800 ergs per g as the tissue dose.

In summing up the LD_{50} data so far obtained it is evident that if the dose is based upon roentgens, the X-ray dose required by subline I is 36 per cent more than the dose of radon and its short-lived decay products. The X-ray dose required by subline II is 71 per cent more than the radon dose. If the dose is based on energy units these percentages change to

Figure 41. Mortality curves of mice given either total-body irradiation with X rays or intravenously-administered radon.

22 per cent and 52 per cent, respectively. Additional experiments are necessary to obtain more significant data but from this tentative data it seems possible to explain these differences on the basis of the certainly existing nonuniform distribution of radon and its short-lived decay products in the body of the mouse.

Physiology

Dr. Harvey M. Patt
Dr. Douglas E. Smith
Mr. Robert L. Straube
Miss Ella B. Tyree
Dr. Robert Conard

PROGRESS REPORT

THE MECHANISM OF CYSTEINE PROTECTION AGAINST X IRRADIATION

by

Harvey M. Patt, Douglas E. Smith, Ella B. Tyree, and
Robert L. Straube

Cysteine effectively reduces sensitivity to lethal amounts of X rays delivered at either high or low dose rates provided that the amino acid is given before the exposure.^(1,2) Glutathione, the cysteine-containing tripeptide, can also diminish radiation toxicity. On the other hand, the related amino acids, cystine and methionine, fail to influence radiosensitivity. These findings indicate first that the critical reactions leading to morbidity are complete when the irradiation is terminated and that these reactions are probably not reversible. Secondly, the presence of a sulfhydryl group distinguishes the substances which do protect from those which do not. It is important to bear in mind, however, that all sulfhydryl-containing materials may not necessarily protect and that other readily oxidizable substrates which are properly distributed may.

Time course of the protection: In earlier reports,^(1,2) it was stated that cysteine administered intravenously was highly effective in protecting rats when given within an hour before exposure to X rays (800 r, 250 kv). Cysteine administration at other times relative to irradiation was without influence. Histograms of the completed data on intravenously-administered cysteine are presented in Figure 42. Acute radiation deaths occur largely from 1 to 3 weeks after exposure with a peak in the second week. The few deaths occurring in the groups given cysteine within an hour prior to irradiation are scattered over this interval. Rather comparable results are obtained when cysteine is given orally. Orally administered cysteine is most effective in both rats and mice when administered 30 to 60 min before exposure (Figure 43). Differences in the protection afforded by the intravenous and oral routes when the amino acid is given 5 min. before irradiation are probably a reflection of the time required for absorption of the material from the gastro-intestinal tract. Under any circumstances orally administered cysteine is not as efficient as intravenously administered cysteine even though the oral dose is twice the latter.

Figure 42. Influence of time of injection of cysteine on sensitivity of rats to total-body X irradiation (cysteine dose, 950 mg per kg, I.V. -- controls received 5 per cent NaCl).

Figure 43. Influence of time of administration of cysteine (orally administered) on the sensitivity of rats and mice to total-body X irradiation (cysteine dose, 1900 mg per kg -- controls received 5 per cent NaCl). Figures on top of bars indicate numbers of animals used.

Cysteine-X-ray dosage relationships: On the basis of one completed experiment the 30-day LD_{50} for rats given cysteine intravenously 5 min. before X irradiation appears to be in the neighborhood of 1150 r. The 30-day LD_{50} for control irradiated rats is around 725 r. These findings are summarized in Figure 44. In Figure 45 are presented the mean survival times of control and cysteine-treated rats dying within 30 days after X irradiation with doses ranging from 800 to 1600 r. A more extensive series that includes a comparison of the effect of different intravenous cysteine doses for each X-ray dose is in progress.

Concerning the influence of the dose of orally administered cysteine, it appears that in both the rat and mouse maximal protection occurs with a dose of 1900 mg cysteine per kg (Figure 46). Higher and lower doses are less effective. With the higher doses (2400 to 2700 mg cysteine per kg body weight) signs of cysteine toxicity are evident.

Influence of related substances: It was hoped that some insight into the mechanism of cysteine protection might be forthcoming from a consideration of the influence of certain related substances on radiosensitivity. The term, related substances, is used in a broad sense and is not meant necessarily to imply either structural or pharmacological similarity. Among the substances evaluated have been the sulfur-containing amino acids, cystine and methionine, glutathione, ascorbic acid, sodium sulfide, and colloidal sulfur. In most instances 2 doses of each substance were used, the maximal tolerated dose and one-half of this dose. In the case of cystine and methionine, dosage was determined largely on the basis of solubility. The results are summarized in Tables 6 and 7. Cystine, unlike its reduced counterpart, cysteine, is not effective either intravenously or orally. Glutathione, on the other hand, can reduce X-ray toxicity when it is given intravenously before the exposure. Rather similar results were obtained with doses of glutathione ranging from 625 to 1250 mg per kg. The reduction in sensitivity to X rays as a result of glutathione is roughly two-thirds that observed with cysteine. However, the maximum intravenous glutathione dose (1250 mg per kg) is equivalent to approximately half of the cysteine dose used for comparison (950 mg per kg) in terms of sulfhydryl content. Orally administered glutathione is not effective in rats or mice even though the oral dose is equivalent to the effective oral dose of cysteine in sulfhydryl content. Methionine is without influence on survival after X irradiation. Ascorbic acid selected because of its reducing properties and its presumed importance in biologic oxidation-reductions is also without effect. The maximum tolerated dose of sodium sulfide that is equivalent to less than 1 per cent of the sulfur contained in the effective cysteine dose likewise does not influence sensitivity to the X rays. Our results with colloidal sulfur are equivocal and further work is necessary to evaluate its influence.

Figure 44. Survival of control and cysteine-treated rats after X irradiation with doses ranging from 600 to 1600 r (cysteine dose, 950 mg per kg, I.V., 5 min. before exposure -- controls received 5 per cent NaCl, I.V.).

Figure 45. Mean survival time of control and cysteine-treated rats after X irradiation with doses ranging from 800 to 1600 r (cysteine dose, 950 mg per kg, I.V., 5 min. before exposure -- controls received 5 per cent NaCl, I.V.).

Figure 46. Influence of orally administered cysteine on sensitivity of rats and mice to total-body X irradiation (cysteine per os 1 hr before exposure -- controls received 5 per cent NaCl). Figures on top of bars indicate numbers of animals used.

8/
Table 6

Influence of related substances on sensitivity of rats (Sprague-Dawley) to 800 r X irradiation

Treatment group	Dose mg/kg I.V.	Number of rats	Per cent survival, weeks after X irradiation				Mean survival time, (days)
			1	2	3	4	
Control	--	33	58	27	21	21	10
Methionine	255	16	56	31	25	25	9
Control	--	15	40	20	20	20	8
Ascorbic acid	500	15	53	13	7	7	9
Control	--	15	47	20	20	20	8
Ascorbic acid	1000	15	53	13	13	13	9
Control	--	15	87	7	7	7	11
Sodium sulfide	2.5	15	73	20	7	7	11
Control	--	15	67	27	13	0	13
Sodium sulfide	5.0	15	73	13	7	7	10
Control	--	15	87	27	0	0	12
Colloidal sulfur	17.5	12	100	67	42	42	15

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Table 7

Influence of related substances on sensitivity of rats (Sprague-Dawley) to 800 r X irradiation

Treatment group	Dose mg/kg	Route	Time of administration before X ray	Number of rats	Per cent survival, weeks after irradiation				Mean survival time (days)
					1	2	3	4	
Control	--			48	67	27	21	21	10
Glutathione	625	I.V.	5 minutes	30	93	73	67	63	13
Control	--			14	93	43	21	21	13
Glutathione	900	I.V.	5 minutes	14	93	64	64	64	10
Control	--			16	31	6	0	0	9
Glutathione	1250	I.V.	5 minutes	10	50	40	40	40	7
Control	--			30	30	7	7	7	7
Glutathione	2000	Oral	1 hour	29	34	7	3	3	8
Control	--			27	52	22	15	15	9
Cystine	280	I.V.	5 minutes	22	68	18	18	18	8
Control	--			15	27	0	0	0	7
Cystine	2500	Oral	1 hour	13	23	8	8	8	7

Work is in progress on other phases of this general problem -- intermediary metabolism of cysteine in the irradiated animal; protective influence of cysteine in the irradiated guinea pig and dog; comparison of the acute, subacute, and chronic changes in control and cysteine-irradiated rats; and influence of cysteine on the radiosensitivity of normal and tumor cells.

LITERATURE CITED

1. Patt, H. M., E. B. Tyree, R. L. Straube, and D. E. Smith: Science (in press).
2. Patt, Harvey M. et al.: Physiology, Quarterly Report, Biological and Medical Divisions, Argonne National Laboratory, February to May 1949, Eds., A. M. Brues and H. Lisco (ANL-4291) p. 44.

**POLONIUM AND CF-1 FEMALE MICE: THE 30-DAY LD₅₀
RETENTION, DISTRIBUTION, AND CONCENTRATION**

by

Miriam P. Finkel, Walter E. Kisielewski,
Gertrude M. Hirsch, and Thomas J. Mulhaney**INTRODUCTION**

The first report of the biological behavior of polonium was published in 1913 by Fernau *et al.*,⁽¹⁾ who studied the local reaction of human and rabbit skin to surface exposure. Since 1924 Lacassagne and his associates have been studying the retention, distribution, and pathological effects of polonium after it has been injected into rabbits and rats.⁽²⁻⁹⁾ Fink, Casarette, and co-workers, at the University of Rochester, have been engaged in biological studies with polonium for the past 5 years, and they have collected a large mass of information concerning the behavior of this metal in dogs and rats in particular. Much of their work has been assembled in completed reports.⁽¹⁰⁻¹³⁾

Although information has now been published concerning the acute toxicity of polonium and the hematological and pathological changes in rats during the first 160 days following injection with 2/5 and 1/5 the 20-day LD₅₀ (0.02 and 0.01 μ c per g), the long term effects of these and smaller doses have not been studied. It was decided to conduct such an investigation in this laboratory. Mice were selected as the experimental animal since large numbers of individuals are required in order to obtain statistically useful results in low dose studies where the latent period for the appearance of pathological changes may exceed 1 or even 2 years. Before long-term studies could be undertaken, it was necessary to determine for the mouse certain facts that already have been established for the rat; e.g., the dose of polonium that kills 50 per cent of the animals in 30 days (30-day LD₅₀), the rate at which the amount of the injected material decreases in the body (retention), and the selective uptake by the various organs and tissues (distribution and concentration).

MATERIALS AND METHODS

The isotope used was polonium²¹⁰ (radium F), which has a half-period of 140 days and decays by the emission of an α particle and a γ ray with respective energies of 5.3 and 0.8 mev. It was injected into one of the lateral tail veins as PoCl_2 in isotonic salt solution at pH 2. The Radiochemistry Group prepared the injection solutions and analyzed the biological samples (p. 103).

Eighty-nine CF-1 female mice, with an average weight of 22 g and ranging in age from 62 to 68 days, were arranged in dose level and disposition groups as shown in Table 8. The animals in groups 1 and 2 were permitted to live until death occurred; the others were scheduled to be killed at 1, 3, 10, 30, 59, 120, or 150 days. Some of the animals of group 3 died before this scheduled date. Separate analyses were done of the liver, spleen, kidneys, ovaries, mesenteric lymph node, lungs, femur (including marrow), gastrocnemius muscle, and remaining carcass of the animals in group 3. The same organs of the animals in group 5, with the exception of the muscle and carcass and the addition of the spine and thymus, were taken for the preparation of radioautographs. Since the latter procedure is still in progress, no statement can be made at this time regarding tissue localization of polonium.

Table 8

Mouse groups

Group	Dose level ($\mu\text{c/g}$)	Number of animals	Disposition
1	0.1	15	Total body analysis at natural death
2	0.05	15	Total body analysis at natural death
3	0.025	15	Organ analyses at timed intervals
4	0.01	15	Total body analysis at timed intervals
5	0.01	15	Organ radioautographs at timed intervals
6	0.005	14	Total body analysis at timed intervals

EXPERIMENTAL RESULTS

The 30-day LD₅₀: A plot on probit paper showed that the survival data closely approximate the normal probability function. The curves that were derived from the probit graphs and the actual survival points are presented in Figure 47; the intervals to 50 per cent and 100 per cent mortality are given in Table 9.

Table 9

Survival

Dose level ($\mu\text{c/g}$)	Mortality	
	50% (days)	100% (days)
0.1	13	20
0.05	22	28
0.025	42	--
0.01	--	--
0.005		

When the logarithm of the number of days required for 50 per cent of the animals to die is plotted against the logarithm of the dose level, a straight line is obtained (Figure 48). From this line it can be estimated that the 30-day LD₅₀ of $\text{Po}^{210}\text{Cl}_2$, when it is administered intravenously to young adult CF-1 female mice, lies between 0.03 and 0.04 μc per g. Fink and his associates⁽¹¹⁾ state that the 20-day LD₅₀ for rats is approximately 0.05 μc per g. This is in close agreement with an interpolation from Figure 48, where the 20-day LD₅₀ for the mouse lies close to 0.06 μc per g.

Retention: The percentage of the injected polonium that is retained by the mouse at increasing intervals after administration (Figure 49) does not vary greatly from the values reported by Fink⁽¹⁰⁾ for the rat. He states that the loss of polonium due to excretion and radioactive decay reduces the body content to 70 per cent at 10 days and 17 per cent at 100 days. In the mouse we found 72 per cent present at 10 days and 9 per cent at 100 days. Fifty per cent of the polonium that was administered to the mouse was eliminated from the body in 33 days; the further reduction by radioactive decay decreased the biological half-period to 26 days.

Figure 47. Survival in CF-1 female mice following intravenous administration of polonium.

Figure 48. Fifty per cent mortality following intravenous administration of polonium in CF-1 female mice.

Figure 49. Retention of polonium in CF-1 female mice following intravenous administration.

Per cent retention did not vary with the dose level; i.e., the proportion of the original dose that was recovered at any specific time after administration from an animal that had received $0.1 \mu\text{c per g}$ was not different from that recovered from an animal that had received only $0.005 \mu\text{c per g}$.

Organ distribution: Three days after injection approximately 20 per cent of the polonium content of the body was found to be present in the liver, 12 per cent in the kidneys, and 2 per cent in the spleen (Figure 50). By 120 days the liver and kidney values had decreased to 13 per cent and 2 per cent, respectively, whereas the spleen value had increased to 6 per cent. Similarly, the ovaries and lungs showed an overall decrease in relative polonium content while the mesenteric lymph node and femur held a larger portion of the total-body content of polonium at 120 days than they had at 3 days. The percentage present in the gastrocnemius muscle remained relatively constant during this period.

Organ concentration: The animals used for this study received $0.025 \mu\text{c per g}$, and the polonium recovered from the total body decreased regularly from that level to $0.002 \mu\text{c per g}$ at 120 days (Figures 51 and 52). (All radioactivity figures given in this discussion do not take into account the reduction due to radioactive decay.) During the 120-day period the spleen, mesenteric lymph node, liver, kidneys, and ovaries maintained specific activities greater than that of the animal as a whole; the values for the lungs and femur remained close to that for the entire animal. The concentration in the gastrocnemius muscle was consistently below the general body concentration.

The uptake per g of tissue by the spleen far exceeded that of any other organ; at 38 days its specific activity was greater than that of the body as a whole by a factor of 50. At the same time the concentration of polonium in the mesenteric lymph node was 13 times greater than it was in the entire body. The kidneys and ovaries had a high initial concentration of polonium, but a fairly steady decrease followed after 3 days. The specific activity of the liver decreased slowly from $3 \frac{1}{2}$ times that of the entire body at 3 days to 3 times at 30 days, after which a temporary increase to a peak of 5 times at 51 days occurred. The slight initial concentration in the lungs was no longer present at 30 days. The femur, which showed no greater specific activity at 3 days than the body as a whole, at 23 days reached a peak 3 times that of the general body level. However, polonium was rapidly lost from the bone thereafter.

Effects on growth: One of the most easily measured criteria of damage by any toxic agent is the effect that it has on the growth rate. In Figure 53 are plotted the weight changes of each group of mice. Those that received more than the 30-day LD_{50} (0.1 and $0.05 \mu\text{c per g}$) lost

Figure 50. Organ distribution of polonium in CF-1 female mice following intravenous administration.

Figure 51. Organ concentration of polonium in CF-1 female mice following intravenous administration.

Figure 52. Organ concentration of polonium in CF-1 female mice following intravenous administration.

Figure 53. Weight change in CF-1 female mice following intravenous administration of polonium.

weight precipitously after the first week, those that received 0.025 and 0.01 μc per g failed to grow during the period of observation, while those that received 0.005 μc per g progressed almost as well as the control animals.

CONCLUSIONS

1. The 30-day LD_{50} of $\text{Po}^{210}\text{Cl}_2$ administered intravenously to young adult CF-1 female mice lies between 0.03 and 0.04 μc per g.
2. The per cent retention of intravenously-injected Po^{210} is independent of dose within the range used (0.1 to 0.005 μc per g).
3. The biological half-period of Po^{210} , under the above conditions, is 26 days. Seventy-two per cent of the injected material was present in the mouse at 10 days and 9 per cent at 100 days.
4. Three days after injection 20 per cent of the Po in the body was present in the liver, 12 per cent in the kidneys, and 2 per cent in the spleen. During the 120-day period, the Po content of the liver, kidneys, ovaries, and lungs decreased, that of the spleen, mesenteric lymph node, and femur increased, and that of the gastrocnemius muscle remained constant.
5. Three days after injection the organs studied showed the following order of decreasing magnitude of specific activity (μc per g): ovary, kidney, spleen, liver, mesenteric lymph node, lung, femur, gastrocnemius muscle. At 38 days, concentration was greatest in the spleen (50 times that of the body as a whole) and the mesenteric node (13 times that of the body as a whole).
6. Doses higher than the 30-day LD_{50} resulted in rapid loss of weight; 0.025 and 0.01 μc per g prevented weight increase during the 120-day period, and 0.005 μc per g had little effect on weight increase.

LITERATURE CITED

1. Fernau, A., Schramek, and Zarzycki: *Strahlentherapie*, 3:333 (1913).
2. Lacassagne, A. and J. Lattes: *Comptes rend. soc. biol.*, 90:352 (1924).
3. _____: *Ibid.*, p. 485 (1924).
4. _____: *Ibid.*, p. 487 (1924).

5. Lacassagne, A., J. Lattes, and J. Lavedan: *J. de radiol. et d'electrol.*, 9:1,67 (1925).
6. Lacassagne, A. and A. Paulin: *Comptes rend. soc. biol.*, 94:327 (1926).
7. Lacassagne, A., C. Levaditi, J. Lattes, and S. Nicolan: *Comptes rend. soc. biol.*, 94:1179 (1926).
- ~~8. Lattes, J.-S. and A. Lacassagne: *J. de radiol. et d'electrol.*, 12:16 (1928).~~
9. Leblond, C. P. and A. Lacassagne: *Am. J. Roentgenol. Radium Therapy*, 50:801 (1943).
10. Fink, R. M., editor: *Biological studies with polonium, plutonium, and radium. Part I. Distribution and excretion of polonium.* AECD-2591 (1947).
11. Fink, R. M., editor: *Biological studies with polonium, plutonium, and radium. Part III, Chapter 1. Toxicity of polonium, plutonium, and radium in rats.* AECD-2593 (1947).
12. Fink, R. M., editor: *Biological studies with polonium, plutonium, and radium. Part III, Chapter 2. Toxicity of polonium, plutonium, and radium in rats.* AECD-2605 (1947).
13. Casarette, G. W.: *A serial study of pathological and hematological effects of injected Po in rats.* UR-42 (1948).

Histopathology

Dr. Arthur M. Cloudman
Dr. S. Phyllis Stearner
Dr. Janet Passonneau
Miss Katherine Hamilton

THE EFFECT OF TOTAL TIME OF TREATMENT UPON
SURVIVAL OF YOUNG CHICKS FOLLOWING X IRRADIATION

by
S. Phyllis Stearner

INTRODUCTION

Recent experiments have shown that the survival of young birds following exposure to X rays is influenced by the dosage rate.⁽¹⁾ Exposure of newly-hatched chicks and ducklings to about 1000 r at dosage rates above 10 to 15 r per min. resulted in a high mortality in the initial period (24 to 48 hr) after irradiation. Following exposures at dosage rates below 10 r per min. few deaths occurred until 5 to 7 days, demonstrating the importance of dosage rate in determining the lethality of X rays for young birds. Actually, these experiments do not make it possible to distinguish between the effects of dosage rate and of total time, since varying the rate necessitated varying the exposure time when the total dose was kept constant. Therefore, in the present study, fractionated dosages were used to determine the relative influence of dosage rate and of total treatment time. In this way it was possible to vary the overall time and maintain a constant dosage rate. The lethality of dosages delivered in 2 and in 4 fractions was compared to that of continuous exposures that extended for the same period of time.

MATERIALS AND METHODS

Unsexed white leghorn chicks were irradiated 2 to 4 days after hatching, and survival was observed for 2 weeks. In all cases the total X-ray dosage was approximately 1000 r, but exposures were made at rates of 6 r, 11.5 r, 18.5 r, and 43 r per min. Exposures at 43 r per min. were either continuous or divided into 2 or 4 fractions, but all exposures at the lower rates were continuous. The arrangement of dosage rates and the interval between exposures are shown in Table 10. From 30 to 90 animals were included in each exposure group.

The physical conditions of irradiation were as follows; 200 kv, 15 ma, and 0.5-mm Cu, and 3.0-mm Bakelite filters. Different dosage rates were obtained by varying the target-field distance.

Table 10

Arrangement of dosage and rates and spacing of exposures

Total treatment time	Number of exposures	Minutes between exposures	Dosage rate
23.2 min.	1	-	43 r per min.
54.0 min.	2	30 min.	43 r per min.
54.0 min.	4	10 min. (X3)	43 r per min.
54.0 min.	1	-	18.5 r per min.
84.0 min.	2	60 min.	43 r per min.
84.0 min.	4	20 min. (X3)	43 r per min.
84.0 min.	1	-	11.5 r per min.
166 min.	2	142 min.	43 r per min.
165 min.	4	47 min. (X3)	43 r per min.
166 min.	1	-	6 r per min.

RESULTS

The effect of total time of the treatment upon survival following 1000 r X irradiation (rate of 43 r per min. in all cases) is shown in Figure 54. In each instance, increasing the total treatment time increased the survival, but not at a proportionate rate. A decrease in overall time from 166 to 84 min. resulted in only a slight increase in mortality, but, when the total time was decreased from 84 to 54 min., there was a three-fold increase in mortality at 14 days, and deaths within 24 hr after exposure increased from 4 per cent to 65 per cent. The decrease in total time from 84 to 54 min. represented an increase in average dosage rate from 11.5 r to 18.5 r per min.

Survival following 1000 r at 43 r per min. fractionated into 2 and into 4 exposures is graphed in Figure 55. For each of the 3 different total treatment times, a dosage given at 43 r per min. but divided into 2 fractions resulted in better survival than did the same total dosage divided into 4 fractions or given in one continuous exposure (overall time of treatment constant). In addition, both fractionated exposure groups showed better survival than did the groups exposed continuously at average rates of 6 r and 11.5 r per min., although the exposure rate of the fractionated dosages was 43 r per min.

Figure 54. Effect of total time of treatment on survival following 1000 r X irradiation (rate = 43 r per min.) The number of animals in each group is indicated in parentheses.

Figure 55. Effect of dosage rate and of fractionation on mortality following 1000 r X irradiation (total time constant). The number of animals in each group is indicated in parentheses.

- A. 166 minutes total time
- B. 84 minutes total time
- C. 54 minutes total time

In groups exposed at an average rate of 18.5 r per min., however, mortality was high during the initial period after irradiation, and there was little difference between the survival of groups that received fractionated dosages at 43 r per min. and those that received continuous exposures at the lower dosage rate. The survival of groups exposed to fractionated and continuous dosages at an average rate of 11.5 r per min. was only slightly less than that of groups exposed at an average rate of 6 r per min. (although the total exposure time was only half as long) and mortality during the initial period was low (compare A. and B. of Figure 55).

DISCUSSION

In radiation therapy it is generally recognized that increasing the exposure time decreases the effectiveness of a radiation dose. By means of fractionating dosages it has been demonstrated that under certain conditions the changes caused by ionizing radiations are reversible. (2,3) When the total dosage is divided into a number of daily exposures, a larger amount of ionizing radiation is required to produce an equivalent effect. Some recovery takes place during each interval and the overall time of treatment influences the total effect caused by the radiation dosage.

In birds, in which survival following X irradiation has been demonstrated to depend upon dosage rate, the effects of exposure to fractionated dosages indicate that overall time of treatment is of greater importance than dosage rate in determining the lethality of X rays. Comparison of survival following fractionated and continuous exposures (treatment time constant) shows that recovery processes operate more efficiently in the interval between exposures than during an exposure. However, for average rates of 6 r and 11.5 r per min. (delivered in a dose rate of 43 r per min.) dosages divided into 2 fractions yielded greater survival than did those divided into 4 fractions, although the total time of the 3 intervals was equivalent to that of the single interval. These findings suggest 1) that the rate of recovery may not be constant but may increase during an interval between exposures or 2) that there may be a lag after each high rate exposure before recovery processes begin to operate. In such a manner, the degree of recovery during one long interval would be greater than the total amount that would result during 3 intervals of $1/3$ the length.

On the basis of results of continuous exposures in newly-hatched birds, early deaths have been reported to occur following exposure rates above about 15 r per min., while "acute radiation deaths" at 5 to 10 days after irradiation are characteristic of exposures at rates below 10 r per min. (1) Although survival was modified by fractionation of the X-ray dosage, the shape of the survival curve was determined by the average dosage rate based on overall treatment time. Exposures at an average rate of 11.5 r per min. produced only a small number of deaths during the initial period

although more deaths occurred than in the groups exposed at an average rate of 6 r per min. A large number of deaths occurred in the initial period in groups exposed to fractionated dosages at an average rate of 18.5 r per min. and survival was similar to that seen in groups exposed continuously at 18.5 r per min. or 43 r per min.

CONCLUSIONS AND SUMMARY

Survival following exposure to fractionated X-ray dosages indicates that total treatment time is of greater importance than dosage rate in determining the lethality of a given total dosage for newly-hatched chicks. Survival is increased as the overall time of exposure is increased, but not at a proportionate rate. Comparison of survival following exposure to 1000 r (rate of 43 r per min.) divided into 2 and into 4 fractions has shown that the dosage divided into 2 fractions results in greater survival than when divided into 4 fractions, although the overall time remains constant. It is suggested that the rate of recovery may increase during an interval between exposures or that there may be a lag after each high rate exposure before recovery processes begin to operate, so that the degree of recovery occurring during 1 interval would be greater than the total occurring in 3 intervals of $1/3$ the length.

Lethal effects of fractionated dosages were usually slightly less than those of continuous exposures to 1000 r delivered at the same average rate. Survival following continuous and fractionated dosages at an average rate of 11.5 r per min. resembled that which follows low rate exposures, while survival after continuous or fractionated dosages at an average rate of 18.5 r per min. were characteristic of that seen after exposures at 43 r per min.

LITERATURE CITED

1. Stearner, S. P.: Histopathology, Quarterly Report, Biological and Medical Divisions, Argonne National Laboratory, February to May 1949, Eds., A. M. Brues and H. Lisco (ANL-4291) p. 90.
2. Ellinger, F.: Radiology, 49:238 (1947).
3. Stearner, S. P., E. L. Simmons, and L. O. Jacobson: Effect of X rays on the Blood and Blood-forming Organs of the Rat. ANL-4001 (1947).

Radiochemistry

Dr. William P. Norris
Mr. Walter E. Kisielecki
Mrs. Blanche Lawrence
Miss Lois Woodruff

A QUANTITATIVE METHOD FOR THE DETERMINATION
OF POLONIUM IN ANIMAL TISSUES

by

Thomas J. Mulhaney, William P. Norris, and Walter E. Kisielecki

INTRODUCTION

Recent interest concerning the metabolism and toxicity of polonium has required the development of a method for the quantitative estimation of polonium in biological material. Chemical work with this material is complicated by the following factors: 1) the volatility of some polonium compounds at temperatures in excess of 300°C, 2) the tendency of polonium to adsorb on glass surfaces under certain conditions,⁽¹⁾ 3) its capacity to form colloids at some conditions of pH, and 4) lack of information concerning its chemical behavior.

In general, polonium analyses have been achieved by virtue of the fact that this material plates out of solution on metals such as copper, iron, and silver. Because of its relative inertness silver has ordinarily been used. Polonium is determined on the plated disc by measuring the α -ray activity of the disc.

METHOD

Whole mice or carcasses remaining after dissection were placed in 500-ml Kjeldahl flasks; tissues were wrapped in filter paper, to prevent sticking, and placed in 100-ml Kjeldahl flasks. For each gram of sample, 2 1/2 ml of concentrated (70 per cent) perchloric acid was added (with a minimum of 2 1/2 ml). A volume of Superoxol (H_2O_2 - 30 per cent Merck) equal to 1/2 the volume of perchloric acid and a volume of water equal to 1/5 the volume of perchloric acid were then added. The flasks were shaken after addition of the reagents to mix the contents. (Where possible, it is desirable to allow the flask to stand at this point for approximately 48 hr since this reduces appreciably the time required for digestion on the rack.)

The flasks were put on a digestion rack in a hood fitted with an explosion screen (Figure 56A). Heating was accomplished by Glas-col mantles

Figure 56. A. Flasks in position in digestion rack.
B. Plating rack.

of appropriate size controlled by a Variac to maintain a flask temperature of approximately 200°C.

After 15 to 20 min. for whole mice (15 to 25 g) or 2 to 5 min. for smaller samples (<1 g) the mixture became clear except for an oily layer on the surface. On further heating the solution became black and began to fume.* At this point 1 to 2 ml of Superoxol was added. The addition of Superoxol caused vigorous boiling which subsided quickly.

Heating was continued with additions of Superoxol as needed whenever blackening occurred and fumes appeared. When a clear white to yellow solution remained which did not blacken on further heating, digestion was considered complete.

The total digestion time for the large samples was 4 to 10 hr, depending on weight and fat content. Small samples required 15 to 60 min.

After cooling, these solutions were transferred to volumetric flasks and sufficient hydrochloric acid was added to make the final solution 1 N.

The size of the aliquot taken for assay was estimated so as to contain approximately 0.01 μ c of polonium (370 d/s). The aliquot was placed in a 100 ml round bottom centrifuge tube (in the plating rack, Figure 56B) and sufficient 1 N hydrochloric acid added to bring the volume to 50 ml. A clean, bright silver disk, 1 inch in diameter and 0.002 inches thick was introduced and the solution stirred at a rate that caused the silver disk to rotate on edge. The tube was heated in a water bath at 90 to 100°C. After 2 1/2 to 3 hr the disk was removed from solution, washed with water and allowed to dry.

Counts were taken of both sides of the disk in a standard 50 per cent geometry parallel plate α chamber.⁽²⁾

Aliquots of dilutions of the injection solutions were plated in the same manner and used as standards. These standards enabled calculations of recovery in terms of injected dose to be made without correction for scatter and radioactive decay; and also minimized error due to day to day variation in counter performance. Counter performance was checked each day with a plutonium standard.

*Care must be exercised to prevent explosion of the digestion mixture at this point. The mixture must not be allowed to become too concentrated, i.e., Superoxol should be added as soon as the solution becomes black and fumes begin to appear.

EXPERIMENTAL AND DISCUSSION

Deposition of polonium on silver: Aliquots of stock polonium solutions were plated from a variety of acids in order to establish the proper method of digestion. The technique of deposition was essentially that described above, except the silver discs were 0.005 inches thick. It was necessary to count the disc on both sides since the count from one side was ordinarily not one-half the total count. Recoveries were based on the count obtained by evaporating aliquots of stock polonium solutions on similar discs. Evaporation in this case was done in air without heating and final drying was accomplished by desiccation over sulfuric acid. The results are summarized in Table 11. Of the acids tested it is apparent that the best results are obtained by plating from hydrochloric, sulfuric, or phosphoric acids. Digestion with phosphoric acid proceeded too slowly for routine use. Attempts at digestion of whole mice with sulfuric indicated that temperatures much in excess of 200°C resulted in losses of polonium by volatilization. The most satisfactory method was that described above using perchloric acid with decomposition of excess perchloric acid with Superoxol.

Table 11

Recovery from various acids (per cent)

Acid	a	b	c
HCl	102	103	
H ₂ SO ₄	99.3	103	
H ₃ PO ₄	103	98.5	
HNO ₃	66	76	
HClO ₄	101.5	52	37
HClO ₄ - HNO ₃	74	99	72

Proper acidity for Po deposition was determined by plating from sulfuric acid at various normalities (Table 12). There is considerable latitude at this point. The midpoint of the range of acid concentrations giving good recoveries was approximated as 1 N and this normality was used in further work.

Table 12Effect of H^+ on deposition of Po from H_2SO_4

H^+	% recovery
0.01	96.6
0.1	99.6
1.0	99.7
6.0	100.0
10.0	96.2
18.0	96.1

The plating time required for 100 per cent recovery was determined by removing the silver disc from the plating solution and counting at intervals during a plating period of 4 hr (Figure 57). A standard time for plating was taken as 2 1/2 to 3 hr. In cases where larger volumes of plating solution are used the time for complete polonium deposition must be increased. One attempt to plate from 500 ml of solution required almost 4 days for complete deposition. About 90 per cent was recovered in 30 hr.

In order to determine recoveries from biological material using the method described, sixteen mice were injected intraperitoneally with a known amount of polonium. The mice were sacrificed immediately and analyzed for polonium content. Recoveries were 100 ± 4 per cent. The results are given in Table 13.

Figure 57. Recovery of polonium from HCl.

Table 13

Recoveries of Po from 16 mice

Animal number	% recovery
1	102
2	100
3	101
4	102.5
5	100
6	96.5
7	96.3
8	96.3
9	96.8
10	102.3
11	101.1
12	98.0
13	104.8
14	104.2
15	100.2
16	97.3

SUMMARY

A method is described for the quantitative determination of polonium in biological material. Tissues were digested in perchloric acid, and polonium was plated on silver from 1 N hydrochloric acid. Recoveries from mice containing known quantities of Po were 100 ± 4 per cent.

LITERATURE CITED

1. English Translation of Gmelin's Handbuch der Anorganischen Chemie. Polonium. N-2167 (1945), p. 152.
2. Jaffey, A. H., T. P. Kohman, and J. A. Crawford: A Manual on the Measurement of Radioactivity. MDDC-386 (1946).

COUNTING AND CALIBRATION OF GEIGER COUNTING APPARATUS

by

William P. Norris and Andrew Macleish

Several months ago it was considered desirable to replace the lead shields and the aluminum Geiger tube mounts that comprise part of the Biology Division's standard equipment for radioactive measurements with mica-end-window, Geiger tubes. Besides replacing old apparatus this move was made in order to attain maximum uniformity in measurement. The equipment, including new aluminum absorbers, has been in use for about 6 months and appears to be giving satisfactory results. It is now proposed to carefully standardize and calibrate counting techniques for various isotopes under conditions imposed by these new setups.

The Geiger tube mount is made entirely of aluminum and is fitted with 6 slots which allow the position of the sample to be varied over a wide range. The dimensions and general construction of the tube mount are shown in Figure 58. The Geiger tube is clamped in position between two jaws, operating on a single screw, which automatically center the tube. Vertical positioning of the tube is produced by means of a ledge that supports the rim of the tube. The ledge also makes it virtually impossible to damage the mica window under ordinary operating conditions. This type of mount will accommodate a wide variety of commercial Geiger tubes.

In operation the tube mount is placed inside a closed cylindrical shield with 1 3/4-inch lead walls lined on the inside with 1/4-inch aluminum as shown in Figure 59. The entire shield is lined on the outside with 1/6-inch stainless steel to give rigidity to the lead and to retain the door without sagging.

Absorbers were stamped from sheets of pure aluminum and weighed to determine the weight in mg per cm². Aluminum less than 34 mg per cm² was clamped in 3/8-inch stainless steel frames to obtain some rigidity and to facilitate handling.

The initial experiment in standardization was one to determine the effect on count of varying the position of a single absorber when the position of the sample and the position of the Geiger tube remain unchanged. In order to obtain accuracy all counts were carried to at least a total of 10,000 which reduces the statistical error to 1.0 per cent. In addition all curves on 3 isotopes were done in duplicate.

The results of this initial experiment can be seen in Figure 60. These curve characteristics were essentially duplicated in 2 other isotopes, P³² and Sr⁹⁰, besides Ce. Absorbers of weights between 1.1 and 19.5 mg per cm² were clamped in the 3/8-inch stainless steel frames while the remaining

Figure 58. Diagram of Geiger tube mounting.

Figure 59. Photograph showing tube mount and shield.

Figure 60. Effect on count caused by varying the position of a single absorber when the positions of the sample and Geiger tube remain unchanged.

ones were rigid enough to make this mounting unnecessary. It will be noticed that this group of curves breaks into 2 families with the break occurring at the point where the use of stainless steel mounting frames is discontinued. The comparatively deep "belly" and upward sweep of the first 5 curves can be attributed to a scatter effect induced by the mounting frames and these characteristics make it difficult to relate these curves to the succeeding group. The "belly" in the next 4 curves decreases until, essentially, a straight line is obtained.

It was then decided to count the same sample while using 1.1 to 19.5 mg per cm^2 absorbers without the steel mounting frames. Results of this experiment can be seen in Figure 61. Here one family of curves is recognizable and, although the first 5 curves exhibit some "belly," the difference between maximum and minimum count for any one absorber is in the neighborhood of 6 per cent rather than the 10 per cent exhibited in Figure 60.

In view of these findings it will now be necessary to do one of two things to rectify this difference in absorber characteristics. Either mount these thinner absorbers in frames of steel or aluminum, but with a frame width no greater than shelf-slot depth (Figure 58), or work out a correction factor for the scatter in the present frames.

The second experiment was one to determine the effect on count by varying both the position of the absorber and the sample with the position of the Geiger tube remaining unchanged. Again, all counts were carried to at least a total of 10,000 thus reducing statistical error to 1.0 per cent. Figure 62 shows the results of this experiment involving two variables. Inconsistencies exist here that can be attributed partially to scatter from the walls of the tube mount and these inconsistencies lend significance to the inclusion of this experiment.

Previous to this type of experiment counting was done with sample and absorber in a standard position, these positions are indicated in the shelf 3 family in Figure 62. Little was actually known about the variations occurring in other sample and absorber positions. It will be noticed that as the distance from the sample to the tube increases the curves become less uniform in relation to each other. This decrease in uniformity is accompanied by a trend that shows an increase in percentage difference between maximum and minimum count for each thickness of absorber as the absorbers and sample move farther away from the Geiger tube. Obviously, the most reliable count can be obtained with the absorber close to the sample.

The information shown by the succeeding graphs indicates the undesirability of forming conclusions short of a standard position. An arbitrary choice of sample and absorber position will not give a valid count unless a correction factor is applied to account for the discrepancies noted in these curves.

Figure 61. Results obtained using 1.1 to 19.5 mg per cm² absorbers without steel mounting frames.

Figure 62. Effect on count caused by varying both the position of the absorber and the sample when the position of the Geiger tube remains unchanged.

Biochemistry

Dr. E. S. Guzman Barron
Miss Betty Gasvoda
Mrs. Jean Gasvoda
Miss Veronica Flood

EFFECT OF X IRRADIATION ON THE ULTRAVIOLET ABSORPTION
SPECTRUM OF CRYSTALLINE BOVINE SERUM ALBUMIN

by
E. S. Guzman Barron and Betty Gasvoda

INTRODUCTION

The effect of ionizing radiations on proteins has been the subject of a large number of investigations (Arnow's review⁽¹⁾). Protein denaturation and precipitation,^(2,3) viscosity decrease,⁽⁴⁾ changes in the absorption spectrum,^(5,6,7) and in the sedimentation constants⁽⁶⁾ have been observed when proteins were irradiated with ultraviolet light, X rays, and α rays. However, the mechanism of these changes is still unknown, and most of the early work was done with impure solutions of protein. Of the different properties of a protein solution, the absorption of ultraviolet light is one which can be measured accurately. Furthermore, it seems that light absorption by proteins, from 2400 Å to 3000 Å, is almost wholly due to the tyrosine present in the molecule.^(8,9) The effect of X rays on the absorption spectrum of proteins has received little attention. Sanigar *et al.*⁽⁶⁾ stated that irradiation of proteins with 29,000 r of soft X rays had no effect at all. Effects similar to those produced by ultraviolet light were found by Arnow⁽⁷⁾ on irradiation with strong doses of α rays from radon. According to him, irradiation at pH values of 4.86 and 1.65 increased absorption of ultraviolet light, while irradiation at pH 6.3 produced a decrease. The results of our investigations on the effect of X rays on crystalline bovine serum albumin are presented in this paper. Contrary to Sanigar's reports, we have found that X rays definitely affect the absorption of light by protein solutions even at such small intensities as 100 r.

EXPERIMENTAL

The crystalline bovine serum albumin used in these experiments was purchased from Armour and Company (Lot No. 43). On electrophoresis it was found to behave as a homogeneous component, although on ultracentrifugation it showed the presence of a second component (about 5 per cent). The X-ray machine used was that of the Marine Biological Laboratory at Woods Hole, Massachusetts.

In all experiments the concentration of protein was 1×10^{-4} M (0.07 g per cent). Irradiation was performed at first at room temperature. When the effect of temperature on protein denaturation was observed, effort was made to irradiate the solutions at low temperature by surrounding the vessels to be irradiated with ice. The protein solutions were irradiated in small vessels, each of which contained 1 cc of solution. The vessels were pooled for the measurement of the absorption spectrum. Irradiation was performed at the isoelectric point (in water), at pH 7.72 (0.01 M phosphate buffer), and at pH 3.0 (0.01 M fluoroacetate buffer). Within these pH values the absorption spectrum of the protein was identical. Sizer and Peacock (9) found that at pH 12 a plateau appeared at 2400 Å, due, according to them, to dissociation of the phenolic OH groups of tyrosine. The absorption spectrum was measured with the Beckman spectrophotometer at room temperature. Because of lack of temperature regulation, the temperature in the absorption cells rose from 12° (temperature of irradiation) to 28° to 30°C 4 to 5 min. after measurements were started. The molecular extinction coefficient at 2790 Å was $4.26 \pm 0.06 \times 10^4$, and at 2510 Å, $1.92 \pm 0.05 \times 10^4$.

X irradiation of water solutions of albumin: The effect of X rays on crystalline bovine plasma albumin was first studied at the isoelectric point of the molecule, i.e., when dissolved in distilled water. In most of these experiments, unfortunately, no attempt was made during irradiation to control the change of temperature, which, though negligible on short exposures, was rather large on exposures above 10 min.

As can be seen in Table 14, X irradiation increased light absorption in the ultraviolet at all wave lengths, from 2360 Å to 3100 Å. The solutions remained clear up to 60,000 r. On irradiation with 71,900 r the solutions gradually became cloudy as the temperature rose. This process can be seen clearly on measuring the absorption of light at 2510 Å. The $\log \frac{I}{I_x}(E)$ values rose steadily with time (Figure 63). In this particular experiment the control solution gave an E value of 0.195. Extrapolation of the irradiated solution to zero time (i.e., immediately after irradiation) would give an E value of 0.190. Rajewski(10) in a study of the effect of radiations on protein denaturation and coagulation found that protein denaturation by ultraviolet light was independent of temperature, while denaturation by X rays was greatly influenced by temperature. The experimental results illustrated in Figure 63 are evidence that the denaturation and subsequent precipitation process after X irradiation are not only temperature dependent but are kinetic processes requiring a rather long time for completion. In Figure 64 we have given the complete absorption curves of the control solution and of a solution irradiated with 50,000 r. It will be shown in our next communication that this increase in the absorption of ultraviolet light is due to the oxidation of the tyrosine component of the protein molecule. Irradiation intensities beyond 50,000 r were difficult to control because of denaturation, a process that is temperature dependent. In fact, protein solutions that become cloudy after 2 hr when irradiated at 10° with 71,900 r remain clear for hours when kept at 3°.

Figure 63. Protein denaturation and precipitation by X irradiation as measured by light absorption at 2510 Å. The E values given ($\log \frac{I}{I_x}$) are the difference between the control and the irradiated solution. Radiation dosage, 71,900 r.

Figure 64. Effect of X irradiation on the ultraviolet absorption spectrum of serum albumin dissolved in water. Ordinate, molecular extinction coefficient $\times 10^5$. Abscissa, wave length in $\text{\AA}$ units $\times 10^3$.

1. Control solution.
2. Solution irradiated with 50,000 r.
3. Solution irradiated with 100,000 r and centrifuged to eliminate the precipitated protein.

Table 14

Effect of X irradiation on the absorption spectrum of crystalline bovine plasma albumin dissolved in H_2O^*

Radiation dosage	$\Delta 2360 \text{ \AA}$	$\Delta 2510 \text{ \AA}$	$\Delta 2790 \text{ \AA}$	$\Delta 3100 \text{ \AA}$	Irradiation temp.
r					
100	0	+0.0075	+0.006	+0.007	room temp.
250	+0.0475	+0.014	+0.013	+0.006	room temp.
500	-0.112	+0.021	+0.017	+0.013	room temp.
1000	+0.25	+0.004	+0.004	+0.002	room temp.
5000	--	+0.014	+0.004	+0.006	room temp.
10,000	--	+0.032	+0.017	+0.009	room temp.
25,000	--	+0.041	+0.016	+0.015	room temp.
50,000	--	+0.142	+0.133	+0.174	room temp.
60,000	--	+0.095	+0.036	+0.036	10°

*Figures given are the differences in the absorption spectrum between the control solution and the irradiated solution.

Evidence that the changes in the absorption spectrum may be independent of the denaturation process is given in Figure 64 (curve 3). The protein solution was irradiated by 100,000 r at room temperature. The solution was cloudy soon after irradiation. It was centrifuged for half an hour, and the absorption spectrum of the supernatant clear fluid was measured. The absorption spectrum of the solution showed a marked increase at 2510 $\text{\AA}$ and 3100 $\text{\AA}$, while it was slightly less than normal at 2790 $\text{\AA}$. The general shape of the curve was similar to that of protein solutions irradiated at pH 3.0.

X irradiation of serum albumin at pH 3.0: When serum albumin dissolved in 0.01 M fluoroacetate buffer was X-irradiated, it was found that even 150,000 r did not produce protein precipitation. The solutions after irradiation remained clear, and no increase in light absorption with time (as in water solutions) was seen. Furthermore, the absorption spectrum curve was entirely different from that observed in protein solutions dissolved in water. The absorption at 2790 $\text{\AA}$ (the maximum value of the tyrosine component of the protein) remained perfectly constant on irradiation from 100 r to 150,000 r, while it increased steadily at 2510 $\text{\AA}$ and 3100 $\text{\AA}$ (Figure 65). As the intensity of irradiation increased, the difference between the E values at 2510 $\text{\AA}$ and at 2790 $\text{\AA}$ decreased, simulating a gradual disappearance of the maximum band

65. Effect of X irradiation on the ultraviolet absorption spectrum of bovine serum albumin dissolved in 0.01 M fluoroacetate buffer. Ordinate, molecular extinction coefficient $\times 10^5$. Abscissa, wave length in $\text{\AA}$ units $\times 10^3$.

1. Control solution.

2. Solution irradiated with 100,000 r.

of tyrosine. The increase in the E values at 2510 Å appeared on irradiation with 250 r and increased steadily as the intensity of irradiation increased (Table 15). This lack of effect on the light absorption at 2790 Å must be due to lack of oxidation of the tyrosine component of the protein. We have no explanation for the increase in light absorption at 2510 Å and at 3100 Å. It is our opinion that these findings will be of help in clarifying our knowledge of the structure of proteins and of the contributions of the peptide bonds to the ultraviolet light absorption by proteins.

Table 15

Effect of X irradiation on the absorption spectrum of crystalline bovine albumin dissolved in fluoroacetate buffer, pH 3.0, 0.01 M.*

Irradiation at 10°. Δ values at three or four wave lengths.

Radiation dosage	Δ2360 Å	Δ2510 Å	Δ2790 Å	Δ3100 Å
r				
100	-.002	-.017	-.003	0
250	-.040	+.004	+.004	0
500	+.007	+.009	+.008	+.026
1000	-.018	+.007	+.003	+.004
5000	-.018	+.013	-.011	0
10,000	-.018	+.023	-.014	+.006
25,000	-.019	+.033	-.034	+.011
50,000	0	+.0715	-.028	+.0285
60,000	+.004	+.105	0	+.047
75,000	+.033	+.111	-.016	+.035
100,000	--	+.159	-.019	+.046
150,000	+.047	+.169	0	+.057

*Figures are the difference in the absorption spectrum between the control and the irradiated solution.

X irradiation of serum albumin at pH 7.72: X irradiation of serum albumin dissolved in 0.01 M phosphate buffer, pH 7.72, produced qualitatively the same alterations in the absorption spectrum as irradiation in water solutions, except that stability of the protein towards denaturation and precipitation

was greatly increased. In fact, even irradiation with 150,000 r failed to precipitate the protein, while in water solution precipitation occurred with half that intensity of irradiation. The increase of light absorption occurred throughout from 2400 Å to 3100 Å (Figure 66). This increase as measured in the far ultraviolet, at 2500 Å, 2790 Å, and 3100 Å, was roughly proportional to the intensity of radiation (Table 16).

Table 16

Effect of X irradiation on the absorption spectrum of crystalline bovine plasma albumin dissolved in .01 M PO_4 buffer, pH 7.72.*

Irradiation at 10°.

Radiation dosage	$\Delta 2360 \text{ Å}$	$\Delta 2510 \text{ Å}$	$\Delta 2790 \text{ Å}$	$\Delta 3100 \text{ Å}$
r				
100	+0.038	+0.003	+0.009	+0.008
250	-0.080	-0.001	+0.0025	+0.001
500	-0.170	+0.006	-0.004	+0.003
1000	--	+0.015	+0.007	+0.005
5000	--	+0.023	+0.0045	+0.011
10,000	--	+0.030	+0.008	+0.012
25,000	--	+0.051	+0.018	+0.026
50,000	--	+0.100	+0.037	+0.046
60,000		+0.142	+0.057	+0.059
100,000	+0.142	+0.182	+0.077	+0.069
150,000	--	--	--	--

*Figures are the difference in absorption spectrum between control and irradiated solution.

These experiments demonstrate that the absorption spectrum of proteins is greatly altered by X irradiation, the electrical charge of the protein having a definite influence in this effect. This is indicated in the experiments shown in Table 17. At all wave lengths, on irradiation with 50,000 r, the increment of light absorption was greater when the protein was dissolved in water. The effect was least when the protein was dissolved in acid solutions, below the isoelectric point.

Figure 66. Effect of X irradiation on the ultraviolet absorption spectrum of serum albumin dissolved in 0.01 M phosphate buffer, pH 7.72. Ordinate, molecular extinction coefficient $\times 10^5$. Abscissa, wave length in $\text{\AA} \times 10^3$.

1. Control.

2. Solution irradiated with 100,000 r.

Table 17

Effect of pH on the absorption spectrum of X irradiated protein solutions.*

Radiation dosage, 50,000 r.

pH	$\Delta 2400 \text{ \AA}$	$\Delta 2510 \text{ \AA}$	$\Delta 2790 \text{ \AA}$	$\Delta 3100 \text{ \AA}$
(In water) 5.5	+0.178	+0.142	+0.133	+0.174
3.0	+0.062	+0.0715	-0.028	+0.0285
7.72	+0.106	+0.100	+0.037	+0.046

* Δ values given are the difference between the control and irradiated solutions at four different wave lengths in $\text{\AA}$.

LITERATURE CITED

1. Arnow, L. E.: *Physiol. Rev.*, 16:671 (1936).
2. Spiegel, Adolf M.: *Biochem. Z.*, 186:181 (1927).
3. Clark, J.: *J. Gen. Physiol.*, 27:101 (1943-44).
4. Fairbrother, J. A. V.: *Brit. J. Radiol. (NS)*, 1:121 (1928).
5. Spiegel, Adolf M. and O. Krumpel: *Biochem. Z.*, 190:28 (1927).
6. Sanigar, E. B., L. E. Krejci, and E. V. Kraemer: *Biochem. J.*, 33:1 (1939).
7. Arnow, L. E.: *J. Biol. Chem.*, 110:43 (1935).
8. Lerner, A. B. and C. P. Barnum: *Arch. Biochem.*, 10:417 (1946).
9. Sizer, I. W. and A. C. Peacock: *J. Biol. Chem.*, 171:767 (1947).
10. Rajewski, I. V.: *Biochem. Z.*, 227:272 (1930).

Microbiology

Dr. E. Lawrence Powers
Mr. Charles F. Ehret
Mrs. Sylvia D. Mayer
Mrs. Carlene M. Raper
Mr. Richard Sato
Mrs. D. Shefner

PRELIMINARY REPORT ON EFFECTS OF HIGH DOSAGES OF X RAYS ON
PARAMECIUM AURELIA

by
E. Lawrence Powers and D. Shefner

INTRODUCTION

In recent reports the effects of a nitrogen mustard and irradiation on Paramecium aurelia have been described. (1,2) In regard to the latter, it was shown that as the dosage of 250 kv X radiation increases, there is a linear increase in the proportion of treated animals unable to survive the intracellular cytological reorganization termed autogamy. It appeared that the amount of death after autogamy reached a high plateau around 12,000 r, and that, in contrast to the results with mustards, very little, if any, vegetative or somatic effects were observable at these dosages that induce significant changes expressed as death after autogamy. The fact that we were unable to observe saturation of the X-ray-sensitive system governing death after autogamy led to the experiments reported briefly below in which very large dosages of X rays were given. As in the mustard experiments, effects of the treatments on fission rate and cell survival were also measured.

IRRADIATION TECHNIQUE

Since the output of the machine used in previous X-irradiation experiments is too low to allow the delivery of the high doses desired in conveniently short periods of time, a Picker high output, long wave-length X-ray machine was used. The doses were delivered at a potential of 50 kv at 40 ma. The drop of culture solution containing the animals was placed on a cover slip that had previously been attached to a thin plastic plate in which a hole slightly smaller than the slip had been drilled. The distance from the drop to the target was 10 cm, and no filters were used. According to the manufacturers performance sheets accompanying the machine, the dose at the drop under the conditions described should be 62,200 r per min. All dosages reported in this paper were calculated from this figure.

BIOLOGICAL TECHNIQUE

Variety 4, mating type VIII of *Paramecium aurelia* was used. The methods of preparation, exposure, isolation, induction of autogamy, and the criteria of effects were the same as in previous experiments.^(1,2)

EFFECTS OF LOW DOSAGES OF 50 KV X RAYS

In order to establish the similarity of effects between 50 kv X rays delivered at 62,200 r per min. and 250 kv X rays delivered at 425 r per min. at similar dosage levels, low dosages were given to a number of cultures and the amount of death after autogamy induced was measured. The first four items of Table 18 show the response of the animals to doses in the low region. Figure 67 depicts the relation between this response and that observed after treatment with 250 kv X rays, the line drawn on the graph being the best line calculated from the numerous data obtained using 250 kv X rays and the points representing the effect of 50 kv X rays. It appears that there is a similarity in response, and, therefore, that the higher dosages of the soft X rays can, for the time being, be considered a valid extension of the results obtained with the harder rays delivered at a much slower rate. Obviously more points are needed in this region to establish with some certainty that a difference in response does not exist.

EFFECTS OF HIGH DOSAGES OF 50 KV X RAYS

Table 18 and Figures 68 and 69 indicate the effects of high doses of 50 kv X rays on death after autogamy, depression of fission rate, and death of the cells before autogamy. Figure 68 demonstrates a regular increase in death after autogamy with increasing dose up to about 60,000 r after which there is a decrease to the 300,000 r region. Beyond this point, the effect seems to rise slowly as the dose is extended into higher regions. With the exception of the second rise, this result is qualitatively similar to that observed⁽¹⁾ when these organisms are exposed to a nitrogen mustard compound for increasing lengths of time, and, with the same restriction, is similar to that observed by other workers who investigated the genetic effects of ultra-violet radiation on several microorganisms.^(3,4) However, it is the first time a decrease in effect with increasing dosage has been observed with X rays, and for the first time a rise in effect with increasing dose beyond that at which the effect is lowered is noted.

Depression of fission rate and death of the cells exposed to 50 kv X rays are pictured in Figure 69. Depression in fission rate was measured with respect to the controls by the relation

$$\% \text{ depression} = 100 - \frac{T'}{T} \times 100$$

Table 18

Dosage in r	Depression of fission rate		Death of cells		Death after autogamy	
	No. cells	%	No. cells	%	No. cells	%
2,592	17	0	17	0	17	16.0
5,183	13	0	13	0	13	49.2
15,550	13	0	13	0	13	72.9
31,100	15	0	15	0	15	97.2
62,200	18	0	18	0	18	97.5
124,400	18	0	18	0	18	92.3
149,280	13	11.6	18	18.8*	X	X
248,800	13	15.1	33	0	33	77.9
398,080	6	9.2	9	25.0*	X	X
497,600	35	35.0	72	30.6	35	79.1
653,100	5	55.5	15	62.5*	X	X
746,400	25	54.6	54	50.0	25	87.7
852,140	5	69.4	27	79.2*	X	X
995,200	6	37.9	27	81.4	6	84.7
1,001,420	--	--	27	100	XX	XX
1,057,400	--	--	27	100	XX	XX

*Corrected for death in controls by Abbott's formula. See text.

X No observation made.

XX All animals died before autogamy.

Figure 67. Comparison of the effect on death after autogamy between 250 kv X rays at 425 r per min. (solid line) and 50 kv X irradiation at 62,200 r per min. (points).

Figure 68. Induction of death after autogamy by 50 kv X rays.

Figure 69. Per cent depression of fission rate of surviving cells (solid points) and per cent death of cells (open points) caused by 50 kv X rays.

in which T' is the number of fissions in 4 days which the treated animals underwent and T is the number of fissions exhibited by the controls during the same period. Death was measured as per cent of the total number observed which were unable to go into autogamy. In one experiment the controls died at the unusually high rate of 11.2 per cent; the experimental values were corrected by means of the Abbott formula(5)

$$P = \frac{P' - C}{1 - C}$$

in which P' is the fractional death of the treated animals, C is the fractional control death, and P is the corrected fractional experimental death.

In Figure 69 depression in fission rate is represented by solid circles, and percentage death is represented by open circles. It is evident that the data on hand allow no distinction between relations of the two responses to dose. According to these results an exposure which kills 50 per cent of the cells within 4 days will be expected to depress the fission rate of the survivors over the 4-day period of observation to 50 per cent of that shown by the controls.

It should be noted from figures that the autogamy response curve, which may be a measure of genetic damage to the micronucleus, begins to decline near the dose (60,000 r) at which the vegetative or somatic effects become prominent enough to be measured significantly by the techniques used. In the case of the mustards, the responses are such that reversal of the autogamy curve could well occur at a corresponding level. Experiments are under way at the present time to define the mustard response curve more precisely to prove the point. For the present, the explanation that animals which would show high death after autogamy if they had gone into autogamy are being removed from the population fast enough to affect the autogamy response curve seems to be indicated in both the mustard and X-ray experiments. In this regard, however, it may be noted that the somatic response curve to X rays seems to cut into the ordinate axis at some point above zero, a fact which appears illogical when the method of calculating the response is considered. A likely explanation of this fact is that the cells are recovering from the damage caused by the X rays at the dosages below approximately 100,000 r at a rate which is sufficiently fast to make the observation of that effect difficult; above the threshold of approximately 100,000 r, recovery is slow enough to allow our noting the effects. Analyses of the data on the lower dosages will undoubtedly shed light on the unexplained upward displacement of the response curve.

LITERATURE CITED

1. Powers, E. L. et al.: Microbiology, Quarterly Report, Biological and Medical Divisions, Argonne National Laboratory, February to May 1949, Eds., A. M. Brues and H. Lisco (ANL-4291) p. 165.
2. _____: Ibid, p. 159.
3. Demerec, M. and R. Latarjet: Mutations in Bacteria Induced by Radiations in Cold Spring Harbor Symposia, MacMillan and Co., Inc., New York (1946), Vol. 11, p. 38.
4. Hollaender, A. and C. W. Emmons: Induced Mutations and Speciation in Fungi in Cold Spring Harbor Symposia, MacMillan and Co., Inc., New York (1946), Vol. 11, p. 78.
5. Finney, D. J.: Probit Analysis. Cambridge (1947).

PLUTONIUM AND YTTRIUM CONTENT OF THE BLOOD, LIVER,
AND SKELETON OF THE RAT AT DIFFERENT TIMES
AFTER INTRAVENOUS ADMINISTRATION*

by

Jack Schubert, Miriam P. Finkel, Marcia R. White,
and Gertrude M. Hirsch

ABSTRACT

Eighteen adult female Sprague-Dawley rats were injected intravenously with a 1 per cent sodium citrate solution containing tracer levels of both $\text{Pu}^{239}(+4)$ and $\text{Y}^{91}(+3)$. They were sacrificed in pairs at 5, 15, 30, and 60 min. and at 2, 6, 12, 24, and 48 hr. A blood sample, one femur, and the liver were analyzed.

Five minutes after injection 50 per cent of the Pu and 80 per cent of the Y had left the blood stream. Within 6 hr almost all of the Y had disappeared from the blood; 48 hr were required for the removal of most of the Pu.

The maximum uptake of both Pu and Y by the skeleton was approximately 45 per cent of the injected dose. This was reached at 24 hr in the case of Pu and at 6 hr in the case of Y.

By the 24th hr the Pu content of the liver reached a maximum of 24 per cent of the injected dose; the Y content reached a maximum of 10 per cent by the 2nd hr and subsequently decreased at a very slow rate.

The fractions of Pu and Y which left the blood within 5 min. were interpreted to be in the form of soluble ions, and the more slowly disappearing fractions to be small colloids. The relative proportions of a cationic radioelement which leave the blood in these 2 forms were considered to be related to the propensity to polymerize, which in turn is a function of the pH at which the hydroxide of a dilute solution of a simple salt of the element will precipitate.

Further, it was assumed that diffusion laws governed the rate of uptake of Pu or Y by the skeleton. The data expressed in terms of the parabolic diffusion law, plotted with the per cent uptake versus the square root of time, gave a linear relationship for Pu up to the plateau value. In the case of Y, the equation held up to 80 per cent of the plateau value.

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TOXICITY OF TRITIUM OXIDE TO MICE

by

Austin M. Brues, Agnes Naranjo Stroud, and Leola Rietz

SUMMARY

Tritium (H^3) is, in common with carbon¹⁴, a radioisotope that may be used to label a large variety of physiologically important compounds. The soft nature of its β ray (mean energy about 5.7 kev) suggests interesting possibilities in the fields of radiobiology and therapeutics that might be achieved by selective localization in living structures. The biological effectiveness of this soft β ray is unknown, but the structures and functions sensitive to density of ionization appear to be predominant in determining radiation toxicity to the higher animals, so that direct experimental evidence regarding the biological effectiveness of tritium is important. Because of the low solubility of tritium or hydrogen gas in body fluids, it may be assumed that tritium water would be a considerably greater health hazard. These experiments were therefore done to establish the lethal dose of tritium water when injected intraperitoneally in physiological saline solution into CF-1 mice. Circumstances of the experimental conditions do not enable us to make rigid comparison of relative biological effectiveness as against better studied radiation, but do indicate clearly that no serious or unexpected correction factor needs to be introduced in calculating short-term tritium water toxicity on physical grounds.

Young female CF-1 mice, whose radiotoxic responses have been widely established in this laboratory, were injected intraperitoneally with 0.85 per cent NaCl solution containing various amounts of tritium water diluted with sterile distilled water. Injection volumes ranged between 0.2 and 0.5 ml, and care was taken that none of the injection solution escaped after removal of the needle. The health precautions used in maintaining the mice after injection and details regarding hematologic and other clinical observations will be detailed in a later communication.

In a pilot experiment dosages between 3 and 200 mc were given to mice weighing between 15 and 18 g; a subsequent experiment utilized mice weighing 20 ± 2.5 g, given dosages between 10 and 30 mc, bracketing the acute lethal range. The mortality results are shown in Table 19.

The tabulated results indicate a 20-day LD_{50} of about 1 mc per g body weight. Autopsy findings and hematologic observations indicate that deaths at and above this level were due to acute radiation disease.

Table 19

Mortality in CF-1 mice following injection of H^3

Experiment T 1: mean weight of mice 16.5 g.				
Total dose	Number of mice	Mice dying in 20 days		Median time to death
		Number	Per cent	
200 mc	2	2	100	90 hours
100 mc	2	2	100	96 hours
30 mc	4	4	100	7.5 days
10 mc	4	0	0	
3 mc	4	1	25	(at 13 days: probably not radiation death)
Experiment T 2: mean weight of mice 20 g.				
30 mc	4	4	100	12 days
25 mc	6	6	100	12 days
20 mc	5	3	60	16 days
15 mc	6	1	17	(at 12 days)
10 mc	6	1	17	(at 16 days)
controls	7	0	0	

Dosage calculations in physical terms would require serial analyses of body water specific activity and of uptake of tritium by organic compounds and can be approximated by measurements of food and water intake. The calculated total water intake (including metabolic water from food) in the mice receiving 20 and 15 mc in the second experiment indicated a rather constant water turnover for the first 5 days of 25 per cent per day, and for the second 5-day period this rose to about 40 per cent per day. Assuming the body to be 75 per cent water and that tritium oxide is excreted at the same rate as water, disregarding the retarding effect of metabolic turnover of hydrogen and tritium, and assuming a mean β -ray energy of 5.7 kev, the calculated dosage to mice receiving 1 mc per g is 350 rep per day at the time of injection; the total accumulated dosage is about 1000 rep during the first 5 days, about 200 rep during the second 5 days, and essentially negligible thereafter. Although no figures are available for estimating

the lethal dose of X or γ rays administered with a time pattern such as this, it may be concluded that no remarkable factor of biological effectiveness exists for tritium β rays in relation to these better known radiations.

It is worth while remarking that dosages of tritium water in the lethal range tend to perpetuate themselves in the body due to the decreased food and water intake characteristic of the acute radiation response. It should also be recognized that the relatively slower water turnover of the human being would result in a greater retention of the isotope and an increased toxicity, perhaps by a factor of 3 or 4, unless appropriate therapeutic measures were used.

Further assay of the solution of tritium oxide used indicates that the concentration of tritium may be substantially higher than by the original assay. This is further evidence that no special biological factor exists for toxicity.

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ERRATUM

The legends on pages 120, 122, and 125 (Figures 64, 65, and 66) should read as follows:

Abcissa, wave length in $\text{\AA}$ units $\times 10^{-3}$.

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