2

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DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH

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Editor

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Medical Research

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TABLE OF CONTENTS

	Page
Administration and Editorial (Abstract)	6
Chronic External Irradiation	10
Factors Influencing Irradiation Treatment of Experimental Lymphoid Tumors. E. Lorenz	10
Progress Report: Biologic Effects of Total-body Irradiation. I. Modification of Acute Irradiation Injury by Heterologous Bone Marrow Injections in Mice and Guinea Pigs. E. Lorenz, D. Uphoff, and C. C. Congdon. . . .	11
II. Modification of Acute Irradiation Injury by Injection of Tissues Originating from Hematopoietic Organs. D. Uphoff, C. C. Congdon, K. Sanford, and E. Lorenz	13
III. Effects of Total-body Irradiation Followed by Bone Marrow Injections on Leukemia in Guinea Pigs. E. Lorenz, D. Uphoff, and C. C. Congdon	15
IV. Septicemia in Irradiated C ₃ Hb Mice and Its Modification by Bone Marrow Injection. F. P. Williams, Jr. and C. C. Congdon	17
Progress Report: Tumor Response to Varying Tumor and Body Doses of X irradiation. J. W. Hollcroft and M. M. Mathews.	24
Neutron Radiobiology	27
Progress Report: Irradiation of Mice with Fast Neutrons and Gamma Rays. H. H. Vogel, Jr., J. W. Clark, C. P. Stroud, and D. L. Jordan	27
Hematology	33
Progress Report: The Effect of Spleen Shielding on the Hematologic Values of Rabbits Following Total-body X irradiation. L. O. Jacobson, E. K. Marks, and E. O. Gaston	33

TABLE OF CONTENTS

	Page
Physiology	47
Temperature Dependence of the Protection of Thymus Cells by Cysteine and Anoxia. M. E. Blackford and H. M. Patt.	47
Progress Report: Effects of X irradiation on Mast Cells. Y. S. Lewis and D. E. Smith.	50
Histopathology	55
Description of a Technique for Ultra-thin Sectioning for Electron Microscopy (Abstract). B. W. Massey.	55
Radium Toxicity.	56
Progress Report: Clinical Aspects of the Radium Tox- icity Program.	56
Experimental Pathology.	57
Progress Report: Toxicity of Sr^{89} in Mice. Design of Experiment and Survival Following Single Injection. M. P. Finkel, A. M. Brues, and H. Lisco	57
The Fate and Pathologic Effects of Plutonium Metal Implanted into Rabbits and Rats (Abstract). H. Lisco and W. E. Kisielewski.	63
Special Problems	64
Progress Report: Blood Pressure Changes in X-irra- diated Chicks. S. P. Stearner, E. J. B. Christian, and J. F. Thomson.	64
Invertebrate Zoology.	68
Progress Report: The Effect of High Energy Neutrons on the Egg Nuclei of Grasshopper Nymphs. T. N. Tahmisian, A. M. Brues, and H. H. Vogel, Jr	68

TABLE OF CONTENTS

	Page
Microbiology	69
Antibiotic Sensitivity of Kappa and the Influence of X irradiation on the Production of Aureomycin- resistant "Killers" in <u>P. aurelia</u> , 51, VIII KK. (Abstract). C. F. Ehret and E. L. Powers	69
Phytoradiobiology	71
Progress Report: The Radiosensitivity of Auxin Biosyn- thesis. R. P. Weber and S. A. Gordon	71
Physiological Chemistry	73
Ethylenediamine - A Carbonate-free Alkali for CO ₂ Absorption. R. W. Swick, D. L. Buchanan, and A. Nakao. . .	73
Organic Chemistry	76
Progress Report: J. H. Pomeroy	76
Physical Chemistry	77
Progress Report: Mechanisms of Reversal of Toxicity of Beryllium, Boron, and Uranium	77
Pharmacology	80
Progress Report: Some Hormonal Factors Governing The Response of Rat Liver Tryptophan Peroxidase- Oxidase to Total-body X irradiation. J. F. Thomson and E. T. Mikuta	80
Theoretical Biology	84
Preliminary Report: A Problem on the Statistical Esti- mation of the Concentration of Microorganisms Using the Dilution Method. J. E. Hall and S. A. Taylor	84
Progress Report: Kinetics of Population Growth. G. A. Sacher	88

TABLE OF CONTENTS

	Page
Biophysics	90
Progress Report: Ionization from Beta-ray Point Sources. R. K. Clark and S. S. Brar	90
The Determination of Photographic Film Exposure by Neutron Activation of Ag ¹⁰⁷ . I. B. Berlman, H. F. Lucas, and H. A. May	91
The Growth of Bacterial Colonies. H. E. Kubitschek	95
Progress Report: A Possible Thermal Neutron Detector of High Efficiency. I. B. Berlman	99
Fluorescent X-ray Analysis. R. E. Rowland	100
Progress Report: Study of the Mechanisms Operating in the Ionization Type Halide Detector. H. A. Schultz and J. G. Dodd, Jr	102
Index	109

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E. Lawrence Powers, Associate Director
Leonidas D. Marinelli, Associate Director
John S. Hood, Assistant Division Director

Editorial Staff: Antreen Pfau
Jane Williams

STAFF ACTIVITIES

In May Dr. Austin M. Brues was elected to membership in the American Association of Physicians and on July 1, he was promoted to the rank of Professor of Medicine at the University of Chicago.

Dr. William B. Looney, who was associated with the radium toxicity program left the laboratory in May to report for active duty in the Navy.

During the month of June, Mrs. Edna K. Marks and Mrs. Phyllis Sudds (née Johnson) left the laboratory to join the staff of the University of Chicago. Mrs. Marks will continue to work with Dr. Jacobson on problems of radiation therapy, and Mrs. Sudds will continue to assist Dr. Barron in investigations of the action of radiations on enzyme systems.

Mrs. Sylvia Mayer, who has been working with Dr. Patt on the modification of irradiation damage by cysteine, left the laboratory in July, and Mr. Rosson L. Cardwell, the Assistant to the Division Director, resigned to accept the position of Executive Director of the Cowles Commission for Economic Research.

On July 1, Mrs. Margaret Blackford, Miss Joan Gurian, Mr. Philip Gustafson, Mr. Akira Nakao, and Mr. Thomas Speckman were promoted from research technicians to junior scientists.

Mr. John Hood, who has been working in the division as an associate engineer, became the Assistant Division Director on July 1. In addition to his new duties, he will continue his work in conjunction with the moving of the division to Building 202.

PUBLICATIONS

Barron, E. S. G.: Effect of Ionizing Radiations on Some Systems of Biological Importance. Symposium on Radiobiology. The Basic Aspects of Radiation Effects on Living Systems. Oberlin College, June 14-18, 1950. Sponsored by The National Research Council of the National Academy of Sciences, Ed., J. J. Nickson, John Wiley and Sons, Inc., New York, 1952. Pp. 216-240.

Brues, A. M. and G. A. Sacher: Analysis of Mammalian Radiation Injury and Lethality. Symposium on Radiobiology. The Basic Aspects of Radiation Effects on Living Systems. Oberlin College, June 14-18, 1950. Sponsored by The National Research Council of the National Academy of Sciences, Ed., J. J. Nickson, John Wiley and Sons, Inc., New York, 1952, pp. 441-465.

Zirkle, R. E.: Speculations on Cellular Actions of Radiations. Symposium on Radiobiology. The Basic Aspects of Radiation Effects on Living Systems. Oberlin College, June 14-18, 1950. Sponsored by The National Research Council of the National Academy of Sciences, Ed., J. J. Nickson, John Wiley and Sons, Inc., New York, 1952, pp. 333-356.

Patt, H. M.: Some Aspects of the Biological Action of High Energy Radiations. *Ann. Rev. Nuclear Science*, I, 495-524, 1952.

Allen, J. G., C. E. Basinger, J. J. Landy, M. H. Sanderson, and D. M. Enerson: Blood Transfusion in Irradiation Hemorrhage. *Science*, 115:523-526, May, 1952.

Brues, A. M.: Nuclear Changes following Irradiation of Tumors. *Am. J. Path.*, 28:547, May-June, 1952 (Abstract).

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Lindenbaum, A., M. R. White, and J. Schubert: Effect of Aurintricarboxylic Acid on Beryllium Inhibition of Alkaline Phosphates. *J. Biol. Chem.*, 196: 273-278, May, 1952.

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Patt, H. M., M. E. Blackford, and R. L. Straube: Effect of X rays on Thymocytes and Its Modification by Cysteine. *Proc. Soc. Exptl. Biol. Med.*, 80:92-97, May, 1952.

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Schubert, J., M. R. White, and A. Lindenbaum: Studies on the Mechanism of Protection by Aurintricarboxylic Acid in Beryllium Poisoning. *J. Biol. Chem.*, 196:279-288, May, 1952.

TALKS

Brues, A. M.: Neutron Activation. Forty-fifth Annual Meeting of the Illinois State Academy of Science, Charleston, Illinois, May 2-3, 1952.

Powers, E. L.: Cellular Effects of Radiations. Forty-fifth Annual Meeting of the Illinois State Academy of Science, Charleston, Illinois, May 2, 1952.

Barron, E. S. G.: Studies on the Mechanism of Action of the Adrenal Cortical Hormones. Sixty-fifth Annual Meeting of the Association of American Physicians, Atlantic City, New Jersey, May 4, 1952.

Jacobson, L. O.: Evidence for a Humoral Factor (or Factors) Concerned in Recovery from Irradiation Injury. Sixty-fifth Annual Meeting of the Association of American Physicians, Atlantic City, New Jersey, May 6, 1952.

Barron, E. S. G.: Studies on the Mechanism of Action of the Adrenal Cortical Hormones. The Sixty-fifth Annual Meeting of the Association of American Physicians, Atlantic City, New Jersey, May 7, 1952.

Looney, W. B.: Pathogenesis of Radium Toxicity. Presbyterian Hospital, Chicago, Illinois, May 9, 1952.

Jacobson, L. O.: Presided at the Senior Scientific Session of the University of Chicago School of Medicine at which Alvan Feinstein presented a paper on Factors Influencing Erythrocyte P^{32} Uptake by A. R. Feinstein, E. L. Gordon, W. F. Bethard, and L. O. Jacobson, Chicago, Illinois, June 11, 1952.

Jacobson, L. O.: Led discussion of a paper by Byron E. Hall of the Mayo Clinic on the Treatment of Nutritional Anemia at the joint sections on Gastroenterology and Proctology and Pathology at the American Medical Association Convention in Chicago, Illinois, June 12, 1952.

Scully, N. J.: Effect of Radiations on the Growth and Development of Plants. Conference on the Use of Isotopes in Plant and Animal Research, Kansas State College of Agriculture and Applied Science, Manhattan, Kansas, June 12-14, 1952.

Skok, J.: Utilization of C^{14} in Plant Research Studies. Conference on the Use of Isotopes in Plant and Animal Research, Kansas State College of Agriculture and Applied Science, Manhattan, Kansas, June 12-14, 1952.

Zirkle, R. E.: Mitotic Aberrations in Individual X-irradiated Cells.
Brookhaven National Laboratory, Long Island, New York, July 22, 1952.

Zirkle, R. E.: Aberrations following Proton Irradiation of Parts of Mitotic Cells. Brookhaven National Laboratory, Long Island, New York, July 24, 1952.

Jacobson, L. O.: The Plan of the Cancer Hospital in the Atomic Energy Program. Directors Meeting of the Atomic Energy Commission, Washington, D. C., June 27, 1952.

FACTORS INFLUENCING IRRADIATION TREATMENT OF
EXPERIMENTAL LYMPHOID TUMORS

E. Lorenz

ABSTRACT

The tumors used in these experiments were a transplantable mouse lymphosarcoma that grows at the site of inoculation and a transplantable guinea pig leukemia that, when inoculated subcutaneously, disseminates rapidly. Both tumors take 100 per cent, do not regress, and kill the host animals within 30 to 40 days. It was found that the growth of the lymphosarcoma in mice was affected profoundly following total-body irradiation, while treatment of the tumor alone had negligible effects on tumor growth. Radiation doses of 2000 r had no more effect on tumor growth than 400 r total-body exposure. These findings suggest a synergistic effect in tumor regression by irradiation. Experimental analysis of this synergistic effect indicates that 1) it is dependent on irradiation of the entire body rather than any one area or organ system; 2) it is maximal when both tumor and body are irradiated simultaneously; and 3) it is mediated through the blood stream since it can be prevented by clamping off temporarily the blood supply to the tumor during total-body irradiation. It seems probable, therefore, that the synergistic factor in total-body irradiation exists in the blood supply, and a modification of tumor radiosensitivity might be accomplished in local tumor irradiation by a chemical or physical alteration in the tumor blood and fluids. It could be shown that starvation of the animals for 20 hours before and during tumor irradiation, or keeping the animals in a high oxygen atmosphere prior to and during irradiation, resulted in a considerably greater tumor regression than in control animals. Modification of radiosensitivity was not evident when starvation followed irradiation, and there was a decreased sensitivity when the animals were kept in a low oxygen atmosphere.

It has been found recently that the lethal effects of irradiation may be largely prevented by the subsequent injection of bone marrow suspensions. This makes it possible to employ large doses of total-body radiation for the treatment of generalized lymphatic leukemia. Experiments of this type are now in progress.

PROGRESS REPORT: BIOLOGIC EFFECTS OF TOTAL-BODY IRRADIATION

I. MODIFICATION OF ACUTE IRRADIATION INJURY BY HETEROLOGOUS BONE MARROW INJECTIONS IN MICE AND GUINEA PIGS

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

It was previously reported⁽¹⁾ that heterologous bone marrow injections into mice from another mouse strain or from guinea pigs gave protection against lethal doses of irradiation. Additional experiments performed since then are listed in Table 1 with the earlier data.

It can be seen from this table that protection afforded by guinea pig bone marrow in mice is erratic. While in an additional experiment good protection was obtained in LAF₁ mice with guinea pig bone marrow, it offered no protection in other experiments, although in occasional animals that died following guinea pig bone marrow injections, recovery of the hematopoietic organs was apparent in comparison with untreated controls. Whether protection against bacteremia was insufficient, thus leading to death, is not known at present (see IV). In the two successful experiments the septicemia, for unknown reasons, may not have been severe enough to cause death. The injected guinea pig bone marrow probably does not stay alive beyond the fifth day as suggested by histologic examination of intraperitoneally injected bone marrow. If we have to deal with a humoral factor in the protective effect, it becomes apparent from this that homologous bone marrow is more effective than heterologous bone marrow. The homologous bone marrow not only stays alive but grows, providing the unknown factor in increasing quantities, while with heterologous bone marrow injections this factor decreases rapidly.

Since septicemia (according to our histologic studies) is absent in guinea pigs, heterologous bone marrow from a hybrid strain (expt. 16) and from family 13 (expt. 15) was injected into family 2 guinea pigs and good survival was obtained. An experiment is under way in which rabbit bone marrow was injected into guinea pigs of family 13, but no data are available at present.

LITERATURE CITED

1. Hollcroft, J., E. Lorenz, and H. Hunstiger. 1950. Effects of ionizing radiations on a transplanted lymphosarcoma. J. Natl. Cancer Inst., 11:1-16.

12

Table 1

EFFECTS OF HETEROLOGOUS BONE MARROW INJECTION ON RECOVERY FROM ACUTE IRRADIATION INJURY

Expt.	Dose (r)	Treatment	Strain	Number animals	Number dying on indicated number of days																% Mortality	Remarks
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16-21		
1	900	None	LAF ₁	38					2	6	4	5	9	6	4					2	100	
2	800	None	LAF ₁	20						2	1	2	4	10				1			100	
3	900	None	A	22		2	12		6	1		1									100	
4	900	None	C ₃ Hb	60				2	4	9	20	8	8	3	6						100	
5	900	IV Fam 2 GP BM	LAF ₁	10								1	1	4							60	
6	900	IV Fam 2 GP BM	LAF ₁	24							2	1	6	7	4	4					100	
7	900	IV Fam 13 GP BM	LAF ₁	29					3	3	2	4	7	7	2	1					100	
8	800	IV Fam 13 GP BM	LAF ₁	10									1	1	1	1					40	
9	800	IV Fam 13 GP BM	LAF ₁	10								1	1	3	1	3		1			100	
10	800	IV Fam 13 GP BM	A	9						4	1	4									100	
11	900	IV Dog BM	LAF ₁	40					13	8	3	4	6	5		1					100	
12	900	IV LAF ₁	C ₃ Hb	18					4	1		2	3		1						61	
13	550	None	Fam 2 GP	11									2	3	2	2			1		91	Last died 25th day.
14	750	None	Fam 13 GP	12								1	5	1	5						100	
15	550	IV Fam 13 GP BM	Fam 2 GP	4																	0	
16	750	IV Albino GP BM	Fam 13 GP	5									1			1				1	60	

II. MODIFICATION OF ACUTE IRRADIATION INJURY BY INJECTION OF TISSUES ORIGINATING FROM HEMATOPOIETIC ORGANS

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

In an attempt to study what cell or cells were responsible for recovery from lethal doses of X radiation after bone marrow injection, we have undertaken a series of experiments in which tissues originating in hematopoietic organs were used. These experiments are listed in Table 2.

In experiments 2-4 spleen brei (approximately 1/2 spleen per mouse) was used. The most effective protection was obtained with immature spleens and spleens that were obtained from animals 3 days after a total-body irradiation with 900 r with the spleen protected. Adult spleen gave less protection. This is to be expected, since the normal adult spleen of LAF₁ mice shows less hematopoietic activity than that of the immature animal. In a previous report⁽¹⁾ data were given showing that adult spleen brei gave no protection. It is probable that improved technique was responsible for the present findings. Since in the protected spleen erythropoiesis is mainly observed during the first few days, it may be thought that erythrocyte precursors have been responsible for the recovery. A reticulum cell sarcoma, however, also gave good protection in one group (expt. 7) and delayed death in another group (expt. 6). Intravenous injection of thymus brei gave indication of improved survival while there was none (expt. 10 and 11) when injections were intraperitoneal. Intravenous thymus brei injections had to be abandoned, because in spite of all precautions in the preparation, too many animals died within a few minutes after injection (not listed) of pulmonary embolism. Brei of a lymphosarcoma originating in the thymus gave no protection (expt. 9). Injection of leukemic blood intraperitoneally into irradiated guinea pigs of family 2 apparently gave no protection, for the leukemia developed so fast in the irradiated guinea pigs that they died before the lethal irradiation effects had fully developed (not listed). Tissue culture cells from immature mouse spleens, which grew luxuriantly and in which the cells looked like macrophages, gave no protection, as did the supernatant of the tissue cultures (expt. 13 and 14).

The conclusions which can be drawn from these experiments are that perhaps all hematopoietic precursors, including the reticulum cell sarcoma, may be effective per se in recovery from irradiation injury.

LITERATURE CITED

1. Lorenz, E., D. Uphoff, T. R. Reid, and E. Shelton. 1951. Progress report: Modification of irradiation injury in mice and guinea pigs. ANL-4625. P. 15.

11

Table 2

EFFECTS ON RECOVERY OF INJECTION OF TISSUES ORIGINATING FROM HEMATOPOIETIC ORGANS

Expt.	Dose (r)	Treatment	Strain	Number animals	Number dying on indicated number of days																% Mortality	Remarks
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16-21		
1	900	None	LAF ₁	38					2	6	4	5	9	6	4					2	100	Spleen stimulated by spleen protection 3 days prior to injection.
2	900	IV adult spleen	LAF ₁	9						1	1			1	1						44	
3	900	IV adult spleen	LAF ₁	16				1			3	1									31	
4	900	IV young spleen	LAF ₁	10											1	1					20	
5	900	None	BXD*	64					1	1	4	6	14	14	16	6	2				100	The early death was attributed to embolism. The early death was attributed to embolism.
6	900	IV Retic. cell sa.	BXD	10						1	1	2	3						1	2	100	
7	900	IV Retic. cell sa.	BXD	15					3	2						1		1			47	
8	900	None	A	22		2	12	6	1			1									100	
9	900	IV Lymphosarcoma	A	16				1		1	3	7	4								100	Tissue culture fluid from spleen culture.
10	900	IV Thymus	A	6	1	1					1										50	
11	900	IV Thymus	A	10		1	1	1	1	1	4		1								100	
12	900	IP Thymus	LAF ₁	20						1	1	1	1	8	2	5	1				100	
13	900	IV Culture fluid	A	10						5		3	2								100	Tissue culture fluid from spleen culture.
14	900	IV Spleen culture	A	20						1	5	2	7	4		1					100	

*F₁ homogeneous hybrid of a C-57 black and dba cross.

III. EFFECTS OF TOTAL-BODY IRRADIATION FOLLOWED BY BONE MARROW INJECTIONS ON LEUKEMIA IN GUINEA PIGS

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

The guinea pig leukemia carried in the family 2 guinea pigs is a true lymphatic leukemia. It takes 100 per cent and is lethal in approximately four weeks. It can be transplanted either by intraperitoneal or intravenous injections of blood or by subcutaneous transplantation of spleen tissue. The subcutaneous transplant usually grows very little and is found at autopsy to be hemorrhagic. The tumor is characterized by an ever increasing lymphocyte count, large spleen (lethal spleen weight 10 to 13 g), and invasion into lymph nodes, bone marrow, and other organs. Detailed histologic data will be given in a subsequent report.

Irradiation of the tumor bearing animals was carried out when the leucocyte count reached 25,000 to 50,000. Control animals transplanted at the same time were usually kept to study the progress of the disease. The first experiment consisted in giving a total-body dose of 550 r, which is lethal, followed by homologous bone marrow injection. After a regression of the leucocyte count to low values for several days, the leukemic blood picture reappeared. A few animals were given at that time a second dose of 550 r followed by bone marrow injection with the same results as after the first treatment.

In subsequent experiments daily doses of 200 r were given until the total cumulative dose reached 800 or 1000 r. The leukemic blood picture reappeared again, later in the 1000 r animals than in those exposed to 800 r, as shown in Figure 1. Finally, a group of four animals were given a total-body dose of 400 r daily until the cumulative dose reached 2000 r. This was followed by intravenous bone marrow injection. One of the animals died a few days after the treatment was finished with a large hemorrhage in the left hind leg in the region of the saphenous vein, which was used for injection of the bone marrow. This may have been the cause of death. The other three are living 45 days after the first treatment with a leucocyte count below normal, as shown in Figure 1. Since in normal animals the leukemic blood picture is apparent two weeks after transplantation, and in animals irradiated with 800 and 1000 r an elevated leucocyte count appears within three weeks after the beginning of the treatment, it may be that the regression of the tumor in the animals exposed to 2000 r is permanent. Additional experiments are in progress.

Figure 1

Leucocyte count in leukemic guinea pigs exposed to daily doses of X radiation.

IV. SEPTICEMIA IN IRRADIATED C₃H_b MICE AND ITS MODIFICATION BY BONE MARROW INJECTION

F. P. Williams, Jr.*† and C. C. Congdon†

Several bacteriologic studies have been carried out on irradiated mice, and Miller et al. have reviewed the literature.⁽¹⁾ The importance of bacterial infection in radiation-induced death in mice has been adequately demonstrated. In the 450 to 600 r range of total-body X irradiation it has been shown that postirradiation treatment with antibiotics, particularly streptomycin, will lower significantly mortality in mice.⁽²⁾

Our primary concern is with the tissue changes resulting from septicemia. The necessary preliminary bacteriologic studies have been made and the results are summarized in this report together with some preliminary observations on tissue changes. In addition, the bacteriologic and pertinent histologic findings (relevant to septicemia) on mice given a bone marrow suspension following total-body irradiation are summarized.

Table 3 shows the incidence of bacterial infection in mice killed at daily intervals after 900 r total-body X irradiation and the incidence of bacterial infection in a similar group of mice that received an injection of bone marrow following the irradiation. The results indicate that bone marrow injection was effective in reducing the incidence of bacterial infection as determined by our techniques. Histologic study on C₃H_b mice irradiated and treated similarly also showed the absence of septicemia in serially killed animals that would have been expected to recover as determined by the degree of hematopoietic regeneration. Mice killed and autopsied 30 days after 900 r total-body exposure and bone marrow treatment have never shown histologic evidence of bacterial infection.⁽³⁾

Tables 4 and 5 show the detailed data from which Table 3 was made. The occasional finding of bacterial growth in liver or kidney cultures in the absence of growth in the spleen and heart blood is of interest, although the possibility of contamination cannot be absolutely eliminated.

A comparison of Table 3 with data that have been published earlier on survival of C₃H_b mice after 900 r total-body X irradiation shows that 51 of 60 irradiated mice died between the 4th and 9th days inclusive.⁽⁴⁾ The remaining 9 mice were dead by the 11th day. This shows the importance of bacterial infection as a cause of death at this dose level as Miller et al. have shown for the 450 to 600 r dose range.

*Biologist with the National Institute of Arthritis and Metabolic Diseases.

†Associated with Dr. Egon Lorenz of the National Cancer Institute.

18
Table 3

INCIDENCE OF BACTERIAL INFECTION IN SERIALLY KILLED C₃H_b MICE FOLLOWING 900 r
TOTAL-BODY X IRRADIATION COMPARED WITH IRRADIATED MICE GIVEN BONE MARROW*

Days after Irradiation	0	1	2	3	4	5	6	7	8	9	10	11	12	Total no. of mice cultured
900r**	†	0	0	5	3	5	3	5	5	3	†	†	†	45
900 r followed by intravenous bone marrow**	0	0	0	1	3	2	2	0	0	0	0	0	0	65

*One group of 5 mice not irradiated and presumably normal was killed and the organs cultured. Bacterial growth was not found.

**The figures refer to the number of mice in the group of 5 killed each day showing bacterial infection.

†Animals were not killed and cultured on these days.

Table 4

TYPES OF ORGANISMS CULTURED FROM SEVERAL TISSUES OF C₃H₆ MICE KILLED
SERIALLY AFTER 900 r TOTAL-BODY X IRRADIATION

Days after irradiation*	Heart blood		Liver	Spleen	Kidney
	Blood agar	Sodium thioglycollate			
3	1 Col. Gram + cocci	**	**	Spore former	**
3	108 Col. Staph.	**	**	Proteus	Proteus
3	5 Col. Gram + cocci	**	**	**	Proteus
3	9 Col. Gram + cocci	**	**	**	**
3	1 Col. Gram + cocci	**	**	**	**
4	1 Col. Spore former	**	Coliform	**	**
4	**	**	**	**	**
4	2 Col. Spore former	**	**	**	**
4	**	**	**	**	**
4	21 Col. Gram + cocci	**	**	**	**
5	60 Col. Alcaligenes	**	Alcaligenes	Alcaligenes	**
5	18 Col. Alcaligenes	**	**	Alcaligenes	**
5	**	**	**	**	Pseudomonas
5	2 Col. Pseudomonas	Anaerobe	**	Pseudomonas	**
5	150 Col. Alcaligenes	**	Alcaligenes	Alcaligenes	Coliform
6	**	**	**	**	**
6	**	**	**	**	**
6	2 Col. Spore former	**	**	**	**
6	**	**	Pseudomonas	Pseudomonas	**
6	125 Col. Alcaligenes	**	Alcaligenes	Alcaligenes	**
7	100 Col. Alcaligenes	**	Alcaligenes	**	**
7	100 Col. Spore former	**	**	**	**
7	100 Col. Spore former	**	**	**	**
7	100 Col. Staph.	**	**	Alcaligenes	**
7	100 Col. Alcaligenes	Anaerobe	Alcaligenes	Alcaligenes	**
8	**	**	**	Staph.	**
8	1 Col. Proteus	**	**	Proteus	**
8	1 Col. Spore former	**	**	**	**
8	**	**	**	Coliform	**
8	**	**	Coliform	**	**
9	3 Col. Spore former	**	Coliform	**	**
9	**	**	Spore former	**	**
9	12 Col. Spore former	**	**	**	**
9	**	**	**	**	**
9	**	**	**	**	**

*All cultures were sterile for days 1 and 2 and were omitted from the table.

**Cultures sterile.

Table 5

10

TYPES OF ORGANISMS CULTURED FROM SEVERAL TISSUES OF C₃H₆ MICE
KILLED SERIALY AFTER 900 r AND INJECTION OF BONE MARROW

Days after irradiation*	Heart blood		Liver	Spleen	Kidney	Bone marrow
	Blood agar	Sodium thioglycollate				
3	**	**	**	**	**	†
3	**	**	**	**	**	†
3	**	**	**	**	**	†
3	**	**	Alcaligenes	**	**	**
3	**	**		**	**	**
4	**	**		**	**	†
4	1 Col. Spore former	**		**	**	†
4	17 Col. Proteus	**	**	**	**	†
4	1 Col. Spore former	**	**	**	**	**
4	**	**	**	**	**	**
5	3 Col. Pseudomonas	**	**	**	**	†
5	**	**	**	**	**	†
5	**	**	**	**	**	†
5	**	**	**	**	**	**
5	12 Col. Spore former	**	**	**	**	**
6	**	**	**	**	**	†
6	50 Col. Spore former	**	Spore former	**	Spore former	†
6	**	**	**	**	**	**
6	1 Col. Staph.	**	**	**	**	†
6	**	**	**	**	**	**

*All cultures were sterile for days 0, 1, and 2 and were omitted from table.

**Cultures sterile.

†Cultures not taken.

Quantitative study of the bacterial infection on heart blood culture given in Table 4 shows the greatest average number of colonies on the 7th day, which was the day on which the greatest number of C₃Hb mice died after 900 r as previously described.⁽⁴⁾

Many histologic examinations have been made on the tissue changes that occur with the septicemia in irradiated inbred mice of strains A, L, and C₃Hb and in the hybrid LAF₁. As in septicopyemia the bacteria may localize in any organ or tissue and produce changes in the surrounding parenchyma. Necrosis of the vessel wall leads to hemorrhage in some tissues. Cells adjacent to the vessel often show necrosis and the retrogressive changes preceding necrosis. Such a lesion in an animal with intact bone marrow would show marked leucocytic infiltration and the formation of a pyemic abscess. The abscess does not develop in the necrotic foci in X-irradiated mice. It seems apparent that the bacterial disease in mice begins as a bacteremia of intestinal origin that quickly progresses to a septicemia and nonpurulent septicopyemia.

Figures 2 and 3 show some of the histologic features of the nonpurulent septicopyemia.

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A

B

Figure 2 A

A focal lesion in the liver showing bacterial masses and some red blood cells in a dilated blood vessel. Bacteria are scattered among dead liver cord cells and in cellular debris beneath the vessel. Adjacent liver cord cells show pyknotic nuclei and vacuolated cytoplasm. Mag., 520 X (hematoxylin and eosin preparation).

Figure 2 B

Focal liver lesion showing many bacteria at the site of necrosis of the vessel wall in the upper right portion of the photomicrograph. Liver cord cells beneath the injured vessel wall show necrosis and retrogressive changes. Some bacteria are spread among the dead cells. Mag., 500 X (hematoxylin and eosin preparation).

A

B

Figure 3 A

Bacterial mass filling heart capillary. Minimal interstitial hemorrhage nearby. Hyalinization with loss of cross striations of three muscle fibers lying next to the bacterial mass. Mag., 270 X (hematoxylin and eosin preparation).

Figure 3 B

Adrenal and kidney showing two foci of bacterial necrosis in the adrenal. The relatively clear circumscribed area in upper left portion of the adrenal cortex shows darkened smudges of bacteria throughout. A large nonpurulent septicopyemic lesion destroys medulla and cortex in the lower right portion of the adrenal. Dark bacterial smudges are present. Darker areas to the left between intact medulla cells represent recent hemorrhage. Mag., 85 X (hematoxylin and eosin preparation).

PROGRESS REPORT: TUMOR RESPONSE TO VARYING TUMOR AND BODY DOSES OF X IRRADIATION

J. W. Hollcroft* and M. M. Mathews*

The role that total-body irradiation plays in the regression of a transplanted lymphosarcoma is a perplexing one.⁽¹⁾ The determination of the size of the body dose necessary to produce maximum response while the tumor dose is held constant might provide an important clue to the problem. Hence the following series of experiments was performed.

Strain A male mice, three to four months of age, bearing a transplanted lymphosarcoma were placed in lucite holders. The irradiation conditions were: 186 kvp and 10 ma; filter, 0.25 mm Cu plus 0.55 mm Al; target-middle-mouse distance, 25 cm. By fitting various copper and lead shields over the holders, the tumors of one group of mice were irradiated with 1000 r while the dose to the rest of the body was varied. Likewise, the bodies of another group were irradiated with 100 r while the tumor dose varied. In addition, the tumors of another group were irradiated with varying doses while the mice were shielded with 5 mm of lead, and a fourth group was given total-body radiation with either bone marrow protection or spleen protection.

When the tumor dose was kept constant at 1000 r a maximum response was noted with 50 r body doses (Figure 4 and Table 6). Greater doses delivered to the body did not increase tumor regression. Furthermore, with a 100 r total-body exposure a maximum effect was noted at 1000 r; no greater response occurred at 2000 r. The tumor-dose curve shows the same leveling at 2000 r.

In total-body exposures spleen protection enhanced tumor regression. Here again the curve levels off as the dose increases above 400 r. Total-body exposures without spleen or bone marrow protection do not appear as effective as those with protection. However, a different substrain of mice was used in this series of experiments. Although the total-body response seems to be greater than the response of 100 r to the body and 900 r to the tumor, the difference may not be significant when the steepness of the curve from 400 r to 1000 r (delivered to the tumor) is considered.

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*Associated with Dr. Egon Lorenz of the National Cancer Institute.

Figure 4

Effects of varying irradiation treatments on tumor regression.

Table 6

16

EFFECTS OF VARYING IRRADIATION TREATMENTS ON TUMOR REGRESSION

Group	Dose in r		No. of animals	$R = \frac{\text{minimum tumor size}}{\text{tumor size at treatment}} \times 100$	100-R
	Tumor	Body			
1	1000	0	19	59.4 ± 28.3	40.6
	1000	50	10	18.4 ± 16.3	81.6
	1000	103	19	21.4 ± 20.5	78.6
	1000	229	10	18.6 ± 17.1	81.4
	1000	380	20	13.4 ± 14.0	86.6
2	400	100	20	85.6 ± 17.0	14.4
	720	100	10	40.5 ± 25.7	59.5
	912	100	7	31.7 ± 19.6	68.3
	2000	100	15	23.5 ± 23.9	76.5
3	400	0	10	86.5 ± 13.0	13.5
	1000	0	19	59.4 ± 28.3	40.6
	2000	0	20	37.2 ± 25.7	62.8
	4000	0	20	41.9 ± 31.6	58.1
	6000	0	10	29.0 ± 26.0	71.0
4	100	100	10	99.4 ± 1.9	0.6
	200	200	10	92.3 ± 6.2	7.7
	300	300	10	81.1 ± 13.1	18.9
	400	400	18	55.4 ± 9.5	44.6
5 Bone marrow protection	100	100	10	99.5 ± 1.6	0.5
	250	250	10	72.5 ± 14.5	27.5
	400	400	10	43.5 ± 13.5	56.5
	600	600	22	18.0 ± 8.7	82.0
	800	800	13	8.7 ± 6.8	91.3
6 Spleen protection	100	100	8	89.0 ± 11.6	11.0
	250	250	8	42.7 ± 6.0	57.3
	400	400	26	20.7 ± 16.0	79.3
	600	600	19	12.7 ± 15.5	87.3
	800	800	16	3.4 ± 5.1	96.6

Neutron Radiobiology

Raymond E. Zirkle
 Howard H. Vogel, Jr.
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 Donn L. Jordan

PROGRESS REPORT: IRRADIATION OF MICE WITH FAST NEUTRONS
 AND GAMMA RAYS

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

During the past three months, in addition to 8 preliminary experiments, carried out to determine dosimetry and lethal ranges, we have completed 62 irradiations of CF#1 female mice as shown in the following table:

1. Fast neutron irradiations⁽¹⁾ (50 mice exposed in each experiment)

<u>Low level</u>	<u>Intermediate level</u>	<u>High level</u>	<u>Total</u>
(CP-3' at 157 kw)	(178 kw)	(188 kw)	
6 runs	7 runs	6 runs	19

2. γ irradiations⁽²⁾ (54 mice exposed in each experiment)

Settings of 18 Co⁶⁰ sources:

21.3 cm from mice (highest exposure)	22.6 cm	24.9 cm	25.9 cm	28.2 cm (lowest exposure)	<u>Total</u>
4 runs	4	3	3	5	19

3. Mixtures (50 mice exposed in each experiment)

50% fast neutrons - 50% γ rays 11 runs	1/3 neutrons - 2/3 γ rays 6 runs	2/3 neutrons 1/3 γ rays 3 runs	<u>Total</u>
			20

In addition to these 58 experiments, in which the irradiation periods were 90 minutes, four experiments have been completed in which mice have been irradiated over a 24-hour period (one γ run, two neutron exposures, and one mixture (50%-50%)).

During this period several miscellaneous fast neutron irradiations have also been carried out:

- 1) Several exposures of grasshoppers to fast neutrons have been completed in collaboration with Dr. Theodore Tahmisian of this division.
- 2) Some preliminary runs were carried out in collaboration with the Air Force Radiation Laboratory to test the effects of fast neutrons on the lungs of various mammals (mice, rats, and guinea pigs) brought to atmospheric conditions simulating high altitudes.
- 3) Preliminary dosimetry work was begun with Dr. Herbert Kubitschek, using his calorimetric measurements for fast neutrons.(3)
- 4) A "recovery experiment" with neutrons was carried out. Mice surviving a preliminary exposure of 40 to 70 neutrons were given a second exposure to the same dose of fast neutrons after a 70-day period.

EXPOSURES

The radiation chamber(2) has been stored in a garage for a two-week period and then driven, on its special trailer(2), to the CP-3' reactor where it remains for an alternate two-week period. Neutron exposures and mixture experiments have been carried out at the thermal column of CP-3', only during the night so as to cause the least disturbance to other investigators and their instruments when our shutter was elevated. Gamma exposures were run, however, during the day.

SURVIVAL CURVES

Complete survival curves are being constructed for these mice after irradiation. These will be published in later reports along with our results on additivity.(1)

Approximately 5000 mice have now been used as experimental and control animals.

CATARACT FORMATION

Several complete cataracts have already been found in CF#1 female mice exposed to fast neutrons:

Expt. No.	Animal No.	Eye	Time first seen as complete opacity	Approximate dose
PE ₁	403	RE	82 days	85 n*
PE ₆	1071	RE	59 days	82 n
PE ₇	1228	RE	62 days	54 n

DOSIMETRY

With the help of Dr. Harald H. Rossi, Assistant Professor of Radiology (Physics), Columbia University, one of our Victoreen condenser chambers has been calibrated in terms of reps (roentgens equivalent physical) when exposed to our fast neutron field. Factors have been obtained to relate the response of our other 10 chambers (25 r, 100 r, and 250 r) to this calibrated chamber in the field of fast neutrons. The response of the chambers to X rays can also be corrected to standard r through a secondary comparison with a standard free air chamber. Thus, after corrections for temperature and barometric pressure, the response of each chamber can be corrected to read our standard "n" (and hence reps) in the neutron field and to standard r by use of the appropriate chamber factor.

By use of films and a carbon chamber Dr. Rossi concluded that the γ contribution to the tissue dose in this neutron field is less than 10 per cent of the dose due to fast neutrons.

It was found by means of a Victoreen rate meter and condenser chambers that the intensity of the neutron beam steadily decreased from the center to the periphery of the animal cage. This variation was also evident in the γ field from the Co⁶⁰ sources, although to a lesser degree. The intensity at each animal position was determined and isodose groups established for both γ and neutron radiations. These groups are illustrated in Figures 5 and 6. The three isodose groups for γ -radiation experiments and the five isodose groups for fast neutron exposures are differentiated in the actual positions of the exposed mice. The black compartments shown in these figures contained no animals and were filled with plastic phantoms during irradiation.

The plastic exposure chamber in which our radiation experiments have been carried out contains three columns (A, B, and C), each with 11 rectangular compartments. Each compartment is subdivided by a diagonal lucite strip so that 66 exposure cells are formed, each of which can

*1 standard n (as measured by our group) = 1.89 reps.

	GROUP 1 (HIGH)	20 MICE
	2 (INTERMEDIATE)- 18 -	
	3 (LOW)	16 MICE
TOTAL: 54 MICE		

Figure 5

"Isodose" groups for γ radiation

	GROUP 1 (HIGH)	6 MICE
2	10 MICE
3	14 MICE
4	12 MICE
5 (LOW)	8 MICE
Turret:		
TOTAL: 50 MICE		

Figure 6

"Isodose" groups for neutron and mixed radiations

hold one mouse. Columns W, X, Y, and Z, shown in the figures, are dosimetry columns in which Victoreen condenser chambers can be inserted during an experimental run.

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PROGRESS REPORT: THE EFFECT OF SPLEEN SHIELDING ON
THE HEMATOLOGIC VALUES OF RABBITS FOLLOWING
TOTAL-BODY X IRRADIATION

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

A single dose of 600 r total-body X radiation, which is near the LD₅₀ for CF #1 female mice, regularly produces a severe anemia, leucopenia, and thrombocytopenia. (1) On the other hand, mice exposed to this dose of irradiation with spleen shielding develop no anemia and a leucopenia and thrombocytopenia of lesser severity and shorter duration. (2) Even with a dose of 1300 r, spleen shielding effectively increases survival and reduces the effect on the blood and blood-forming tissue below that expected of 600 r without spleen shielding. (3) Extension of the spleen shielding technique to rabbits revealed that the effect on survival and on the recovery of blood forming tissue was not as great as that noted in mice. In this report data are presented on the survival of rabbits and on the recovery of the hematologic constituents of the peripheral blood of rabbits exposed to 800 r total-body X radiation with spleen shielding. Data on the peripheral blood of rabbits exposed to 800 r with appendix shielding are added for comparison.

EXPERIMENTAL PROCEDURES

The experimental procedures have been described in detail previously. (4) Five groups of rabbits were studied. Group I consisted of 10 untreated control rabbits. Group II consisted of 9 operated control animals similar to the animals of groups III and IV but not exposed to X radiation. Groups III and IV (27 and 29 rabbits, respectively) were exposed to 800 r total-body X radiation, but during the irradiation the surgically exteriorized spleens of the rabbits in group IV were placed in lead shields* one-quarter inch thick. The surgically exteriorized spleens of the rabbits in group III were placed in gauze moistened with warm saline during irradiation. Group V (10 rabbits) consisted of animals exposed to 800 r total-body X radiation only. The hematologic studies included the following:

hemoglobin in grams per 100 cc (Evelyn photoelectric method)
erythrocytes per cu mm (Evelyn photoelectric method)
hematocrit in per cent (Kato micro method)
leucocytes per cu mm

* Designed by Mr. Albert Pearl of the Central Shops, Argonne National Laboratory. See ANL-4451, pages 30-31, 1950.

differential leucocytes
plasma platelet counts
reticulocytes (in per cent of erythrocytes)

RESULTS

Figures 7, 8, and 9 show, respectively, the results of the erythrocyte counts, hematocrit determinations, and the hemoglobin values. No apparent difference can be noted in the anemia that occurred in the three irradiated groups.

The platelet values are similarly reduced in the irradiated groups (Figure 10).

The reticulocyte values (Figure 11) are comparable in the irradiated groups through the first 12 days. By 16 days the reticulocyte values of the rabbits that had spleen shielding during irradiation are increased markedly as compared with the rabbits that received only X radiation and those irradiated with the spleens exteriorized but not protected.

The leucocyte values (Figure 12) in the irradiated groups are similarly reduced by 3 days. The one striking difference in the three groups is apparent between the 7th and 14th days when the rabbits with spleen shielding during irradiation fail to show the "abortive rise" commonly seen following exposure to single total-body doses. Recovery, however, proceeds in the same manner in all the irradiated groups after the 14th day. The lymphocyte (Figure 13) and heterophil values (Figure 14) parallel those of the leucocytes.

The 28-day survival of the rabbits with shielding of the exteriorized spleen (Figure 15) is 72 per cent as compared with 50 per cent survival for the animals with sham shielding of the spleen or animals irradiated without surgical manipulation.

DISCUSSION

These findings are of interest from the standpoint of the difference in species response to shielding or related techniques. First, it is obvious that the rapidity of recovery of blood-forming tissue and the enhancement of survival are spectacular in spleen-shielded mice after exposure to dosages up to and including 1300 r. As is obvious from the data presented on

Figure 7

Effect of 800 r total-body X irradiation on the erythrocyte values of rabbits with and without shielding of the exteriorized spleen during irradiation.

Average All Animals to Date July 1952
800 r Rabbits

Figure 8

Effect of 800 r total-body X irradiation on the hematocrit values of rabbits with and without shielding of the exteriorized spleen during irradiation.

Figure 9

Effect of 800 r total-body X irradiation on the hemoglobin values of rabbits with and without shielding of the exteriorized spleen during irradiation.

Average All Rabbits
800 r 1946 - July 1952

Figure 10

Effect of 800 r total-body X irradiation on the platelet values of rabbits with and without shielding of the exteriorized spleen during irradiation.

Figure 11

Effect of 800 r total-body X irradiation on the reticulocyte values of rabbits with and without shielding of the exteriorized spleen during irradiation.

Figure 12

Effect of 800 r total-body X irradiation on the leucocyte values of rabbits with and without shielding of the exteriorized spleen during irradiation.

Figure 13

Effect of 800 r total-body X irradiation on the lymphocyte values of rabbits with and without shielding of the exteriorized spleen during irradiation.

Figure 14

Effect of 800 r total-body X irradiation on the heterophil values of rabbits with and without shielding of the exteriorized spleen during irradiation.

Figure 15

Effect of 800 r total-body X irradiation on the survival of rabbits with and without shielding of the exteriorized spleen during irradiation.

rabbits in this report, spleen shielding exerts a minimal effect on 1) enhancement of survival, and 2) recovery of the hematologic constituents of the peripheral blood. Appendix shielding hastens recovery of the lymphocyte values of the peripheral blood somewhat more than does spleen shielding (Figure 16). Preliminary histologic study of spleen-shielded and appendix-shielded rabbits tends to bear out this latter finding, namely, that lymphatic tissue recovers more rapidly in appendix-shielded than in spleen-shielded rabbits. This difference in hematopoietic recovery between spleen-shielded and appendix-shielded rabbits is perhaps too slight to warrant comment. However, the appendix is more than twice the size of the spleen by weight, and this may account for the observed difference.

Of interest is the finding that the capacity of rabbits, with appendix or with spleen shielding, to produce antibodies in response to an intravenously administered particulate antigen is retained, whereas in animals without spleen shielding this capacity is suppressed.⁽⁴⁾ In fact, the capacity to produce antibodies to an injected antigen is retained in rabbits even though the originally shielded spleen is removed 24 hours after the shielding-irradiation procedure and the antigen is given 48 hours after the irradiation (24 hours after splenectomy).

The antigen was administered in the experiments referred to above (both in the experimental and control animals) when the (cellular) hematologic constituents of the peripheral blood were very low. Cell death and phagocytosis of hematopoietic tissue continued and no evidence of regeneration had appeared. Nevertheless, antibody formation was initiated in the spleen-shielded, splenectomized animals but not in the nonshielded animals.

More recently we have obtained preliminary data indicating that leaving the originally shielded spleen intact in the circulation for three hours after total-body X irradiation of the rabbit is sufficient to restore significantly the capacity to produce antibodies.⁽⁵⁾

These findings in rabbits when compared with data from mice indicate that the mechanism of recovery from radiation is probably the same in these two species. If early initiation of recovery of blood-forming tissue and antibody formation are related to a humoral substance or substances coming from the shielded tissue, then one must conclude that either the shielded tissue in the rabbit is not producing enough of the material to initiate an early widespread recovery or utilization of the material and recovery of tissue proceeds at a slower pace in the rabbit. Comparative studies on several species are indicated since such studies would be especially revealing if a specific cellular function, such as antibody formation, could be used as the criterion of recovery.

Figure 16

Effect of 800 r total-body X irradiation on the lymphocyte values of survivors with and without shielding of the exteriorized appendix.

CONCLUSIONS

The only apparent difference in the hematologic values of rabbits following 800 r total-body X irradiation with and without spleen shielding is the absence of the "abortive rise" in the leucocyte values between the 7th and 14th days following irradiation. Rabbits that had shielding of the exteriorized spleen during total-body exposure to 800 r X radiation have a 72 per cent survival as compared with 50 per cent for those without spleen shielding.

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Physiology

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TEMPERATURE DEPENDENCE OF THE PROTECTION OF
THYMUS CELLS BY CYSTEINE AND ANOXIA

M. E. Blackford and H. M. Patt

Cysteine has been shown to protect thymic cells against X irradiation; the degree of protection depends not only on the concentration of cysteine and the time of administration relative to exposure but also on the temperature of the cell suspension. (1, 2) Further experiments have been carried out using the methods described earlier. The more recent studies confirm temperature dependence and indicate that chilling of the suspension in an ice water bath (2°C) for 15 minutes either immediately before or after X irradiation largely diminishes the protective action. The effects of chilling become progressively less marked with time after exposure. For example, chilling is without appreciable effect 60 and 90 minutes after irradiation. (Figure 17).

As reported previously, oxygen deprivation affords comparable protection to thymic cells. (2) It also appears to depend on temperature. (Table 7). Protection is not seen when the cell suspension is chilled in an ice bath while being equilibrated with nitrogen. This is the case whether the equilibration period is 15 or 30 minutes. When the suspension is nitrogenated for 15 minutes at room temperature and chilled for a period of 15 minutes immediately after X irradiation, only partial protection is observed. The difference between chilling and no chilling either before or immediately after irradiation is statistically significant ($p < .01$).

There is reason to believe that the protective action of cysteine may be a consequence of hypoxia, or at least that a common mechanism is operating, since cysteine does not protect thymic cells in the absence of oxygen. The temperature dependence of the protective phenomenon observed for both cysteine and hypoxia suggests that mechanisms other than the immediate oxidative reactions induced by activated water may be involved. Temperature coefficients for protection with cysteine and anoxia are being determined.

Figure 17

Effect of chilling on the protection of thymic cells by cysteine.

Table 7

EFFECTS OF CHILLING ON PROTECTION AFFORDED X-IRRADIATED
THYMIC CELLS BY ANOXIA

No. of experiments	Time of nitrogenation*	Time chilled at 2°C*	Survival eosin- resistant cells at 22 hr (%)	
			<u>Nitrogen</u>	<u>Control</u>
13	-15		56	20.5
5	-15	-15 to 0	23	20
9	-15	0 to + 15	39	21

*Time in minutes relative to X irradiation with 200 r.

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PROGRESS REPORT: EFFECTS OF X IRRADIATION ON MAST CELLS

Y. S. Lewis and D. E. Smith

Since the tissue mast cells are considered to be a source of heparin, a series of studies was initiated to note the reaction of these cells to irradiation and the possible significance of this to the hemorrhagic syndrome following total-body exposures.

Whole mounts of the hamster cheek pouch and mesentery were examined after treatment with toluidine blue. Fifty-two hamsters were given total-body X radiation of 600 r (250 kv, 15 ma, 0.5 mm Cu and 3.0 mm Bakelite, and 217.14 r per minute), and animals were sacrificed at two- to three-day intervals. Nonirradiated but otherwise similarly treated controls were sacrificed and studied.

Within three to five days after irradiation, many of the mast cells exhibited specific cytoplasmic and granular alterations. The metachromatic granules conglomerated forming irregular masses within the cell, and the cytoplasm became distended with vacuoles. The cells were distorted and were very often held intact by fragmentary cell boundaries. Free mast cell material, i.e., granules and granular clumps, were to be found dispersed throughout the tissue. (Figures 18 to 21). Between seven to ten days, the changes were greatest. Cell counts on cross sections of the cheek pouch showed decided decreases in the numbers of mast cells present at this time. The number of abnormal cells appeared to start tapering off at about 12 to 14 days and the normal picture was restored at 30 to 35 days at which time cell counts in cheek pouch cross sections from irradiated and control hamsters were the same.

Similar abnormal mast cells have been noted after other treatments and have been considered to represent secretory activity.^(1,2,3)

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Figure 18

Hamster cheek pouch of control
animal. Toluidine blue. Mag. 750 X.

Figure 19

Hamster cheek pouch seven days
postirradiation. Toluidine blue.
Mag. 750 X.

Figure 20

Hamster cheek pouch seven days postirradiation. Note the conglomeration of granules and degree of vacuolization. Toluidine blue. Mag. 1800 X.

Figure 21

Hamster cheek pouch seven days postirradiation. Note the free mast cell material and granule scattering. Toluidine blue. Mag. 1800 X.

DESCRIPTION OF A TECHNIQUE FOR ULTRA-THIN SECTIONING
FOR ELECTRON MICROSCOPY

B. W. Massey

ABSTRACT

The preparation of ultra-thin tissue sections for electron microscopy has posed a major problem. Neither the method of Pease and Baker⁽¹⁾ nor of Newman et al.⁽²⁾ has proved to be routinely reliable in this laboratory, and, hence, the best features of each have been combined and modified to devise a technique that appears to be effective and highly dependable.

Fixation is accomplished by perfusion of the whole animal with neutral formalin or alcohol formalin, and the pieces that are to be examined are immersed in neutral osmium tetroxide. The embedding medium is a mixture of equal parts of n-butyl and ethyl methacrylate polymerized by ultraviolet light. Sectioning is done by means of a glass knife on an International ultra-thin microtome set at 0.1 micron. The sections are floated on warm water to spread and then placed on Formvar-coated grids, dried, and put into toluol to dissolve the plastic. The specimens, which are then ready for study, are ultra-thin and have only minimum distortion.

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Radium Toxicity

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PROGRESS REPORT: CLINICAL ASPECTS OF THE
RADIUM TOXICITY PROGRAM

During the past quarter, a 65-year-old woman was referred to the group by her physician in Chicago for a determination of radium content because of an unexplained necrosis of the head of her left femur. Determinations by γ -ray and expired radon method revealed a total-body content of $6.8\mu\text{g}$. The patient is unaware of having ever been administered radium, either parenterally or orally, but the history would indicate that she possibly received radium during hospitalization in Chicago in 1925. Roentgenography reveals the previously described minimal lesions of bone resorption in the tibia, fibula, radius, and ulna, and a marked destruction of the head of the left femur and the left acetabulum.

Previous studies by Dr. Andrew Stehney and his group indicate that the drinking water collected from deep wells in the Lockport-Joliet area is higher in radium content than Lake Michigan drinking water (the source of Chicago's water supply) by at least a factor of ten. Arrangements have been made to carry out clinical studies on several thousand inmates of the Stateville Penitentiary if pilot studies, using the exhaled radon and total-body γ -ray method, reveal a significant accumulation of radium in the bodies of inmates who have been confined in the penitentiary and hence have drunk only this water for periods of twenty to forty years.

Experimental Pathology

Miriam P. Finkel
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PROGRESS REPORT: TOXICITY OF Sr^{89} IN MICE. DESIGN OF
EXPERIMENT AND SURVIVAL FOLLOWING SINGLE INJECTION

M. P. Finkel, A. M. Brues, and H. Lisco

In order to gain some knowledge of the acute and latent toxicity of a bone-seeking fission product with high carcinogenic potencies, approximately 3000 mice were injected with strontium 89.* One-third of the mice received a single injection, one-third received monthly repeated injections, and one-third served as controls. The total experiment was divided into five equal and identical parts, each started at a different time within a four-month period. The mice employed were CF #1 females obtained from Carworth Farms and ABC male and female hybrid mice supplied by the Roscoe B. Jackson Memorial Laboratory. These three groups were equally represented within the experiment.

The animals were received in the laboratory at five to six weeks of age and were injected about a month later. The radiostrontium was given intraperitoneally as SrCl_2 in normal saline at neutral pH. The control animals received similar injections without radiostrontium. Moribund animals were sacrificed and all animals were examined after death for gross evidence of pathologic change; tissues were taken for histological study when necessary. Total-body roentgenograms were made in all cases.

A re-evaluation of the amounts of strontium 89 injected into the singly treated animals has been completed. Because of errors in injection schedules and in estimates of the activity of the injection solutions, it was found that the five replicates could not be combined. These and other errors became evident when the β emanations from each animal were counted by placing the animal, confined in a cellulose acetate container, under a Geiger-Müller tube at 20 days and again at 129 days after injection. The discharges per second were converted to total contained microcuries by means of factors that had been determined by correlating the external counts and ash counts of a large series of animals. This method of estimating the radiostrontium content of living mice proved to have an error of about ten per cent.⁽¹⁾ An estimate of the strontium injected into each animal was derived by making the necessary corrections for excretion and radioactive decay

*The Sr^{89} solution was slightly contaminated with Sr^{90} .

from twenty days to the day of injection. The dose in terms of μc per g was calculated individually with the body weight of each animal on the day before injection.

The doses, which ranged from 0.01 to 8.7 μc per g, were arbitrarily divided into seven groups and the animals arranged accordingly (Table 8). It is felt that this distribution of the 1056 animals that received a single injection of radiostrontium is sufficiently accurate to warrant further analysis of the experiment.

Table 8

DESIGN OF SINGLE DOSE EXPERIMENT

Group	Dose ($\mu\text{c/g}$)		Number of animals			
	Range	M	CF#1 F	ABC F	ABC M	Total
1	7.3 -8.7	7.8	1	6	4	11
2	3.8 -6.8	5.0	9	12	17	38
3	1.3 -3.7	2.5	21	22	18	61
4	0.8 -1.2	1.0	13	23	21	57
5	0.4 -0.7	0.5	42	52	65	159
6	0.1 -0.3	0.2	124	130	118	372
7	0.01-0.09	0.05	142	110	106	358
8	control	0	320	320	322	962
Total			672	675	671	2018

Examination of the survival of the control animals showed that the ABC females lived the longest, the ABC males fell behind the females after 400 days, and the percentage of survival among the CF#1 females after 200 days was consistently seven per cent lower than that of the ABC females (Figure 22). The differences in life expectancy between the ABC males and females were maintained throughout; after 600 days the CF#1 females and ABC females showed similar life expectancies (Figure 23). In contrast, a comparison of the survival of the ABC males and females at the various dose levels does not demonstrate greater survival of the latter. In fact, in all dose level groups but one the ABC males equalled or surpassed the survival of the ABC females. The nature of the control ABC male survival curve may be a function of the number of animals in a cage at a given time and the pugnacious habits of these animals. The slightly shorter life span of the control CF#1 females was consistent but not striking. Since the difference was so small and since greater validity is gained with larger numbers, the three kinds of mice were combined for further analysis of the survival of treated versus control animals.

59

Figure 22

Survival of control mice.

Figure 23

Expectation of life of control mice.

The times to various mortality percentages are plotted against dose in Figure 24. The dotted lines represent the control data. Doses of $0.5 \mu\text{c}$ per g and lower had no effect on survival, but the duration of life was shortened progressively with increasing doses above $2.5 \mu\text{c}$ per g. The results following the administration of $1 \mu\text{c}$ per g are equivocal.

Figure 24

Times to various mortality percentages plotted against dose. The dotted lines represent the control data.

At the present state of the analysis of this experiment the following conclusions can be drawn:

- 1) The survival curves of the ABC male and female mice were similar through 400 days; after that time the curve for the males fell more rapidly than that for the females. This difference in survival after 400 days may be due to fighting among males caged together.

- 2) CF#1 female mice have a somewhat shorter life span than ABC hybrid females.

3) Doses of 2.5 μc per g and more of radiostrontium significantly decreased the survival of both strains of mice.

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THE FATE AND PATHOLOGIC EFFECTS OF PLUTONIUM METAL IMPLANTED INTO RABBITS AND RATS

H. Lisco and W. E. Kisielewski

ABSTRACT

Plutonium metal, when implanted subcutaneously into rabbits and rats, disintegrates into many small fragments soon after introduction into the tissues. The pieces were found to be relatively inert locally, and only small amounts of plutonium were absorbed from the implants during an observation period extending up to 1048 days. The implantation sites became heavily calcified in all animals (7 rabbits and 3 rats).

Retention, organ distribution, and excretion studies gave evidence that rabbits absorbed more than rats. The majority of the animals appeared to suffer no ill effects from the local implants or from the absorbed plutonium. One rat died with an osteogenic sarcoma of the spine produced by the deposition of plutonium in the skeletal tissues. The data indicate that both species can harbor considerable quantities of unmetabolized plutonium in the subcutaneous tissues without showing evidence of this in the excreta.

Special Problems

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PROGRESS REPORT: BLOOD PRESSURE CHANGES IN
X-IRRADIATED CHICKS

S. P. Stearner, E. J. B. Christian, and J. F. Thomson

In mammals hypotension has been demonstrated as one of the early reactions that follows X irradiation.⁽¹⁻⁴⁾ The initial response to exposures at rates above ten r per minute is more profound in the bird and has been postulated to be associated with marked changes in blood pressure. The newly hatched chick is normally hypotensive, and at three to four days after hatching the mean pressure in the left ventricle is less than 30 mm Hg. A similar condition has been reported in the newborn of certain mammalian species.^(5,6) At birth the rabbit has a systolic pressure (left ventricle) of 30 to 40, while in the newborn dog it is about 50 mm. The adult level for the dog is not reached for about six weeks and in the rabbit about two months are required.^(7,8)

Three- to four-day white leghorn cockerels were exposed to single doses of 1000 r total-body X radiation at 43 r per minute. Mean blood pressure was determined by direct puncture of the left ventricle with a 22-gauge needle connected to a saline mercury manometer. The lower pressures were measured against a carbon tetrachloride column and values converted to mm of mercury.

The changes in mean blood pressure that followed X irradiation and their correlation with the physiological condition of the bird are shown in Table 9. Animals have been grouped according to estimated physical condition and severity of gross pathology instead of time after treatment because of the difference in speed of response following exposure. This classification has reduced variation within each group. A fall in mean ventricular pressure occurred within one-half hour after exposure, although there was no depression of normal activity at this time nor was any gross pathology seen at autopsy. As the chicks became very inactive (at least three hours after irradiation) there was a marked drop in blood pressure. Such animals showed severe multiple hemorrhages in the viscera (especially in the liver, proventriculus, and kidneys), ascites, and generalized edema; and urate crystals in the kidneys were occasionally seen. Birds in this condition usually died within two hours after irradiation.

65

Table 9

BLOOD PRESSURE CHANGES IN 3- to 4- DAY CHICKS
FOLLOWING X IRRADIATION

	Control	1000 r, 43r/minute		
Physical condition	active	active	slightly inactive	marked depression (very inactive)
Time after X ray: Average Range	-- --	1 1/4 hr 1/2 - 2 hr	2 hr 1 - 3 1/2 hr	3 1/2 hr 3 - 4 hr
Gross pathology		negative	Few petechiae in viscera, esp. kidneys.	Ascites, severe hemorrhage in liver, kidneys and proventriculus. Urates in kidneys occasionally seen.
Mean blood pressure (mm Hg)	28.9	23.5	20.3	11.0
% of control mean	--	81.3	70.2	38.1
Number of animals	47	35	30	25

Factors contributing to the rapid and pronounced fall in blood pressure include a decrease in circulating blood volume. This is indicated by the accumulation of ascitic and edematous fluid and confirmed by increased hematocrit values. The effective circulating volume is further decreased by the extensive hemorrhaging that is apparent within three to four hours after exposure. Further investigation is required, however, to determine the importance of the numerous and more subtle factors that have been reported to influence postirradiation hypotension in mammals.

The relation between observed blood pressure changes and early X-ray-induced death is not clear. The more precipitous fall in pressure does not occur soon enough after irradiation to indicate that it is a primary response, but it does occur some time before death and must be assumed to be an important contributing factor.

SUMMARY

The normal blood pressure (mean left ventricular) of three- to four-day chicks is less than 30 mm Hg. Within one-half hour after X irradiation there was a 20 per cent fall, although at this time there was no clinical evidence of radiation damage. A marked drop (to less than one-half of normal) was seen within three to four hours and was associated with marked depression, ascites, and extensive hemorrhages in the viscera.

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PROGRESS REPORT: THE EFFECT OF HIGH ENERGY NEUTRONS
ON THE EGG NUCLEI OF GRASSHOPPER NYMPHS

T. N. Tahmisian, A. M. Brues, and H. H. Vogel, Jr.

^{2/10} Eggs of Melanoplus differentialis nymphs ^{and imago} as well as those of the imagoes have the capacity to synthesize deutoplasm at a very high rate. An egg cell that is originally 35 to 50 μ in diameter increases to 3500 x 1000 μ in diameters after deutoplasm deposition. During this process the ovary and the ovariole are infiltrated with extensive anastomoses of tracheae and tracheoles, indicating that deutoplasm formation is dependent upon a rapid oxygen-carbon dioxide exchange, which, in turn, is evidence of a high rate of energy metabolism.

In several earlier communications the sequence of irradiation damage to the metabolic processes of the cell and to cell division and cell differentiation were discussed.^(1,2) Functional differentiation and anabolism were found to be highly susceptible to X irradiation. Since both of these phenomena are inherent in egg formation in fourth and fifth instar nymphs and in the imagoes, the animals were used to study the effects of fast neutrons in dosages ranging from 5 n* to 57.6 n.

~~X~~ High energy neutron bombardment above 21.4 n was found to inhibit deutoplasm deposition.³ Dosages of lesser magnitude resulted in total inhibition of deposition in some ovarioles, but in others the primordial oocytes divided but did not differentiate to a degree necessary to deposit yolk. In the latter case, the ovariole was filled with a large number of oocytes rather than one, which is typical.

Because grasshopper eggs in ovario are highly susceptible to X irradiation as well as to neutron bombardment we hope to obtain a roentgen-neutron ratio of effectiveness, and attempts will be made to use these eggs as biological irradiation "meters."

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*1 standard n (as measured by our group) = 1.89 reps.

Microbiology

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ANTIBIOTIC SENSITIVITY OF KAPPA AND THE INFLUENCE OF
X IRRADIATION ON THE PRODUCTION OF
AUREOMYCIN-RESISTANT "KILLERS" IN
P. AURELIA, 51, VIII KK

C. F. Ehret and E. L. Powers

ABSTRACT

The effect of chloramphenicol, terramycin, and aureomycin in eliminating kappa particles from the cytoplasm of "killer" animals in Paramecium aurelia 51 VIII KK has been studied as a function of antibiotic concentration and duration of exposure in lettuce-Aerobacter medium. With reference to relative toxicity toward Paramecium, aureomycin was most efficient in eliminating kappa, and chloramphenicol least efficient. In contrast to Brown's results⁽¹⁾ it was difficult to obtain permanently sensitive animals with chloramphenicol, even the most effective conditions (1.28 mg/ml for 24 hours) resulting in deleterious sequelae. Aureomycin and terramycin were both effective in eliminating kappa in the range of 0.04 to 0.16 mg/ml, with 24-hour exposures, but terramycin was more toxic to Paramecium. The most efficient conditions for aureomycin were 0.08 mg/ml for 24 hours after which all animals tested remained sensitive to paramecin. Although kappa particles spontaneously resistant to aureomycin were not detected, the literature on antibiotic resistance suggests that a low spontaneous frequency of change might be increased to the level of detection by X irradiation. On such an assumption, one portion of a killer clone was irradiated with 6900 r, the other portion was retained as nonirradiated controls. Both groups were exposed to aureomycin (0.08 mg/ml for 28 hours). Each group was divided into 20 aliquots averaging 100 animals per aliquot. After a 10-day incubation period, animals were re-exposed to aureomycin, reincubated, and then tested for the presence of kappa by killing tests. None of the nonirradiated animals retained kappa; all were sensitive. Among irradiated animals clear-cut evidence of the presence of kappa was not immediately evident, for most of the animals were killed by normal killers; however, death within clones and killing of 186 VII KK sensitives indicated the probable presence of kappa in 15/20 aliquots, verified by two to eight separate killing tests, some after a third passage through aureomycin. That some of the death observed was correlated with postirradiation second and third autogamies was indicated by incomplete survival observed in isolation cultures. However, quantitative and qualitative aspects of most of the killing observed clearly implicate paramecin as

the major agency of death. Only one of the induced-killer cultures showed killing in all tests; in isolation, this too yielded sensitives and self-killing also continued. The results may be interpreted to indicate the presence of kappa particles resistant to aureomycin, but having an unusually low fission rate ($\leq 2/\text{day}$).

The number of "aureomycin-resistant" kappa particles present in each aliquot just after irradiation may be estimated from

$$P_0 = e^{-m}$$

where P_0 is the fraction of aliquots containing no kappa particles and m is the mean number of "resistant" particles per aliquot. The observed fraction 0.25 indicates an average 1.39 kappa particles per aliquot. Taking into consideration the fact that X rays inactive kappa, one can calculate a frequency of induction for aureomycin-resistant kappa of the order of 10^{-8} per r per original kappa exposed, assuming an original concentration of 10^3 particles/animal.

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PROGRESS REPORT: THE RADIOSENSITIVITY OF AUXIN BIOSYNTHESIS

R. P. Weber and S. A. Gordon

It has been shown previously that growth hormone production in the plant is sharply curtailed by low doses (25 to 100 r) of ionizing radiation. The enzyme that converts the immediate precursor of auxin, presumably indoleacetaldehyde, to the auxin, indoleacetic acid, appears to be the major locus of radiosensitivity.^(1,2)

Since pure indoleacetaldehyde was not available for the experiments referred to above, neutral fractions of cabbage ether extracts were used as a crude source of the aldehyde. A recent report by Jones *et al.*⁽³⁾ indicated, however, that the principal neutral component with auxin activity in cabbage is indoleacetonitrile. To clarify whether the nitrile rather than the aldehyde was the substrate of the radiosensitive enzyme, we re-examined the cabbage neutral material. We found that 1) the cabbage neutral component is inactivated by the aldehyde specific reagent "dimedon," 2) synthetic indoleacetonitrile* does not react with "dimedon," and 3) reactivities of the synthetic nitrile and the neutral fraction from cabbage to the auxin-forming enzyme are dissimilar. These observations indicate that the cabbage neutral activity was not caused by the nitrile but by the aldehyde.

Indoleacetic acid is considered to arise from tryptophan as the primary precursor:

We have shown previously that conversions I to III, I to IV, and III to IV occur in the mung bean plant.⁽⁴⁾ The remaining enzymatic sequences are being investigated to determine whether the radiosensitivity of transformation III to IV is relatively unique in the over-all reaction. Infiltration of tryptamine into mung bean tissues and incubation of the amine with cell free extracts

*Synthesized by Dr. Robert E. Stutz.

indicate that this plant, unlike the pineapple and grass coleoptile, is unable to utilize tryptamine as an intermediate in auxin formation. Indolepyruvate is now being synthesized to study the alternative pathway of the sequence.

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ETHYLENEDIAMINE- A CARBONATE-FREE ALKALI
FOR CO₂ ABSORPTION

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Complete recovery of purified CO₂ from "wet" oxidations, acidified carbonates, and from other reactions has been obtained in a vacuum apparatus by the use of a proper sequence of cold traps and heated reaction tubes.⁽¹⁾ However, in many reactions that yield CO₂, gaseous impurities are evolved which, like CO₂, do not condense in a Dry Ice trap but only at the temperature of liquid nitrogen. Most of these are removed best by trapping the gas mixture in alkali and subjecting the alkaline carbonate to a vacuum before acidifying to release the CO₂. Despite careful preparation and handling, solutions of alkali usually contain measurable quantities of carbonate.

A simple and effective method of eliminating the undesired blank is to select an alkali that can be separated from its carbonate by distillation. Some amines such as ethanolamine and diethanolamine, which are used industrially for CO₂ scrubbing,⁽²⁾ are not sufficiently alkaline to prevent the release of some CO₂ during distillation. Furthermore, these compounds have relatively low volatility. Ethylenediamine, a more alkaline and more volatile compound, can be readily purified by vacuum distillation.

Figure 25 shows the partial pressure of CO₂ (32°) which equilibrates with a 20 per cent solution of the base at various stages of neutralization. It is apparent that the highest purity may be obtained by distilling small quantities of ethylenediamine from a sizeable volume of the commercially available compound. Also, it is obvious that only one of the two equivalent amino groups can be relied on to bind carbonate firmly, as would be anticipated from the pH neutralization curve.⁽³⁾

Ethylenediamine (Eastman, 95 to 100 per cent) and water are distilled from reservoirs on the vacuum manifold and frozen into a vessel fitted with a stopcock. The stopcock is closed and the mixture warmed to room temperature. Evacuation then removes neutral and alkaline gases. Subsequent acidification with H₂SO₄ followed by heating releases CO₂ quantitatively. With an excess of water HCl, present as a contaminant, is not evolved in measurable quantities, but SO₂, being a weaker acid, is not retained upon acidification. Other acid gases probably behave as these do, their retention depending upon their acid strength.

Figure 25

Equilibrium partial pressure of CO₂ in 20 per cent ethylenediamine in water at 32°.

The measured blank of vacuum distilled ethylenediamine was 0.001 per cent of its CO₂ binding capacity. To test completeness of recovery five purified CO₂ samples were measured manometrically and then absorbed in a tenfold excess of a 1:4 ethylenediamine-water mixture. After evacuation the samples were acidified with a twofold excess of 50 per cent H₂SO₄, heated to about 80°C, and the evolved CO₂ collected and again measured. The mean recovery was 99.8 ± 0.2 per cent (standard error). When the solutions were heated or first heated and allowed to cool before collecting the CO₂, the recovery was low, usually about 2 to 4 per cent.

It is suggested that ethylenediamine, because of the ease of purification, might find wide usefulness as an absorbing agent in chemical and radioactive analyses requiring the collection of CO₂.

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PROGRESS REPORT

Indolyl-3-pyruvic acid (IPA) has been prepared by using the general procedure of Ellinger and Matsuoka⁽¹⁾ as modified by Berg.⁽²⁾ Indolyl-3-carboxaldehyde was condensed with hippuric acid in the presence of acetic anhydride and sodium acetate to give an azlactone that was hydrolyzed with 40 per cent sodium hydroxide to give the sodium salt of IPA. While it is not too difficult to obtain reasonable yields of crude IPA, losses in purification are high because of the instability of IPA. The use of this material in plant test experiments will be reported by Gordon and Weber at a later date.

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Physical Chemistry

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PROGRESS REPORT: MECHANISMS OF REVERSAL OF TOXICITY OF
BERYLLIUM, BORON, AND URANIUM

Earlier studies on the reversal of beryllium-induced inhibition of plasma alkaline phosphatase by aurin tricarboxylic acid (ATA), and salicylic acid, and sulfosalicylic acid have been continued. The relationship of chemical structure to capacity to effect reversal has been investigated in more than 65 analogous compounds. As a result of this work it now appears certain that the primary requisite of any substance used to reverse the toxic effects of beryllium must be the possession of functional groups capable of chelating with beryllium ions.

The stability of complexes of beryllium with salicylic and sulfosalicylic acids has been investigated. These complexes were found to be less stable at lower concentrations with respect to ability to reverse alkaline phosphatase activity than was the Be-ATA complex. This is thought to be a reflection of fundamental differences in the dissociation constants of the enzyme-compound complexes.

A fuller report of this work is now being prepared for publication.

Salicylic acid injected into mice 1 or 4 hours, but not 8 hours, after the injection of an LD₉₅ of beryllium sulfate prevents death in approximately 75 per cent of the animals. Sulfosalicylic acid similarly protects Be-poisoned animals when injected 1 hour, but not 4 or 8 hours after the Be. In order to explain this difference the excretion and tissue distribution of radioberyllium is being studied following the injection of these substances either 1, 4, or 8 hours after Be⁷ injection using two levels of carrier beryllium sulfate -- a nonlethal amount of 0.3 mg Be per kg and 0.7 mg Be per kg (LD₉₅).

The use of complexing agents to reduce the toxicity of boron in mice has been unsuccessful so far (Table 10). Female CF#1 mice were injected intraperitoneally with 550 mg boron per kg (LD₅₀ = 400-500 mg boron per kg) in an aqueous solution of boric acid adjusted to a pH of ~7.3 with sodium hydroxide. The lack of protection is ascribed to the fact that a pH of about 9 or greater is necessary to obtain the maximum complexing action with the compounds studied.

Table 10

28

**EFFECT OF DIFFERENT SUBSTANCES ON THE SURVIVAL OF MICE ACUTELY
POISONED BY AN INTRAPERITONEAL INJECTION OF BORIC ACID**

Mg B/kg	Treatment					No. mice	Survival (%)	Time to 50% death (hr)	Mean time of death (hr)
	Substance	Dose (mg/kg)	No. of inj'ns	Time	Route				
550	--	--	--	--	--	20	0	4.20	7.58
550	α -hydroxy butyric acid	210	1	Immediately before boron	I.V.	10	10*	4.15	5.70
550	Salicylic acid	600	1	10 minutes after boron	I.P.	10	0	1.67	2.27
550	Sulfosalicylic acid	1600	1	10 minutes after boron	I.P.	10	0	3.43	4.12
550	Catechol disulfonic acid	700	1	10 minutes after boron	I.V.	10	10	17.58	15.01
550	Catechol disulfonic acid	860	1	Immediately before boron	I.P.	10	0	4.58	6.95
550	Catechol disulfonic acid	4140	5	Immediately before B and hourly there-after	I.P.	10	0	~ 15.5	~ 15.
None	Catechol disulfonic acid	4260	5	Hourly	I.P.	10	100	--	--

*Survivor moribund and sacrificed at 36 hours.

Injection of rats with single or multiple doses of either salicylic acid or sulfosalicylic acid or a single dose of aurin tricarboxylic acid 30 minutes after the injection of an approximate LD₉₅ of uranyl nitrate did not significantly increase their survival.

A compilation of beryllium toxicity data for mice has been made (Table 11).

Table 11

BERYLLIUM TOXICITY DATA

BeSO₄, aqueous solution, injected intravenously into
female CF#1 mice

Dose of Be (mg/kg)	Mice (No.)	Survival at 14 days (%)	Approximate time to 50% death (days)
0.48	10	90	
0.52	18	39	5.5
0.56	18	17	3.5
0.60	18	11	2.5
0.64	18	6	2.5
0.68	18	0	2.0
0.70	410*	2.48	2.26
0.85	10	0	1.8
1.00	20	0	1.8
1.30	15	0	1.5

*Includes 90 mice reported by Finkel and White, Proc. Soc. Exptl. Biol. Med., 1952, 79:672.

PROGRESS REPORT: SOME HORMONAL FACTORS GOVERNING THE
RESPONSE OF RAT LIVER TRYPTOPHAN PEROXIDASE-OXIDASE
TO TOTAL-BODY X IRRADIATION

J. F. Thomson and E. T. Mikuta

The increased activity of rat liver tryptophan peroxidase-oxidase (TPO) following 1000 r total-body X irradiation occurred in hypophysectomized as well as in normal rats.⁽¹⁾ Further studies have shown that the magnitude of the radiation-induced increase diminished with increasing intervals between hypophysectomy and exposure, an observation presumably referable to progressive atrophy of the adrenal cortex. This point is currently under investigation.

That the adrenals are involved in the activation of this enzyme system after radiation is beyond question, since the increase following X-ray exposure is completely prevented in adrenalectomized rats. Adrenal demedullation was previously found to diminish but not inhibit entirely the effect of irradiation on TPO.⁽¹⁾ The adrenal medulla seems to be of dubious importance, since pretreatment of rats with drugs that are known to alter the pharmacologic effects of epinephrine or nor-epinephrine, e. g., priscoline, ephedrine, cocaine, and tetraethylammonium chloride, failed to affect consistently the response of TPO to X rays; these compounds by themselves produced an increase in enzyme activity. The same situation obtained in the case of other drugs acting on the autonomic nervous system. It is likely that the diminished effect of radiation observed after medullectomy was attributable to the removal of appreciable amounts of cortical tissue. Similarly, decreased adrenal cortical function is a likely reason for the diminution in demedullated rats of the TPO response to the medullary secretions, epinephrine, and nor-epinephrine.⁽¹⁾

"Adaptation" to tryptophan⁽²⁾ was strikingly diminished in hypophysectomized rats, 2 g per kg of dl-tryptophan evoking only a 4-fold increase in activity, in contrast to the 15-fold rise seen in normal rats. Since the increase after tryptophan injection is also pronounced in adrenalectomized and adrenal-demedullated animals, the lessened response seen after hypophysectomy would seem to be referable to some role of the anterior pituitary other than its adrenocorticotrophic function. It was noted incidentally that this dose of tryptophan produced an average decrease of 4.7°C in the body temperature of normal rats. Spinal cord section at C₆, which also lowers body temperature, increased TPO to about 5 times the normal assay. Exposure of normal rats to 3°C for 6 hours failed to alter the TPO activity.

Intramuscular injection of 17-hydroxycorticosterone-21-acetate (Compound F) produced a marked increase in TPO in rats. Both hypophysectomized and adrenalectomized rats showed as great a response as did unoperated animals. It is of interest that the TPO of guinea pig liver was increased neither by X ray nor by injection of this steroid. In vitro experiments with Compound F and rat liver slices have been inconclusive thus far.

It seems probable that the increase in TPO of rat liver after irradiation is a consequence of an effect on the adrenal cortex, not necessarily mediated through the pituitary. Although activation of the pituitary-adrenal system may be the mechanism of increase in TPO following injection of epinephrine or histamine⁽²⁾, the hypophysis seems not to be an obligatory participant in determining the response of this enzyme system to total-body irradiation.

Data relevant to this discussion are presented in Tables 12 and 13.

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Table 12

82

THE EFFECT OF VARIOUS DRUGS ON THE RESPONSE OF RAT LIVER
TRYPTOPHAN PEROXIDASE-OXIDASE TO TOTAL-BODY IRRADIATION

Treatment	Dose (mg/kg)	Control			4 hr after 1000 r		
		No.	Av.	Std. dev.	No.	Av.	Std. dev.
None	--	43	7.7	1.5	10	13.7	2.6
Atropine sulfate	200	4	14.3	2.2	4	19.6	3.3
Physostigmine salicylate	0.5	2	13.0	2.3	2	16.0	0.9
Diphenhydramine hydrochloride	5	4	7.0	2.0	2	12.5	0.7
Tetraethylammonium chloride	50	4	16.7	3.3	4	18.7	2.3
Ephedrine sulfate	100	6	12.7	2.6	6	15.9	4.6
Cocaine hydrochloride	10	2	11.0	1.1	2	12.8	1.7
Priscoline hydrochloride	20	2	11.2	0.2	2	16.9	0.6
Cysteine hydrochloride (I.V.)	1200	3	18.9	1.2	3	23.4	0.3

Values given as micromoles of kynurenine formed per hour per mg dry weight.

Compounds given subcutaneously 30 minutes before exposure unless otherwise indicated.

Table 13

TRYPTOPHAN PEROXIDASE-OXIDASE ASSAYS IN NORMAL AND OPERATED ANIMALS FOLLOWING
PRETREATMENT WITH VARIOUS DRUGS

Pretreatment	Time (hr)	Dose (mg/kg) and route	Normal			Adrenal- ectomized			Adrenal demedullated			Hypophy- sectomized			Spinal (C ₆)		
			No.	Av.	Std. dev.	No.	Av.	Std. dev.	No.	Av.	Std. dev.	No.	Av.	Std. dev.	No.	Av.	Std. dev.
None	---	---	43	7.7	1.5	14	7.7	2.1	20	8.3	1.5	16	5.1	1.5	7	34.7	7.8
X ray, 1000 r	4	---	10	13.7	2.6	11	7.0	1.3	19	11.0	1.9	14	6.8 [†]	1.4	4	36.7	7.1
17-hydroxycortico- sterone-21-acetate (Compound F)	4	I.M. 25	3	18.6	4.0				2	18.4	12.2						
		5	4	16.7	3.8	2	17.1	4.0	4	12.1	2.3	4	23.0	5.3			
		2	4	10.8	2.0												
		1	2	11.0	4.1												
		0.5	4	10.8	2.1												
1-Epinephrine bitartrate	6	2 * SC	3	16.7	1.3	4	5.4	0.6	2	10.2	0.7						
dl-nor-Epinephrine hydrochloride	6	2.5* SC	3	23.6	8.8				3	11.3	2.8						
Sodium ascorbate	6	1000 IP	3	20.9	0.3	2	6.6	0.0	3	9.7	0.5	5	9.6	2.5			
Nicotine chloride	6	8 SC	2	20.6	2.3				3	12.4	1.3						
Histamine diphosphate	6	100 SC	Twofold inc. (Knox, ref. 2)						3	13.6	1.6						
dl-Tryptophan	6	2000 IP	9	121	18		**		7	74	25	4	25.2	12.4			
	4		2	65	12	4	60	13									

*Doses represent 0.006 mM/kg of the 1- isomers.

**Tryptophan lethal to adrenalectomized rats within 5 hours.

†Averages include rats exposed between 7 and 24 days after hypophysectomy.

PRELIMINARY REPORT: A PROBLEM ON THE STATISTICAL
ESTIMATION OF THE CONCENTRATION OF MICROORGANISMS
USING THE DILUTION METHOD

J. E. Hall and S. A. Tyler

The problem of estimating the concentration of microorganisms in solution is usually handled by estimating the parameter on the dose-response curve as follows:(1)

Assume a unit volume of solution contains an unknown concentration (λ) of organisms. Choose several dilution factors x_i ($i = 1, 2, \dots, k$) and correspondingly effect k distinct dilutions of concentration $x_i \cdot \lambda$. Under the assumption that each organism is capable of growth,* we sample n_i unit volumes from each dilution and score the results as a dichotomy; i.e., record 0 or 1 accordingly as no growth or growth is observed. In repeated sampling of unit volumes at concentration λx_i , the Poisson law,

$$p(k; \lambda, x_i) = \begin{cases} \frac{e^{-\lambda x_i} (\lambda x_i)^k}{k!} & k = 0, 1, \dots \\ 0 & \text{otherwise} \end{cases} \quad (1)$$

defines the probability distribution of individual cells. From expression (1) the proportion of growths expected from samples at dilution x_i is

$$P_i = 1 - e^{-\lambda x_i} \quad (2)$$

It follows that $Q_i = 1 - P_i = e^{-\lambda x_i}$ and

$$\ln Q_i = -\lambda x_i \quad (3)$$

Expression (3) is analogous to a dosage response curve and is employed to estimate λ by plotting the natural logarithm of the sample proportion of no growth (Q_i) for several of the x_i . For this process to be meaningful (in general) the x_i 's must be chosen so that at least one growth but not all

*The prerequisite experimental conditions for growth are assumed.

growth occurs in each sample of n_i . A straight line is passed through the charted points by eye and its estimated slope serves as an estimate of λ . Estimates of the error of $\hat{\lambda}$ have been computed when three dilutions are used. (2) This is a rather "casual" procedure for estimation and more powerful methods are available for estimating the slope of the line. These methods give rise to an estimator converging to that of the maximum likelihood at the expense of increased numerical computations. In this note a procedure is developed for estimating λ using a single dilution. This method permits, very simply, the construction of exact confidence intervals about λ and gives rise to an asymptotic expression for the variance of $\hat{\lambda}$.

Let us assume that the Poisson density, expression (1), holds and that a satisfactory dilution factor x has been obtained. Consider the population of microorganisms S obtained by dilution a unit volume of solution whose concentration is λ by the factor x . From S a random sample of n unit volumes is taken. The sample may be arrayed as $(z_1, z_2, \dots, z_n)$ where z_i is the i th sample item. Each z_i takes the values of 0 or 1 depending on the observance of no growth or growth, respectively. The probability distribution of z_i is

$$\phi(z_i; P) = \begin{cases} P^{z_i}(1-P)^{1-z_i} & \text{for } z_i = 0, 1 \\ 0 & \text{otherwise} \end{cases}$$

Since the maximum likelihood estimator of Q is $\hat{Q} = 1 - \frac{1}{n} \sum_{i=1}^n z_i$ it follows

from equation (3) that the similar estimator of λ (i.e., $\hat{\lambda}$) is

$$\hat{\lambda} = -\frac{1}{x} \ln \hat{Q} \quad (4)$$

Expression (4) is easily verified by maximizing the likelihood

$$L(\lambda) = \prod_{i=1}^n P^{z_i}(1-P)^{1-z_i}$$

with respect to λ .

No mathematical expressions in closed form are available for the expectation and variance of $\hat{\lambda}$; however, meaningful asymptotic approximations are readily obtained. Expanding $1 - Q$ as powers of $(Q - \hat{Q})$ by Taylor's formula gives

$$\hat{\lambda} = -\frac{1}{x} \left(f + f'(Q - \hat{Q}) + \frac{f''}{2!} (Q - \hat{Q})^2 + \frac{f'''}{3!} (Q - \hat{Q})^3 + \dots + \dots \right) \quad (5)$$

from which the expectation of $\hat{\lambda}$ is found.

$$E(\hat{\lambda}) = -\frac{1}{x} (f + f' E(Q-\hat{Q}) + \frac{f''}{2!} E(Q-\hat{Q})^2 + \frac{f'''}{3!} E(Q-\hat{Q})^3 + \dots + \dots) \quad (6)$$

where $f = \ln Q$ and $E(\)$ denotes the operation of mathematical expectation. Reduction of (6) gives

$$E(\hat{\lambda}) = \lambda + \frac{1}{x} \left(\frac{P}{2Q \cdot n} - \frac{P(1-2P)}{3Qn^2} + \frac{3P^2}{4Q^2n^2} \pm \dots \right) \quad (7)$$

It follows from (7) that as $n \rightarrow \infty$, $E(\hat{\lambda}) = \lambda + 0(1)$. This implies that in reasonably large size samples the average of the $\hat{\lambda}$'s from repeated sampling will approach the true concentration λ .

For the variance of $\hat{\lambda}$ ($\text{Var}(\hat{\lambda})$), expand $\hat{\lambda}^2 = \frac{1}{x^2} (\ln Q)^2$ into powers of $(Q-\hat{Q})$ by Taylor's formula and obtain

$$\hat{\lambda}^2 = \frac{1}{x^2} (g + g'(Q-\hat{Q}) + \frac{g''}{2!} (Q-\hat{Q})^2 + \frac{g'''}{3!} (Q-\hat{Q})^3 + \dots + \dots) \quad (8)$$

Consequently,

$$E(\hat{\lambda})^2 = \frac{1}{x^2} \left(g + g' E(Q-\hat{Q}) + \frac{g''}{2!} E(Q-\hat{Q})^2 + \frac{g'''}{3!} E(Q-\hat{Q})^3 + \dots + \dots \right) \quad (9)$$

where $g = (\ln Q)^2$. Squaring expression (6) gives

$$E^2(\hat{\lambda}) = \frac{1}{x^2} \left(f^2 + f \cdot f' E(Q-\hat{Q}) + \frac{f \cdot f''}{3} E(Q-\hat{Q})^3 + \left(\frac{f \cdot f'''}{12} + \frac{f''}{4} \right)^2 E(Q-\hat{Q})^4 + \dots + \dots \right) \quad (10)$$

and by subtracting (10) from (9) the following results

$$\text{Var}(\hat{\lambda}) = \frac{f'}{x^2} (f' E(Q-\hat{Q})^2 + f'' E(Q-\hat{Q})^3 + \frac{f'''}{3} E(Q-\hat{Q})^4 + \dots) \quad (11)$$

or

$$\text{Var}(\hat{\lambda}) = \frac{1}{x^2} \left(\frac{P}{Qn} + \frac{P(3P-1)}{Q^2n^2} + \frac{P(1-6P+6P^2)}{3Q^3n^3} + \dots \right)$$

Thus as $n \rightarrow \infty$

$$\text{Var}(\hat{\lambda}) = \frac{P}{x^2 Q \cdot n} + 0\left(\frac{1}{n}\right) \quad (12)$$

Precise $(1 - \alpha)\%$ confidence intervals about λ are obtained in the following manner. Form the functions

$$\sum_{\hat{P}=\gamma_1}^n b(\hat{P};P) = \alpha_1 \quad (13)$$

and

$$\sum_{\hat{P}=0}^{\gamma_2} b(\hat{P};P) = \alpha_2 \quad (14)$$

where $b(\hat{P};P) = \frac{n!}{(n-x)!x!} P^y(1-P)^{n-y}$ with $y = \sum_{i=1}^n z_i$ and $\alpha_1 + \alpha_2 = \alpha$.

Expressions (13) and (14) imply the probability statement

$$\Pr(\gamma_2 < \hat{P} < \gamma_1) = 1 - \alpha \quad (15)$$

Substituting the estimated P (which is computed from the sample), say $\hat{P}_*$, for $\hat{P}$ in (13) and (14) and finding the corresponding P 's for chosen values of α_1 and α_2 gives P_L and P_U where P_L and P_U are defined as the lower and upper confidence limits of P , respectively, and satisfy the equality

$$\Pr(P_L < P < P_U | \hat{P}_*) = 1 - \alpha \quad (16)$$

Since the λ 's are single-valued functions of the P 's, we put $P_L = 1 - e^{-\lambda_L x}$ and $P_U = 1 - e^{-\lambda_U x}$ into statement (16) and simplify to obtain

$$\Pr(\lambda_L < \lambda < \lambda_U | \hat{P}_*) = 1 - \alpha \quad (17)$$

Thus $(1 - \alpha)\%$ confidence intervals are constructed for which λ_L and λ_U bracket the true concentration λ $(1 - \alpha)\%$ of the time in repeated sampling.

ACKNOWLEDGMENT

The authors would like to thank Mr. Howard Ducoff for bringing this problem to their attention and aiding them with the biological interpretation.

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PROGRESS REPORT: KINETICS OF POPULATION GROWTH

G. A. Sacher

Construction and testing of a continuous culture apparatus has proceeded in the past quarter. Component parts have been tried and found satisfactory but operation of the complete system has not yet been attempted. The operating principle is illustrated in Figure 26.

Liquid nutrient flows very slowly through the sintered glass plug (P) at a rate that is determined by the height of the liquid column in the stand-pipe (S). The height of this liquid column is held constant within 2 mm by the constant leveling tube (L) in the neck of the reservoir flask (R). The nutrient falls by drops into the culture space (V) in which the microorganisms are growing. The fluid volume in the culture space is held constant by continuously aspirating through (A) into the collecting flask (F). The culture is aerated by forcing air through the sintered plug (Q). This air is made isothermal and saturated in the train (C), which is immersed in the same waterbath (B) as the culture vessel.

Flow rates have been found to be highly uniform for long periods when the sintered plug regulator is employed. The rate of flow is measured by counting drops. Since the nutrient is a weak electrolyte, drops can be counted easily and inexpensively by making each drop complete an electrical circuit (E) that actuates a register (Re) through an electronic relay. Drop size is highly uniform (variation of weight of single drops less than 0.3 per cent) when conditions are kept constant, so that drop counting gives an accurate measure of flow rate.

It can be seen that this form of culture apparatus has no moving parts, and will provide a uniform rate of flow that can be measured with an error considerably smaller than 1 per cent. Since the nutrient reservoir can be replaced easily, operation can continue indefinitely. One remaining problem is to devise an assembly that can be sterilized conveniently. When this is done, the apparatus described here should prove superior to existing devices in simplicity and accuracy. It does not have all the flexibility needed for studies of population dynamics, but it is a necessary stage in the development of the more complex systems that are required.

89

Figure 26. Simplified diagram of apparatus for continuous culture of microorganisms

R nutrient reservoir	C train for warming and saturating air
L leveling device	N flow-meter
P,Q sintered glass plug	M manometer
S standpipe, to regulate nutrient flow by hydrostatic pressure	Ve air vent
V culture space	E electrical contact
A capillary tube, for aspiration	Re register

Biophysics

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PROGRESS REPORT: IONIZATION FROM BETA-RAY POINT SOURCES

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All corrections and sources of error have now either been determined or shown to be negligible, including those mentioned in the previous report.⁽¹⁾

Studies of the ionization distribution from P^{32} , Y^{91} , Tl^{204} , and RaE have been completed. In addition, the absolute volume of the grid chamber has been determined (indirectly) in terms of a radium standard calibrated by the Bureau of Standards. This makes possible, in combination with absolute activity measurements, the determination of dosages in standard units (such as mr per mc) as a function of distance for these isotopes.

It is expected that the final report on this work can be made within the next few months.

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THE DETERMINATION OF PHOTOGRAPHIC FILM EXPOSURE BY NEUTRON ACTIVATION OF Ag^{107}

I. B. Berlman, H. F. Lucas, and H. A. May

It is a well established fact that the amount of reduced silver in a photographic emulsion exposed to radiation is a function of the exposure. The most widely used method of studying this relationship consists in optical densitometric measurements, which are usually limited to densities ≤ 3.0 and are definitely impractical at densities ≥ 6.0 . [This report demonstrates the feasibility of using the neutron activation of the reduced silver as an extension of the more conventional procedures.]

Two sets of du Pont #510 film were exposed to radium γ radiation, in the range of 10.7 to 1154 r and developed according to the manufacturer's recommendations. One set of films was used for the densitometer readings and the other set was used for the activation determinations. The density (~~minus the log of the transmission~~) of films in one set was read on an Ansco-Sweet Densitometer with density ranges of 0 to 3 and 3 to 6. An exposure of 80 r was found to produce the maximum readable density. It was possible, however, to extend the range of this double-emulsion film by reading the density of each emulsion separately. This was done by clamping the film between two plates that masked all but a small portion of one side of the film. The unprotected emulsion was softened with hot NaOH solution (0.1 N), loosened by gentle swabbing, and washed away. A portion of emulsion was removed from each side of the film at adjacent but not overlapping spots. Total density was computed from the sum of the densitometer readings for each emulsion layer, less a small correction for the film base. The crosses of Figure 27 indicate a nearly linear relationship between density and exposure to approximately 200 r.

The high activation cross section of Ag^{107} and the short half-life (2.3 m)(1) of Ag^{108} make the neutron activation method very convenient, since it allows short irradiation time and gives high counting rate with essentially no increase in the background of the film caused by impurities present in the film. In the activation procedure each film was inserted individually at a fixed position into the Argonne standard graphite pile, using Ra-Be source #38 for the same length of time. The time between removal of the film from the source and beginning of radioactive measurements was 80 ± 1 seconds in each case. Each film was counted for 900 seconds, approximately six half-lives. A shielded β -proportional counter with a background count of approximately five counts per minute was used. The number of counts obtained from a blank film similarly treated was taken as background. The data obtained from the activated films are shown in Figure 28. The agreement between the densitometer and neutron activation measurements at low exposures is shown in Figure 27. Circles indicate the

Figure 27

Calibration of film; neutron activation method.

Figure 28

Comparison of film calibrations; photometric and neutron activation methods.

radioactivities at various exposures normalized at 86 r. It should be noted that variations in types of emulsions, in emulsion thickness, and in film development will affect the activation calibration curve; moreover, the densitometer readings become less accurate at the higher densities, whereas the activation method becomes more accurate with higher exposure and with use of stronger neutron sources.

We wish to acknowledge the services of Mr. G. O. Payne in exposing the film and to Mr. J. E. Rose for helpful advice.

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THE GROWTH OF BACTERIAL COLONIES

H. E. Kubitschek

Several attempts have been made to describe the growth of bacterial cells as a stochastic process, notably by Kendall⁽¹⁾ and Bellman and Harris.⁽²⁾ Kendall has attacked the problem by assuming that a bacterium must go through k successive states before division is possible and has shown that the population size at large time increases exponentially. However, his considerations are for the age-independent branching process: the chance for any bacterium to pass from one state to the next is independent of the time the bacterium has spent in that state. Although this procedure may turn out to be a valid approximation, no solution has been found for the probability distribution of the population size as a function of time except for $k = 1$, where the solution obviously does not conform to observation.

On the other hand, Bellman and Harris, describe colony growth more generally, and perhaps more realistically, as an age-dependent process. But their approach is inconvenient for our purposes, involving as it does, the evaluation of a generating function given through the solution of a nonlinear integral equation. As with their approach, our method allows the division time to have an arbitrary distribution, but dispenses with the explicit evaluation of a generating function.

If we assume that the probability that a cell divides by a time t after "birth" is given by the cumulative density function $p(t)$ sometimes referred to as the life-length distribution. "Birth" is taken to be that time at which, according to the opinion of the investigator, the parent cell has produced a new and distinct individual, or is replaced by new individuals. Then the rate of increase of probability for cell division is dp/dt . Thus, for example, in a culture of N parent cells the number of divisions between times T_1 and T_2 is

$$N \int_{T_1}^{T_2} \frac{dp}{dt} dt = N \int_{t=T_1}^{t=T_2} dp(t)$$

When a number of successive divisions occur and each follows the same cumulative density function $p(t)$, as, for example, in the case of a bacterial population in the logarithmic phase, we may determine the probability $\Pr(G \geq g, t)$ that g or more divisions occur by time t . Let the time of the k^{th} division be t_k . Obviously,

$$0 \leq t_1 \leq t_2 \leq \dots \leq t_k \leq \dots \leq t_g \leq t \tag{1}$$

Then the differential probability that the first division occurs between t_1 and $t_1 + dt_1$, the second between t_2 and $t_2 + dt_2$, etc., is

$$d_g \Pr(G \geq g, t) = dp(t_1) \cdot dp(t_2 - t_1) \cdot dp(t_3 - t_2) \dots dp(t_g - t_{g-1}) \quad (2)$$

The change in time base for each successive probability density dp indicates that the life-length distributions are defined to be nonexistent until the appropriate previously completed division; that is, they vanish for t negative.

According to condition (1) every division is conditional upon occurrence of a previous division, which is taken into account in the following integration by constraining the lower time limit of any division to be the time of the previous division:

$$\begin{aligned} \Pr(G \geq g, t) &= \int_0^t \frac{d}{dt_1} p(t_1) \int_{t_1}^t \frac{d}{dt} p(t_2 - t_1) \int_{t_2}^t \frac{d}{dt_3} p(t_3 - t_2) \dots \int_{t_{g-1}}^t \frac{d}{dt_g} \\ &p(t_g - t_{g-1}) dt_{g-1} \dots dt_1 = \int_0^t \int_{t_1}^t \int_{t_2}^t \dots \int_{t_{g-1}}^t dp(t_g - t_{g-1}) \\ &dp(t_{g-1} - t_{g-2}) \dots dp(t_2 - t_1) dp(t_1), \end{aligned} \quad (3)$$

As an example of the application of (3), we consider the classical deterministic expression for the growth of a bacterial culture in logarithmic phase having the population N at the time t when the initial population is N_0 . Then,

$$\frac{dN}{dt} = \lambda N \text{ or } N = N_0 e^{\lambda t}$$

where λ is the growth rate. (For $N_0 = 1$, these equations require interpretation in terms of probabilities for division.) Since the parent population N_1 decreases at the rate of division equal to the growth rate λ ,

$$\frac{dN_1}{dt} = -\lambda N_1$$

or

$$\frac{N_1}{N_0} = e^{-\lambda t} \quad (4)$$

indicates their survival.

We may define

$$q = \frac{N_1}{N_0}$$

as the probability against division of the parental cells. In this case cumulative density function for division of parental cells is

$$p = 1 - q = 1 - e^{-\lambda t}$$

This distribution is fundamental to the assumptions of Kendall⁽¹⁾ in the examination of cellular divisions as a stochastic process, and it is also implicit in Lea and Coulson's⁽³⁾ derivation for the distribution of mutants in a population. The differential is

$$dp = \lambda e^{-\lambda t} dt,$$

so (3) becomes

$$\begin{aligned} \Pr(G \geq g, t) &= \lambda^g \int_0^t \int_{t_1}^t \dots \int_{t_{g-1}}^t e^{-\lambda t_g} dt_g dt_{g-1} \dots dt_1 \\ &= 1 - e^{-\lambda t} \left\{ 1 + \lambda t + \frac{\lambda^2 t^2}{2!} + \dots + \frac{(\lambda t)^{g-1}}{(g-1)!} \right\} \end{aligned}$$

Now the probability $\Pr(g, t)$ that exactly g divisions take place is

$$\begin{aligned} \Pr(g, t) &= \Pr(G \geq g, t) - \Pr(G \geq g + 1, t) \\ &= \frac{(\lambda t)^g}{(g)!} e^{-\lambda t} \end{aligned}$$

Thus we see that the probability for any number of divisions g has a Poisson distribution under the simple assumption above that each subsequent division of the parent cell has uniform probability, λ , throughout time. Consequently, the mean number of divisions at time t will be (λt) , which agrees, as it must, with the deterministic expression.

In point of fact, however, it is well known that the cumulative density function of (4) does not represent the behavior of individual cells, since these must have a growth period before being able to undergo a second division. The true cumulative density function p is not known for any organism, although an approximation could be obtained from the experiments of Kelly and Rahn⁽⁴⁾ on Bacterium aerogenes. This will not be done here since we plan more extensive experiments.

In the event that environment or heredity changes for each successive generation, there will be new cumulative density function for each generation. Allowing p_k to be the density function for the k^{th} generation, the probability for g or more divisions by time t is generalized:

$$\Pr(G \geq g|t) = \int_0^t dp_1(t_1) \int_{t_1}^t dp_2(t_2 - t_1) \int_{t_2}^t dp_3(t_3 - t_2) \dots \int_{t_{g-1}}^t dp_g(t_g - t_{g-1}) \quad (5)$$

It should also be remarked that the representation of equations (3) and (5) holds for populations of low viability, and is reflected in the cumulative density function approaching a value less than unity as $t \rightarrow \infty$. Thus this treatment corresponds to the simple birth-and-death process of the stochastic method. The factor of emigration cannot be put into the cumulative density function, but can be handled as an independent probability function for immigration, say f , operating from the time of initial immigration, I . The probability that an immigrant arrives and undergoes g or more divisions by time t is

$$P_I(\geq g, t) = \int_I^t f dt \int_I^t \int_{t_1}^t \int_{t_2}^t \dots \int_{t_{g-1}}^t dp_g(t_g - t_{g-1}) \dots dp_1(t_1)$$

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PROGRESS REPORT: A POSSIBLE THERMAL NEUTRON DETECTOR OF HIGH EFFICIENCY

I. B. Berlman

Several materials^(1,2) have been incorporated recently into liquid scintillators to make them sensitive to thermal neutrons. Among these are lithium and boron. These elements, however, on capturing a neutron emit a heavy ionizing particle for which the luminescence efficiency of the solution is not very good. An attempt has been made to determine the effectiveness of gadolinium introduced into a terphenyl-xylene solution.

Five hundred ml of a gadolinium-terphenyl-xylene solution containing more than 4 g of gadolinium per liter, 4 g terphenyl per liter, and 7.8 per cent mono-2-ethylhexyl phosphate was prepared by Dr. D. F. Peppard and his associates in the Chemistry Division. Preliminary results indicate a favorable response of this solution to thermal neutrons for the pile. Further research is in progress.

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FLUORESCENT X-RAY ANALYSIS

R. E. Rowland

Part I

Some tests have been made to determine the possibilities and limitations of fluorescent X-ray analysis by means of proportional counters. Three basic approaches have been investigated:

1. The minimum detectable quantity of an element
2. The possibility of quantitative analysis for one element under fixed conditions
3. The problem of determining the spectrum of an X-ray beam

In the first problem, only uranium has been investigated thus far. It appears that the minimum quantity of uranium detectable with the present equipment is somewhere in the region of 1 to 10 μg . To improve on this figure, it will be necessary to pay particular attention to the radiations scattered by and excited from the collimating structures. Since the instrument in its present form is sensitive to characteristic X radiations of all elements with atomic numbers greater than 15, low atomic number materials should be used in the apparatus itself. In the analysis for uranium the $M\alpha$ line at 3.2 kev was used, thus if the sample contains chlorine or any other element with a K or L line near this value, the minimum amount detectable will be somewhat greater.

The proportional counter has been employed to determine the spectrum of an X-ray beam, in order to determine the effectiveness of various primary filters. The method is successful, but the results require interpretation, since the efficiency of the counter is a function of photon energy. In order to obtain a photon flux small enough to be counted, pinhole collimation of the beam is required.

Part II

The conventional optical densitometric methods of reading dose from films exposed to radiations have a relatively small useful range. This shortcoming is not due to the film, for it is capable of yielding information covering a relatively large range, but rather to the method of interpreting the information carried by the film. In an effort to extend the range of usefulness of "overexposed films," i.e., those too dense to be read by a densitometer, several techniques have been developed.⁽¹⁾

The problem of obtaining meaningful results from exposed and developed films consists in measuring the amount of reduced silver in the photographic emulsion. This can be accomplished very readily by means of fluorescent analysis. A beam of soft X rays is directed upon the developed and fixed emulsion, and the characteristic X rays excited are examined by a single channel pulse height analyzer. The number of characteristic silver X rays detected is a measure of the amount of silver present. The shape of the calibration curve thus obtained agrees substantially with that obtained with neutron activation by Berlman.¹

The experimental equipment consisted of a 25-kvp Machlett X-ray tube that is used to excite the L X rays of silver. These X rays, of 2.99 kev, are then detected by a proportional counter with a 0.005-inch beryllium window. The primary spectrum obtained has a peak at this energy with a full width at half maximum of 30 per cent. For counting purposes, only a small central region of this peak was recorded. The same calibration films employed by Berlman were used, having a range from 10 to 1100 r.

The results indicate that the range of du Pont no. 510 film can be extended to about 250 r with equipment normally available to most laboratories.

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PROGRESS REPORT: STUDY OF THE MECHANISMS OPERATING IN THE IONIZATION TYPE HALIDE DETECTOR

H. A. Schultz and J. G. Dodd, Jr.

INTRODUCTION AND SUMMARY

A series of experiments has been under way to evaluate the possibility that the formation of alkali halides is an intermediate step in the process of Freon detection, is preceded by thermal decomposition of the stable organic halides to form reactive halogen compounds, and followed by the decomposition of the alkali halides on a heated surface to give positive ions of the alkali metals. It is now known that the conditions of operation of the ionization type halide detector are favorable to each of these steps. It is concluded, therefore, that this mechanism probably accounts for at least part, and possibly all, of the sensitivity of this type of halide detector. In order to obtain a reproducible, stable response by this mechanism, it is necessary to arrange a proper source of alkali atoms, to provide a geometry that will allow the steps to occur efficiently in the proper sequence, and to minimize the recycling of gaseous halogen compounds. It has been found that coatings of alkali compounds on warm surfaces of certain other materials make promising sources. The presence of iron or copper in the base material appears to be particularly favorable to the reaction. It has been learned that certain alkali compounds show a strong tendency to creep along warm surfaces of the type used, and it is thought that this may be important to the process.

THERMAL DECOMPOSITION OF FREON

It is well known that organic fluorides are extremely difficult to decompose. Dozens of different methods have been published; each of them requires either high temperature or considerable time.⁽¹⁾ Freon 12, dichloro-difluoro-methane, was chosen as an atmospheric tracer largely because of its great stability under ordinary conditions.

A combustion furnace was set up in the laboratory to study the decomposition of Freon in air under conditions of time, temperature, and contact materials similar to those existing in Freon detectors. The gaseous reaction products were led into a gas washing bottle containing a solution of sodium carbonate. The concentration of Freon in the air stream was 3 per cent instead of a few parts per million as in Freon detectors. It was found, by using a second bottle in series, that the amount of Cl not trapped by such a wash bottle was negligible compared with the amount trapped.

To test for Cl, a small amount of AgNO_3 was added to a diluted sample of the carbonate solution. The yellowish precipitate that formed contained Ag_2CO_3 , which was reconverted to AgNO_3 by adding HNO_3 to drive off all the CO_2 . If a small concentration of Cl was present the solution that remained was turbid due to AgCl . The concentration of AgCl in such a solution was estimated by visual comparison of its turbidity with that produced when standards made up with Cl_2 gas were subjected to the same treatments at the same time. Concentration ratios of 2 to 1 could be separated without doubt, and ratios of 2 to 1 appeared to be distinguishable, but smaller increments were not considered to be distinguishable by this method of inspection. Neither Freon nor fluorine makes any contribution to the turbidity.

In one test a dry air-Freon mixture was passed at the rate of 50 ml per minute through a quartz tube with a platinum liner containing spongy palladium. The reaction zone was 13 mm in diameter by about 80 mm in length. Tests were run at six different temperatures in the range of 670 to 1030°C and the results of the turbidity method of estimation indicated that yields between 1/2 and 2 atoms of Cl per molecule of Cl_2CF_2 were obtained at temperatures above about 900°C. Qualitative tests indicated that fluorine was also present in the solutions when chlorine was present.

It was reported previously⁽²⁾ that the number of ions per molecule of Freon could be increased by preheating the air-Freon mixture in a quartz combustion tube, provided the flow was fast enough, but that the number of ions per molecule could be decreased if the flow was very slow. One possible interpretation is that the decomposition products, which are known to be highly reactive, attacked some of the materials along the path to the detector, and formed solid halogen compounds, which were then deposited out on the walls, and it is probable that this occurred to some extent. Another possibility is that the Freon decomposition was a two-step process, and that the first product was more reactive in the detector than the second. Biesalki⁽³⁾ found that pyrolysis of CCl_4 in air produced phosgene, $\text{O} = \text{CCl}_2$, which might subsequently yield Cl_2 .

FORMATION OF ALKALI HALIDES

The results of tests indicate that halogen gases in air probably react at high temperature with certain compounds of the alkali metals to produce alkali halides.

In one experiment it was shown that a stream of air passed over Li_2O at 1000°C in a platinum combustion tube did not carry any appreciable amount of lithium downstream, but that the addition of 3 per cent Freon to the air stream caused a white deposit to form downstream. This deposit gave a positive test for Li, Cl, and F. The residue left in the combustion tube also contained Li, Cl, and F. Similar results were obtained when chlorine was used

instead of Freon, the white deposit and the residue containing Li and Cl. The Li_2O was obtained by thermal decomposition of Li_2CO_3 .⁽⁴⁾ When LiF was used instead of Li_2O , and either Freon or chlorine was used in the air stream, both the downstream deposit and the residue in the tube were found to contain Li and Cl.

The search has been far from exhaustive, but results obtained thus far indicate that alkali halides are formed when Freon-air mixtures are passed through Freon-sensitive detectors. So far as is known, every Freon sensitive detector tested in this laboratory has contained alkalis, existing on the electrodes either as impurities or as additives.

In another series of experiments, alkali salts were coated upon cylindrical spirals of chromel A (Ni-Cr alloy) wire, and the spirals were heated electrically. The spirals, about 10 mm in diameter, were mounted coaxially around a platinum tube, about 3 mm in diameter, which was used as a gas-air burner. The presence of alkalis in the region of the flame caused a color change, red for lithium, yellow for sodium. In most cases, the base of the flame was just above the top of the spiral. The burner and spiral were surrounded by a glass chimney about 25 mm in diameter.

It was found that after the spiral had been treated with certain alkali compounds, the intensity of the coloring of the flame was greatly increased whenever Cl_2 , or Cl_2CF_2 was injected into air at the base of the chimney. In some tests with Cl_2 , a white deposit was formed inside the chimney, above the flame. This deposit was water soluble and gave a positive test for Cl. In some cases a second flame was held above the top of the chimney, and it yielded a positive flame test for the alkali whenever Cl_2 was introduced at the base of the chimney.

It may be important to have only a thin coating on the spiral. If it was coated directly (with Li_2CO_3 for example), the effect of Cl_2 was most spectacular after much of the Li had gone. If only the lead-ins were coated, the Li was found to spread to the hotter zones of the spiral, where it was quite responsive to chlorine.

It was desired to test the effect of iron in this arrangement, since it had been learned that a base of Nichrome (Ni-Fe-Cr alloy) could be used in making Freon sensitive alkali "sources" for a series of detectors to be described later in the report, while chromel-based (Ni-Cr alloy) sources were insensitive. It was found that a persistent Cl_2 sensitive source for flame tests could be made by coating a chromel wire spiral with $\text{Fe}(\text{OH})_3$ that had been precipitated by reacting aqueous solutions of $\text{Fe}(\text{NO}_3)_3$ and Li_2CO_3 . Lithium was carried down by the hydroxide.⁽⁵⁾ This source continued to respond to high concentration "puffs" of chlorine over a much longer period of time than any other tried so far. A test for iron was then made on a sample of chromel from the lot used in making filaments for the flame test, and a trace was found, so unfortunately no spiral completely free of iron has yet been used as a control.

IONIZATION AND COLLECTION

The production of positive alkali ions from alkali halides on surfaces of high work function has been known for at least a decade⁽⁶⁾ and has recently been used to construct a very potent source of alkali ions.⁽⁷⁾ Tests run in this laboratory indicate that the reaction will take place in the presence of air, but one is of course more limited in the choice of a base material because of the possibility of oxidation. To collect the ions once they are formed, one must provide an electric field of sufficient strength to remove the space charge.⁽⁸⁾

DESIGN OF PRACTICAL ALKALI-SENSITIZED DETECTORS

There are very good reasons to suspect that alkali halides serve as intermediates in the process of Freon detection, since the three major steps of (1) Freon decomposition, (2) alkali halide formation, and (3) dissociation with release of positive ions are all known to occur under conditions that exist somewhere in each Freon-sensitive detector. If this is really the major process, then it appears that all that is required is to put together sufficient components to do just these things efficiently. Conditions that should be satisfied are that the alkali source should not be readily depleted and that the Cl and F left on the ionizing filament should be removed as fast as possible so that they will not recycle and therefore prevent a sharp cut-off when the flow of Freon is stopped. While it appears that Freon detectors in general may operate as outlined here, it has not always been possible to predict in advance which designs would be sensitive. There have been indications that geometrical factors are involved.

The study of a series of experimental Freon detectors for the "part per million range" has preceded and run concurrently with the tests described above, involving several per cent of Freon. Numerous consistencies between the two different approaches have been noted. It was learned by empirical methods that Freon sensitive detectors could be built provided they included three elements; a hot filament, a warm "source," and a collector, arranged as mentioned earlier⁽⁸⁾ and as described in more detail here. The filament is made of Chromel A, wound into a flat spiral. The source consists of a wire, or cup that is sensitized with an alkali compound and iron in some form, or copper instead of iron in a few cases. One part of this source is very close to the filament, and is therefore warm, while the other end is cooler. The collector is a flat electrode parallel to the filament and held at a negative potential relative to it. All of the alkali metals, Li, Na, K, Rb, Cs, have been found to produce Freon sensitivity. The absolute sensitivity, measured in microamperes per part per million is greatest with Cs, and least with Li. The relative sensitivity, measured in ion current per ppm divided by the background current, depends upon the anion attached to the alkali metal, and even more upon the geometry of the detector, but hardly

at all on the alkali metal itself. This suggests that the background ion current is due to vapor of the sensitizing alkali compound. It has been found that newly constructed sources using alkali compounds other than the oxides give high backgrounds and little or no Freon sensitivity, until they have been operated overnight in air (about 16 hours), which lowers the background and makes the detector sensitive to Freon. It is thought that this may be due to the formation of oxides. There may also be a redistribution of the alkali over the iron- (or copper-) containing source, as has been observed on the spiral used in the flame tests described above. No sensitivity has been obtained without iron or copper in the source; however, no exhaustive search for other materials has been made. Also, it has been found that certain geometrical arrangements of elements similar to those described above show no sensitivity to Freon.

Iron has been used in positive ion sources since Kunsman's work⁽⁹⁾ on the positive ion emission properties of certain catalytic mixtures used in the synthesis of ammonia from nitrogen and hydrogen. A mixture made from Fe_3O_4 with 1 per cent Al_2O_3 and $\frac{1}{2}$ per cent alkali or alkaline earth was found to be a good positive ion source. Other mixtures of iron and alkali were tried with success. However, no reference has been found to the possibility of chlorine sensitivity of such sources. In an X-ray study of a catalyst of this type ($\text{Fe}_3\text{O}_4 + \text{Al}_2\text{O}_3 + \text{K}_2\text{O}$) Wycoff and Crittenden⁽¹⁰⁾ concluded that the role of the alkali and the aluminum was to prevent the growth of large crystals. Perhaps this has a bearing on the present problem.

Looking back over previous data for references to iron or copper, it is noted that a stainless steel collector was used with some success quite early in the work.⁽¹¹⁾ The spectrographic results that have been obtained on nitric acid washings of the surfaces of the electrodes of modified commercial detectors show that copper has been present in all cases, especially on the collector, or cooler electrode. Of course, a dozen or more other elements have been found, especially Si, Al, K, and sometimes Na, in amounts comparable to that of Cu, and also Ca and Mg in lesser amounts. In some cases one or more of these were found: Fe, Ni, or Pb, and traces of still others, including Li. It should also be noted that the brass case of the modified commercial detector was quite warm during operation, so that it might have served as a copper-bearing source.

MULTI-STEP PROCESS

The three steps, thermal decomposition, formation of alkali halide, and decomposition of alkali halide into ions are known to take place under conditions that exist at different places in Freon detectors. However, a number of aspects of the problem are not yet understood, such as: the role of iron, the details of the transportation of alkali halide molecules from the site of their origin to the site of ionization, and the details of the effects of electrode shape and arrangement.

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INDEX

A		B	
	Page		Page
Air Force Radiation Laboratory	28	Bacteria, continuous culture apparatus	88
		growth of colonies	95
Altitude, effects on mammals exposed to fast neutrons	28	<u>Bacterium aerogenes</u>	97
American Association of Physicians	6	Barron, E. S. Guzman	6
Anemia	33	Berlman, Isadore B.	90, 99
Anoxia, temperature dependence of protective effect	47	Beryllium, reversal of toxic effects	77
Antibodies, production in spleen-shielded animals	44	Beta rays, from Sr ⁸⁹ ionization from point sources	57 90
Apparatus, continuous culture system	88	Biophysics	90
Argonne standard graphite pile	91	Blackford, Margaret	6, 47
Aureomycin, effects on kappa particles in <u>P. aurelia</u>	69	Blood, anemia	33
Aurin tricarboxylic acid	77	influenced by spleen shielding during X ray	33
Auxin, radiosensitivity of biosynthesis	71	leukemia	10, 13, 15
		pressure changes in X-rayed chicks	64
		Bone marrow, therapeutic effects following X ray	10, 15
		therapeutic effects of heterologous injections	11
		therapeutic effects on septicemia	17
		Boron, use of complexing agents to reduce toxicity	77
		Brar, Sarmukh S.	90

INDEX

	Page		Page
B			
Brues, Austin M.	6, 56, 57 64, 68	Cobalt 60, γ -ray source	27
Buchanan, Donald L.	73	Columbia University	29
Building 202	6	Congdon, Charles C.	11, 13 15, 17
C			
Carcinogenesis, Sr ⁹⁰	57	Cowles Commission for Economic Research	6
Cardwell, Rosson L.	6	Cysteine, temperature dependence of protective effect	47
Cataracts, induced in mice by fast neutrons	29	D	
Central Shops	33	Detectors, for thermal neutrons	99
Chemistry Division	99	Freon	102
Chicks, blood pressure changes following X ray	64	Dosimeters, calibration of Victoreen condenser calorimetric for fast neutrons	29 28
Chloramphenicol, effects on kappa particles of <u>P. aurelia</u>	69	grasshopper egg nuclei for fast neutrons	68
Christian, Emily J. B.	64	Ducoff, Howard H.	64, 87
E			
Chronic External Irradiation	10	Ehret, Charles F.	69
Clark, John W.	27	Electron microscopy	55
Clark, Robert K.	90	Ethylenediamine, CO ₂ absorp- tion	73

INDEX

	Page		Page
Experimental Pathology	57	Grasshoppers, effects of fast neutrons on egg nuclei	28, 68
Eyes, cataract formation following fast neutrons	29	Guinea pigs, factors influencing radiation treatment of tumors therapeutic effects of tissues from hemato- poietic organs following X ray	10 13
F		Gurian, Joan	6, 84
Fast neutrons, calorimetric dosimetry	28	Gustafson, Philip	6
effects on grasshopper egg nuclei	68	H	
effects on lungs of mammals	28	Hall, James E.	84
effects on mice	27	Hamilton, Katherine F.	64
induction of cataracts	29	Hamster, effects of X ray on mast cells	50
Finkel, Miriam P.	57	Hasterlik, Robert J.	56
Fixation, technique for ultra- thin sectioning	55	Hematology	33
Freon, ionization type halide detector	102	Heparin	50
thermal decomposition	102	Hirsch, Gertrude M.	57
G		Histopathology	55
Gadolinium-terphenyl-xylene solution, thermal neutron detector	99	Hollcroft, J. W.	24
Gamma irradiation, effects on mice	27		
Gaston, Evelyn O.	33		
Gordon, Solon A.	71		

INDEX

	Page		Page
Hood, John S.	6	Leucopenia	33
Hormones, effects on TPO		Leukemia	13, 15
X-ray response	80	factors influencing treatment	10, 15
I			
Indoleacetic acid, radio-sensitivity of bio-synthesis	71	Lewis, Yvette S.	50
Indolyl-3-pyruvic acid, preparation	76	Lindenbaum, Arthur	77
Invertebrate Zoology	68	Lisco, Hermann	57, 63
J			
Jacobson, Leon O.	6, 33	Lockport-Joliet, Ra content of deep wells in area	56
Jordan, Donn L.	27	Looney, William B.	6, 56
K			
Kappa particles, effects of aureomycin, chloramphenicol, and terramycin	69	Lorenz, Egon	10, 11, 13, 15, 17, 24
Kisielewski, Walter E.	63	Lymphosarcoma	24
Kubitschek, Herbert E.	28, 90, 95	factors influencing treatment	10
L			
Lake Michigan, radium content of water	56	therapeutic effects of brei following X ray	13
Lesko, Roman	77	M	
		Marinelli, Leonidas D.	6, 56, 90
		Marks, Edna K.	6, 33
		Massey, Barbara W.	55
		Mast cells, effects of X ray	50
		Mathews, M. M.	24
		Mayer, Sylvia	6

INDEX

	Page		Page
Mice, cataracts following		O	
fast neutrons	29		
effects of bone marrow		Organic Chemistry	76
on septicemia	17		
effects of γ rays and		Osteogenic sarcomas	63
fast neutrons	27		
use of complexing		P	
agents to reduce toxicity			
of boron	77	<u>Paramecium aurelia</u> , antibiotic	
Microorganisms, statistical		sensitivity of Kappa	69
estimation of concentration	84	effects of X ray on production	
Microbiology	69	of aureomycin-resistant	
Mikuta, Eleanor T.	80	killers	69
Miller, Charles E.	56	Passonneau, Janet V.	68
Mung bean, auxin bio-		Patt, Harvey M.	6, 47
synthesis	71	Payne, George O.	94
N		Pearl, Albert	33
Nakao, Akira	6, 73	Peppard, D. F.	99
National Bureau of		Pfau, Antreen	6
Standards	90	Pharmacology	80
National Cancer Institute	17, 24	Phosphorus 32, β -ray point	
National Institute of		source	90
Arthritis and Metabolic		Physical Chemistry	77
disease	17	Physiological Chemistry	73
Neutron Radiobiology	27	Physiology	47
Neutrons, activation of Ag^{107}	91	Phytoradiobiology	71
Norris, William P.	56	Pile, Argonne Standard	
		graphite	91

INDEX

	Page		Page
Plutonium, effects of metallic form in rabbits and rats	63	Rats, pathologic effects of Pu implants	63
Point sources, β ray	90	Reactor, CP-3'	27
Pomeroy, John H.	76	Rose, John E.	90, 94
		Rossi, Harald H.	29
Powers, E. Lawrence	6, 69	Rowland, Robert E.	90, 100
Proportional counters, fluorescent X-ray analysis	100	S	
Publications, by staff members	6	Sacher, George A.	88
R		Salicylic acid, reversal of Be toxicity	77
		Sanford, K.	13
Rabbits, effects of spleen shielding during X ray on blood	33	Sarcoma	13
pathologic effects of Pu implants	63	Schubert, Jack	77
		Schultz, Harvey A.	90
Radium, clinical aspects of toxicity program	56	Septicemia	11
content of drinking water	56	modified in mice by bone marrow	17
γ exposure of du Pont film	91	Septicopyemia	21
pilot studies on human subjects	56	Shefner, Deborah S.	69
Radium E, β -ray point source	90	Silver 107, neutron activation	91
Radium Toxicity	56	Simmons, Eric L.	33
Raper, Carlene A.	69	Smith, Douglas E.	47, 50
		Special Problems	64

INDEX

	Page		Page
Speckman, Thomas	6	Terramycin, effects on kappa particles in <u>P.</u> <u>aurelia</u>	69
Spleen, therapeutic effects of brei following X ray	13	Thallium 81, β -ray point source	90
Stateville Penitentiary, Ra content of inmates	56	Theoretical Biology	84
Stearner, S. Phyllis	64	Thomson, John F.	64, 80
Stehney, Andrew F.	56	Thrombocytopenia	33
Straube, Robert L.	47	Thymus, temperature dependence of protection by anoxia and cysteine	47
Strontium 89, toxicity to mice	57	therapeutic effects of brei following X ray	13
Stroud, Agnes N.	64	Tryptophan	71
Stroud, Clyde P.	27	Tryptophan peroxidase- oxidase system, hormonal factors governing response to X ray	80
Stutz, Robert E.	71	Tumors, leukemia	10, 13, 15
Sudds, Mrs. Phyllis	6	lymphosarcoma	10, 13, 24
Sulfosalicylic acid, reversal of Be toxicity	77	osteogenic sar- coma	63
Sutton, Harriet A.	10	regression following variation in X-ray dose to body and	
Swick, Robert W.	73	lymphosarcoma	24
		reticulum cell sarcoma	13
T		Tyler, Sylvanus A.	84
Tahmisian, Theodore N.	28, 68		
Talks, by staff members	8		
Temperature, effects on protection of thymus cells by anoxia and cysteine	47		

INDEX

	Page		Page
U		X irradiation (Contd)	
University of Chicago	6	effects of varying dose to body and tumor	24
Uphoff, Delta	11, 13, 15	effects on blood pressure of chicks	64
Uranium, reversal of toxic effects	77	effects on mast cells	50
V		effects on production of aureomycin-resistant killers in <u>P. aurelia</u>	69
Vogel, Howard H., Jr.	27, 68	factors influencing treatment of leukemia and lymphosarcoma	10
W		hormonal reaction governing response of rat liver	
Wallace, Herschel D.	47	TPO	80
Wassermann, F.	55	septicemia incidence in mice	17
Water, radium content of Lake Michigan and Lockport-Joliet wells	56	therapeutic effects of heterologous bone marrow transplants	11
Weber, Robert P.	71	X rays, fluorescence analysis	100
White, Marcia R.	77	Y	
William, F. P., Jr.	17	Yttrium 91, β -ray point source	90
Williams, Jane	6	Z	
X		Zirkle, Raymond E.	27
X irradiation, antibody formation in spleen-shielded animals	33		
biologic effects in mice and guinea pigs	11, 13, 15		
bone marrow therapy	10, 11, 15, 17		
effects of spleen shielding on blood of rabbits	33		

- Blood Pressure -- effects of radiation on, in chicks
- Paramecium -- antibiotic sensitivity of kappa particles, effects of radiation
- Plant Metabolism -- effects of radiation on ^{auxin biosynthesis} the concentration of, using the
dilution method and kinetics of population growth
- Plutonium -- tissue distribution and pathological effects of implanted, in rats
and rabbits
- Metabolism -- effects of radiation on, in egg nuclei of grasshopper nymphs
- Strontium -- ^{toxicology} toxicity for mice
- Microorganisms -- statistical estimation of the concentration using the
dilution method and kinetics of population growth
- Beryllium -- ^{toxicology} toxicity and effects of chelating agents on
- Boron -- ^{toxicology} toxicity, effects of complexing agents
- Enzymes -- hormonal factors governing the effects of radiation on
- Photographic Film Detectors -- reading after neutron activation of Ag¹⁰⁷
- Populations -- of microorganisms, statistical analysis
- Radiation -- effects on biosynthesis of auxins, blood pressure of chicks, and
enzyme systems
- Thermal Neutrons -- gadolinium-terphenyl-xylene solution in liquid scintillators
for detection and measurement of
- Halide Detectors -- ionization type, performance
- French -- ionization type detector for

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REPORT REVISION

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Page 64 }

Delete the last sentence: "Birds in this condition usually died within two hours after irradiation."

Insert the following: "Irradiated birds usually died within two hours after this physical condition was reached."

Page 103-
Line 9

Insert square root sign to read:

"and ratios of $\sqrt{2}$ to 1 appeared to be"

Hoylande D. Young

Hoylande D. Young, Director
Information Division

2/17/53