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Argonne National Laboratory

QUARTERLY REPORT

MAY, JUNE, JULY, 1950

DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH

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Chicago 80, Illinois

DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH

QUARTERLY REPORT, MAY, JUNE, JULY, 1950

Editor

Austin M. Brues, Director, Division of Biological and
Medical Research

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Administration

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

On July 1, 1950, all research in biology, medicine, and radiological physics was placed under the administration of the Division of Biological and Medical Research, directed by Dr. Austin M. Brues. Dr. Hermann Lisco, Mr. Leonidas D. Marinelli, and Dr. E. Lawrence Powers have been appointed Associate Directors.

At the same time, a Program Committee was appointed to establish and maintain liaison among the various groups in the Laboratory and those associated with the AEC that are engaged in radiobiological investigations. The committee will serve in an advisory capacity and is comprised of Dr. Austin M. Brues, Chairman, Dr. Robert J. Hasterlik, Dr. Hermann Lisco, Dr. Franklin C. McLean, Mr. Leonidas D. Marinelli, Mr. John E. Rose, Dr. Raymond E. Zirkle, and Dr. E. Lawrence Powers, Executive Secretary.

Mr. Rosson L. Cardwell, a medical economist, has joined the staff as Assistant to the Division Director, and replaces Mrs. Josephine Graff, who terminated her employment with the Laboratory during this quarter. Mr. Cardwell received an A.M. degree in economics from the University of Chicago in 1949 and is currently completing a dissertation for the doctoral degree in the School of Business. In addition to his administrative duties he will carry on research in the field of administration of scientific research organizations. For the past two years Mr. Cardwell has been Assistant to the Dean of the School of Arts and Sciences, Roosevelt College, Chicago, Illinois.

Dr. William B. Looney, a graduate of the University of Virginia Medical School, has joined the Special Problems Group as a post-doctoral AEC Fellow. He will work with Dr. Brues on problems concerned with the long-term toxicity of radium.

Dr. Robert A. Conard, Commander, M.C., U.S.N., who was on loan to the Division during the past year, has been assigned to the Naval Medical Research Institute, Bethesda, Maryland.

Editorial

Mrs. Antreen Pfau
Miss Helen Supert

During the period covered by this Quarterly Report the following papers were prepared for ANL reports:

Allen, J. Garrott. Progress report: Abnormal bleeding. ANL-4474 (unclassified).

Barron, E. S. Guzman and Veronica Flood. The effect of X irradiation on cytochrome C. ANL-4467 (unclassified).

Tyler, Sylvanus A. and Joan Gurian. Determination of the LD₅₀ by use of probit, angular, and logit transformations. ANL-4486 (unclassified).

Lists of exhibits, publications, and talks by staff members are presented below.

EXHIBITS

Brues, A. M. and A. N. Stroud. Action of Tritium on Tissue Cultures. Chicago Cancer Research Group, Argonne National Laboratory, Chicago, Illinois, May 18, 1950.

Jacobson, L. O., E. K. Marks, E. O. Gaston, M. J. Robson, E. L. Simmons, and W. F. Bethard. Studies on the Function of Mammalian Spleen. Illinois State Medical Society, Springfield, Illinois, May 23-25, 1950, and at the Annual Meeting of the American Society of Medical Technologists, Houston, Texas, June 11-15, 1950.

PUBLICATIONS

Jacobson, L. O., E. L. Simmons, E. K. Marks, M. Robson, W. F. Bethard, and E. O. Gaston: The Role of the Spleen in Radiation Injury and Recovery. J. Lab. Clin. Med., 35 746, May 1950.

Miller, C. P., C. W. Hammond, and M. Tompkins: The Incidence of Bacteremia in Mice Subjected to Total Body X-radiation. Science, 111 541, May 1950.

Schubert, J. and M. R. White: The Effect of Different Dose Levels of Zirconium on the Excretion and Distribution of Plutonium and Yttrium. J. Biol. Chem., 184 191, May 1950.

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Snyder, R. H. and W. E. Kisielewski: The Relative Biological Effectiveness of Beta and Roentgen Radiation as Shown by the Radiotoxicity of Na^{24} for Mice. *Radiology*, 5 743, May 1950.

Miller, C. P., C. W. Hammond, and M. Tompkins: Reduction of Mortality from X radiation by Treatment with Antibiotics. *Science*, 111 719, June 1950.

Schubert, J. and M. R. White: Effect of Citrate Salts and Other Chemical Factors on the Distribution and Excretion of Beryllium. *J. Lab. Clin. Med.*, 35 854, June 1950.

Huff, R. L., W. F. Bethard, J. F. Garcia, B. M. Roberts, L. O. Jacobson, and J. H. Lawrence: Tracer Iron Distribution Studies in Irradiated Rats with Lead-shielded Spleens. *J. Lab. Clin. Med.*, 36 40, July 1950.

Russell, E. R., R. C. Lesko, and J. Schubert: A Direct Method for Determining Radium in Exposed Humans. *Nucleonics*, 7 60, July 1950.

Schubert, J., E. R. Russell, and L. S. Myers, Jr.: Dissociation Constants of Radium-Organic Acid Complexes Measured by Ion Exchange. *J. Biol. Chem.*, 185 387, July 1950.

TALKS

Brues, A. M.: Practical Aspects of Radiation Biology. Wayne University College of Medicine, Detroit, Michigan, May 5, 1950.

Scully, N. J.: The Use of C^{14} in Plant Studies. Forty-third Annual Meeting of the Illinois State Academy of Science, Rock Island, Illinois, May 5-6, 1950.

Tahmisian, T. N.: New Method for Staining Cells with Cobalt and BAL. Forty-third Annual Meeting of the Illinois State Academy of Science, Rock Island, Illinois, May 5-6, 1950.

Tahmisian, T. N.: The Biological Effects of Radiation. The First Presbyterian Church, La Grange, Illinois, May 8, 1950.

Scully, N. J.: The Use of Atomic Energy in Biological Studies. Advanced Group Studying Ecology. Morton Arboretum, Naperville, Illinois, May 9, 1950.

Hasterlik, R. J.: Business Machine Methods of Recording Medical Data. Oak Ridge National Laboratory, Oak Ridge, Tennessee, May 10, 1950.

Brues, A. M.: The Place of Radioactive Isotopes in Therapy. American Gynecological Society, White Sulphur Springs, West Virginia, May 11, 1950.

Allen, J. G.: The Role of Research in the Medical School. University of Kansas Medical Center, Kansas City, Kansas, May 12, 1950.

Brues, A. M.: Studies on Mammalian Chromosomes. Zoology Club, University of Chicago, Chicago, Illinois, May 17, 1950.

Brues, A. M.: The Question of Resting Chromosomes. Chicago Cancer Research Group, Chicago, Illinois, May 18, 1950.

Hall, B. V.: Dynamics of Heterotransplantation. Chicago Cancer Research Group, Chicago, Illinois, May 18, 1950.

Allen, J. G.: Studies on Bleeding and Coagulation. Institute of Radiobiology and Biophysics, Chicago, Illinois, May 22, 1950.

Scully, N. J.: The Growth and Development of C^{14} -containing Plants. Plant Physiology Club, University of Illinois, Urbana, Illinois, May 25, 1950.

Powers, E. L.: Gene Analysis with Radioactive Elements. Northwestern University, Zoology Department, Evanston, Illinois, May 26, 1950.

Straube, R. L.: The Impact of Atomic Energy on Biology and Medicine. The Air Force Reserve Officers Squadron 9628 TH, Oak Park Village Hall, Oak Park, Illinois, June 6, 1950.

Stutz, R. E.: Organic Acids in Photosynthesis by Higher Plants. Biology Conference on CO_2 Assimilation Reactions in Biological Systems, Brookhaven National Laboratory, Upton, New York, June 8, 1950.

Marks, E. K.: The Role of the Spleen in Radiation Injury and Recovery. American Society of Medical Technology, Houston, Texas, June 12-15, 1950.

Sanderson, M.: Recent Trends in Bleeding and Clotting Times of Whole Bloods and Plasma. American Society of Medical Technology, Houston, Texas, June 12-15, 1950.

Skirmont, Ellen: The Effects of P^{32} on Polycythemia Induced by Cobalt. American Society of Medical Technology, Houston, Texas, June 12-15, 1950.

Brues, A. M.: Analysis of Radiation Injury. National Research Council Symposium on Radiobiology, Oberlin, Ohio, June 14-18, 1950.

Schubert, J.: Analytical Applications of Ion Exchange Separations. Division of Analytical Chemistry Third Annual Summer Symposium, American Chemical Society, Ohio State University, Columbus, Ohio, June 16, 1950.

Hasterlik, R. J.: Symposium on Occupational Medicine. Annual Meeting of the American Medical Association, San Francisco, California, June 25-30, 1950.

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Jacobson, L. O.: Studies on Hematopoietic Recovery from Radiation Injury. Fifth International Cancer Congress, Paris, France, July 21, 1950.

Jacobson, L. O.: The Therapeutic Uses of Radioisotopes. Sixth International Congress of Radiology, London, England, July 25, 1950.

Marinelli, L. D.: Ionization in the Air Surrounding Beta-ray Sources. Sixth International Congress of Radiology, London, England, July 28, 1950.

Warren, S. and A. M. Brues: Protection Against Radiation Hazards. Sixth International Congress of Radiology, London, England, July 28, 1950.

Biometrics

Mr. Sylvanus A. Tyler

PROGRESS REPORT: THE EXCRETION OF Sr^{89} BY GOATS

by

S. A. Tyler and Joan Gurian

INTRODUCTION

Several years ago, research using goats as experimental animals was conducted by M. N. Swift, C. L. Prosser, and E. S. Mika.⁽¹⁾ Radiostrontium* (specific activity $\approx 50\mu\text{c}/\mu\text{g}$), obtained from the Clinton Laboratories, was injected intramuscularly into 14 animals after a control period of one to two weeks, and the resulting toxicity, hematological, and pathological changes were studied in an attempt to determine species parameters which, together with relevant information already available for other laboratory animals,⁽²⁾ would aid in predicting the effects of this isotope in man. The Sr^{89} content in the urine and feces was determined daily for periods ranging from 7 to 17 days after injection and single measurements were recorded up to 32 days postinjection. Of the information gathered, daily excretion data on 13 goats are sufficiently complete to analyze statistically the clearance of Sr^{89} from the body. Data from 11 of this number permitted this study of total daily excretions over a ten-day collection period.

In an effort to investigate in greater detail the time-distribution of Sr^{89} excretions for goats, an analysis of the time course of excreted radiostrontium for individual goats was made and the inter-relationship between individual patterns noted. Daily excretions (in per cent of the injected dose) were assumed to follow the empirical model

$$E_t = E_1 t^b \quad (1)$$

Where E_t is the per cent of the injected dose excreted during day t , t = time in days after injection, and E_1 and b are empirically determined constants. Thus the correspondence

$$Y = \log E_t, X = \log t \quad (2)$$

was adopted as the rectifying transformation. The excretion data were corrected for decay on the assumption that all activity consisted of Sr^{89}

*Radiostrontium was injected in saline dilution of pH 5.0 to 7.0. Total injected amounts ranged from 10 to 15 cc per goat.

with a half-life of 55 days. The least square parameters of the characterizing linear equation* based on daily excretions from day 2

$$\log E_t = \log E_1 + .b \log t, \quad (3)$$

are tabulated in Tables 1, 2, and 3. The scatter diagram of paired slopes (urine versus feces) is presented (Figure 1) and the dependence of urinary excretion slope on age of animal is illustrated (Figure 2).

DISCUSSION

Considerable dispersion exists between individual urinary and fecal excretion slopes which range from -0.575 to -2.411 and from -0.908 to -2.547, respectively. The only two male goats in this group (numbers 10 and 16) are among the three animals possessing urinary excretion slopes greater than -1. The variation among individual slopes for total excretions is somewhat less than that found in the separate excretion components. Total excretion slopes range from -1.038 to -2.478. Fecal slopes, on the average, are higher than urinary slopes although this order is not preserved for all animals. The scatter diagram of urinary versus fecal slopes (Figure 1) does not show positive evidence of a systematic ordering of the paired slope-points since the positive correlation ($r = +0.346$) between this paired set does not differ significantly from zero ($.20 < p < .30$). This behavior is in contrast with the order existing between similar quantities in both strontium and plutonium-injected (I.M.) dogs⁽³⁾ for which a significant negative correlation was found. A significant relationship exists between urinary slopes and corresponding ages of animals under four years; but, a dependence upon age is not evident for fecal slopes (Figure 2). Both urinary and fecal slopes for animals over four years of age vary indiscriminately. This result supports the thesis that age and retention of a metabolizing element are negatively correlated during the growth period.

With due cognizance of the variability existing between patterns of excretion for individual goats, let us assume that the time-pattern of excretion characterizing the group is approached by the distribution in time of the average values of the marginal distributions** at times t_i .

*Excretions during the first 24-hour period were not used in the fitting process because of their departure from the log - log trend which characterizes, sufficiently well, the distribution of values at later times.

**Measured excretions are assumed described by

$$E_t = F(t) + \eta_t$$

Where $F(t)$ is some function of the time (t) after injection and η_t is a random (error) variable.

Table 1

PARAMETERS OF THE LOG - LOG LINEAR REGRESSION OF
PER CENT DAILY URINARY EXCRETION VERSUS TIME FOR
INDIVIDUAL GOATS

Goat no.	No. of collections n	Last coll. day t_f	Slope $b \pm \sigma_b$	Day 1 intercept E_1	$\sigma_{E.t^2}$
1	18	19	-2.306 ± 0.226	18.3	0.083
2	16	17	-2.121 ± 0.317	5.1	0.140
3	9	11	-1.773 ± 0.292	6.3	0.051
4	10	14	-2.411 ± 0.224	13.2	0.041
5	13	25	-1.950 ± 0.217	7.4	0.077
6	13	23	-1.471 ± 0.201	3.3	0.061
7	17	30	-0.575 ± 0.219	3.4	0.116
8	16	31	-1.229 ± 0.257	14.1	0.158
9	13	32	-1.360 ± 0.528	5.0	0.373
10	10	16	-0.869 ± 0.087	0.2	0.008
15	15	16	-1.563 ± 0.529	2.7	0.353
16	13	15	-0.805 ± 0.126	0.8	0.017
24	13	14	-1.679 ± 0.245	4.8	0.060

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Table 2

PARAMETERS OF THE LOG - LOG LINEAR REGRESSION OF
PER CENT DAILY FECAL EXCRETION VERSUS TIME FOR
INDIVIDUAL GOATS

Goat no.	No. of collections n	Last coll. day t_f	Slope $b \pm \sigma_b$	Day 1 intercept E_1	$\sigma_{E.t^2}$
1	13	16	-2.091 ± 0.358	24.9	0.142
2	15	17	-2.547 ± 0.339	77.5	0.153
3	7	10	-0.908 ± 0.293	10.4	0.034
4	9	14	-1.858 ± 0.401	27.8	0.126
5	11	21	-1.942 ± 0.212	20.11	0.057
6	10	21	-2.039 ± 0.175	35.7	0.037
7					
8					
9	10	16	-1.287 ± 0.163	23.0	0.027
10	11	17	-1.889 ± 0.335	15.0	0.136
15	15	16	-1.575 ± 0.184	15.2	0.042
16	13	14	-1.519 ± 0.006	11.3	0.00004
24	6	7	-1.395 ± 0.698	29.4	0.134

Table 3

PARAMETERS OF THE LOG - LOG LINEAR REGRESSION OF
PER CENT DAILY TOTAL EXCRETIONS VERSUS TIME FOR
INDIVIDUAL GOATS

Goat no.	No. of collections n	Last coll. day t_f	Slope $b \pm \sigma_b$	Day 1 intercept E_1	$\sigma_{E \cdot t^2}$	Age
1	13	16	-2.008 ± 0.151	37.1	0.037	~ 3 yrs
2	15	17	-2.478 ± 0.293	82.5	0.114	> 4 yrs
3	9	10	-1.038 ± 0.175	15.4	0.017	> 4 yrs
4	11	14	-1.994 ± 0.271	42.8	0.064	> 4 yrs
5	10	12	-2.012 ± 0.131	31.2	0.012	> 4 yrs
6	10	21	-2.049 ± 0.134	44.6	0.012	> 4 yrs
7					}	4 yrs (twins)
8						
9	12	16	-1.154 ± 0.203	24.1	0.014	24 mo +
10	12	16	-1.650 ± 0.268	11.4	0.077	13 mo
15	15	16	-1.547 ± 0.150	17.2	0.028	27 mo
16	13	14	-1.260 ± 0.098	10.9	0.097	17 mo
24	6	7	-1.462 ± 0.043	35.9	0.001	29 mo

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Figure 1. Scatter diagram of urinary and fecal excretion slopes for Sr^{89} -injected goats.

Figure 2. Scatter diagram of age versus excretion slope for Sr^{89} -injected goats (36 months of age or less).

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Upon this assumption 11 goats were separated into two groups:

- 1) Animals for which maximum total Sr^{89} excretion occurred during the first 24 hours ($E_1 > E_2$) after injection,
- 2) Animals with maximum total Sr^{89} excretion recorded for the 24- to 48-hour period ($E_1 < E_2$).

The time dependence of total average excretions over 48-hour periods was calculated for both groups (Figure 3). A significance test (Table 4) of the log - log regression parameters shows a nonsignificant difference between time patterns for these groups. This result implies that the distribution of strontium excretions in these groups is essentially the same. A possible explanation for the apparent shift in maximum excretion periods is that the amount excreted during the periods 1 to 24 hours and 24 to 48 hours largely depends on the phase of the individual normal excretion cycle in which injection takes place. The establishment of an adequate hypothesis concerning individual excretion patterns requires a more extensive discussion than is given above. A test of the validity of this hypothesis demands a more powerful statistical approach.

PROSPECTUS

Much information still awaits extraction from the data. Two phases of this study, yet to be investigated, are 1) the total isotopic elimination from the body and 2) the retention function. The problems involved in the determination of total excretion are discussed by G. Sacher,⁽³⁾ and solutions have been attempted by both Sacher⁽⁴⁾ and S. A. Tyler.⁽⁵⁾ Due to losses of excreted materials, mainly through absorption by the surface area of the metabolism cages, total recovery of injected materials through excretions is not possible. The recovery range for goats is expected to be lower than the range for dogs (80 to 90 per cent) since the surface area of the cages in which the goats were housed was much greater than that of cages used for dogs. Thus, the loss from surface absorption is, therefore, expected to be greater for goats.

Figure 3. Plot of average per cent two-day excretion versus time in days after injection.

Table 4

PARAMETERS OF THE LOG - LOG LINEAR REGRESSION OF
AVERAGE PER CENT TWO-DAY EXCRETIONS VERSUS TIME

	N	Day 1 intercept $a \pm \sigma_a$	Slope $b \pm \sigma_b$	$\sigma_{y \cdot x^2}$
Group 1				
$E_1 > E_2$	5	1.610 ± 0.101	-1.398 ± 0.148	0.0124
Group 2				
$E_1 < E_2$	5	1.590 ± 0.110	-1.667 ± 0.160	0.0147

Note: Group 1 consisted of goats no. 1, 3, 4, 5, 6, 9, 15.

Group 2 consisted of goats no. 2, 10, 16, 24.

LITERATURE CITED

1. Swift, M. N., C. L. Prosser, and E. S. Mika. 1946. Effects of Sr^{89} and X radiation on goats. CH-3888 (declassified).
2. Anthony, D. et al. 1947. Radiotoxicity of injected Sr^{89} for rats, mice, and rabbits. I. Introduction: Methods. CH-3844 (declassified). II. Metabolism and organ distribution. CH-3846 (declassified). III. Lethal action and clinical changes. CH-3845 (declassified).
3. Sacher, G. 1948. The excretion, retention, distribution, and clinical effects of strontium⁸⁹ in the dog. II. Statistical analysis of excretion and retention of strontium⁸⁹ for individual dogs. CH-3866 (declassified).
4. Sacher, G. Unpublished data.
5. Tyler, S. A. and J. Gurian. On the determination of retention behavior and expected total metabolic excretion of radioisotopes from daily excretion data. In press.

Hematology

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

ANL-4488

PRELIMINARY REPORT: NEMBUTAL DOSAGES FOR EXPERIMENTAL
ANIMALS

by

E. L. Simmons and Edna K. Marks

Many investigators are reluctant to use Nembutal anesthesia because of difficulty in finding dosages that will permit full anesthesia without resulting in death. Since we have had considerable success with the drug, using carefully graded dose-weight scales, we believe that the dosages that we have established may be of value to others. It is quite likely that slight modifications will have to be made, depending upon strains of animals, physical state, etc.

Veterinary Nembutal (pentobarbital sodium, Abbott) in fluid form, containing 60 mg per cc, is diluted with isotonic sodium chloride. Following administration of the proper dose, a period of ten minutes should pass for deep anesthesia to ensue.

For Sprague-Dawley male rats dilute 1:10 and administer intraperitoneally. Do not give old, heavy rats an additive dose based upon the injection scale for younger animals (i.e., a 320-g rat should not be given twice the dose for a 160-g rat). In such cases the dose should be reduced slightly. Preliminary experiments suggest that a lower dosage should be used for Sprague-Dawley female rats than for males. The doses given in Table 5 have proved successful for a 30-minute period of deep anesthesia (0.039 mg Nembutal per gram weight).

For CF#1 female mice dilute 1:10 and administer intraperitoneally. Dosages (0.072 mg per g) that induce a 30-minute period of deep anesthesia are given in Table 6.

Male and female guinea pigs are injected intraperitoneally but the dilution is one half (0.168 cc per g), (Table 7). Again, the period of anesthesia is about 30 minutes.

No dilution is made for male and female Swift's snuffle-free rabbits. Full strength veterinary Nembutal (0.65 cc per kg) is injected slowly into the ear vein. This dosage is sufficient to keep the animals anesthetized for 50 minutes or less, but for longer experiments an additional 0.5 cc is injected intravenously after the first 35 or 40 minutes have elapsed.

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Table 5

NEMBUTAL DOSAGE FOR RATS (1:10 DIL. SALINE)

140 grams	-	0.91 cc
145	-	0.94
150	-	0.97
155	-	1.00
160	-	1.04
165	-	1.07
170	-	1.10
175	-	1.14
180	-	1.17
185	-	1.20
190	-	1.23
200	-	1.30
205	-	1.33
210	-	1.36
215	-	1.39
220	-	1.43
225	-	1.46
230	-	1.49
235	-	1.52
240	-	1.56
245	-	1.59
250	-	1.62
255	-	1.65
260	-	1.69

Table 6

NEMBUTAL DOSAGE FOR MICE (1:10 DIST. H₂O OR
PHYSIOLOGICAL SALINE)

10 grams	-	0.13 cc
11	-	0.14
12	-	0.15
13	-	0.17
14	-	0.18
15	-	0.19
16	-	0.2
17	-	0.22
18	-	0.23
19	-	0.24
20	-	0.25
21	-	0.26
22	-	0.28
23	-	0.3
24	-	0.31
25	-	0.32
26	-	0.34
27	-	0.35
28	-	0.36
29	-	0.38
30	-	0.39

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Table 7

NEMBUTAL DOSAGE FOR GUINEA PIGS
(DILUTE ONE-HALF WITH H₂O OR PHYSIOLOGICAL SALINE)

200 grams	-	0.24 cc
225	-	0.27
250	-	0.30
275	-	0.33
300	-	0.36
325	-	0.39
350	-	0.42
375	-	0.45
400	-	0.48
425	-	0.51
450	-	0.54
475	-	0.57
500	-	0.60
525	-	0.63
550	-	0.66
575	-	0.69
600	-	0.72
625	-	0.75
650	-	0.78
675	-	0.81
700	-	0.84
725	-	0.87
750	-	0.90
775	-	0.93
800	-	0.96
825	-	0.99
850	-	1.02
875	-	1.05
900	-	1.08

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EFFECT OF RADIOPHOSPHOROUS ON RATS WITH COBALT-INDUCED POLYCYTHEMIA

by

W. F. Bethard, Ellen Skirmont, and L. O. Jacobson

ABSTRACT

These experiments have been undertaken to learn more of the nature of the difference in response of normal animals and polycythemic animals to radiation.

Seventy-eight, female, Sprague-Dawley rats, averaging 250 g in weight, were divided into five experimental groups and treated as follows:

Group 1 - cobalt only

Group 2 - cobalt only for 12 weeks and then both cobalt and P^{32}

Group 3 - normal controls

Group 4 - P^{32} only

Group 5 - cobalt and P^{32} started simultaneously.

Cobalt was administered as $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. Each of the treated animals received 1.25 mg per kg of cobalt ion subcutaneously five times per week. Severe polycythemia invariably appeared by the end of 12 weeks. Radiophosphorous was used in the dose of $1.5 \mu\text{c}$ per g intraperitoneally every three weeks. Hematocrit, hemoglobin, heterophil, and lymphocyte determinations were performed every 3 weeks.

An initial polycythemia appeared in animals of group 2. Groups 2 and 4 responded similarly to radiation. Hematocrit and hemoglobin values began to fall after a total P^{32} dose of $4.5 \mu\text{c}$ per g had been given (22nd week). The drop continued gradually until the 28th week, after which a level considerably lower than normal was maintained. Rats in group 5, however, developed polycythemia despite simultaneous administration of P^{32} and they tolerated a total P^{32} dose of $9.0 \mu\text{c}$ per g before hematocrit and hemoglobin values began to fall. The drop was then more rapid than, but quantitatively similar to, those of groups 2 and 4.

It would appear that no significant difference in sensitivity of the blood-forming tissues to P^{32} exists between normal rats and rats with an established cobalt-induced polycythemia. In contrast, the initial bone marrow hyperactivity following cobalt administration apparently affords some protection against the destructive effects of P^{32} when the two are begun simultaneously.

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It has been postulated that one mechanism of both radiation injury and cobalt-induced polycythemia is inactivation of -SH containing enzymes or substrates. If this were true, one would expect no protective action; rather the destructive effects might be additive. It would seem from this experiment, therefore, that such a mechanism is not the only possible explanation for either radiation injury or cobalt-induced polycythemia.

PRELIMINARY REPORT: FURTHER STUDIES OF THE PROTECTIVE EFFECTS OF SHIELDING

by
E. L. Simmons

Data from experiments now in progress show that a marked increase in survival occurs in both male and female rats when the intestine is surgically mobilized and lead-protected during total-body X irradiation. This protective action seems to be almost as effective as that exerted by the lead-protected spleen in the rat. Histologic findings of the effects of such shielding upon hemopoietic recovery will be presented at a later date.

Initial studies of survival of the X-irradiated, spleen-shielded rat show less dramatic results than those obtained when mice were irradiated with spleen protection. The question was raised as to whether the rat spleen might be stimulated to greater activity by administering a sub-lethal "priming" dose of X rays to the body of a spleen-protected animal and then waiting five to seven days for such activity to be at its height before administering the massive acute dose.

Preliminary experiments now in progress have failed to show any increased capacity of the spleen to aid survival following X irradiation when priming doses of 100 r, 200 r, or 300 r were given before 800 r. However, there was an increase in mortality with increase in the total dose administered as shown in Table 8.

Table 8

EFFECT OF PRIMING DOSES ON SURVIVAL

No. of rats	Treatment	% surviving at 30 days
8	Single dose of 800 r - sham operation only	11.1
8	Single dose of 800 r - spleen protected	87.5
8	100 r + 800 r - spleen protected both times	75.0
8	200 r + 800 r - spleen protected both times	37.5
7	300 r + 800 r - spleen protected both times	14.3

U-67-27

ELECTRON MICROSCOPE STUDY OF THE MICROSCOPIC NETWORK
OF FIBRILS AS A COMPONENT OF CONNECTIVE TISSUEby
F. Wassermann

ABSTRACT

The network of fibrils in the ground substance, first shown in Figures 63-68 of the previous quarterly report⁽¹⁾, was studied with improved technique. We obtained electron micrographs in which the contrasts are much greater than those in the earlier paper (Figures 4, 5, and 6). The fibrils show the typical periodic structure of collagen (Figures 5 and 6) and can be measured. It was found that the solitary fibrils have diameters of about 500 Å, identical with those of the fibrils that were isolated from tendons and skin by Schmitt and co-workers⁽²⁻⁵⁾, Wolpers,⁽⁶⁾ Ingelmark,⁽⁷⁾ and others. These are the same fibrils that were found during the formation of collagen fibers in tissue cultures.⁽⁸⁾ Thus the demonstration of the collagen fibril is nothing new. However, our technique of preparing connective tissue spreads makes it possible to show a network of these fibrils as solitary structures in the connective tissue of adult animals (mouse) (Figure 4). Although these are present regularly in the tissue of animals of different age groups, it may be that the fibrils are particularly abundant in young animals and in pregnant females. Should this be true, it would not contradict the statement that the network of submicroscopic fibrils is a regular constituent of connective tissue, and, as a matter of fact, this author found these to be present in the tissues of adult mice before young and pregnant animals had been used for this work.

There is no doubt that the fibril is the structural unit of the fiber (Figure 4). The finest fibers visible in the light microscope in silver preparations have widths of about 2000 Å, while other "silver" fibers measure about 5000 to 8000 Å. All of these fibers are bundles of fibrils having the same widths as the solitary fibrils. The difference between fibers of different diameters is, therefore, but a difference in the number of fibrils contained. From the microscopic point of view the distinction of two kinds of fibers, the reticulin and collagen, would seem to be no longer justified inasmuch as the submicroscopic fibrils are cross-banded and evenly spaced at approximately 270 Å, according to Porter and Vanamee.⁽⁸⁾ However, Heringa⁽⁹⁾ recently referred to differences between reticulin and collagen in regard to sulfur content, resistance to pepsin, and X-ray diffraction pictures. We may say, therefore, that the "generally accepted" opinion that the reticulin is the precursor of collagen, which was found to be justified

Figure 4. Electron micrograph (4200 X) showing fine fibers and network of solitary fibrils in formalin-fixed spread preparation of subcutaneous fascia of young mouse. Note the fraying of fibers in the upper part of the picture and in lower left corner at a diagonal.

Figure 5. Electron micrographs (9400 X) of the same tissue, same fixation as Figure 4. Solitary fibers partly showing periodic structure. Cytoplasmic strand extends diagonally through the picture. Notice fine gray filaments in the ground substance.

Figure 6. Electron micrograph (30,000 X) showing same tissue taken from a pregnant mouse. Solitary fibers partly showing cross-banding.

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also by this author,⁽¹⁰⁾ must be held in suspense until such evidence as proposed by Heringa is checked against new observations by the aid of the electron microscope.

Although the microscopically visible fibers are certainly compounds of fibrils, they are likely to be more than a bundle of units since the binding of the fibrils, either by a cementing substance or by surface forces, makes them the resistant structures they are. As far as the mechanical function of connective tissue is concerned, the fibrils are not likely to play any role in this respect. Their considerable extensibility, shown by Schmitt and co-workers, was demonstrated also by this author in the previous report. From the purely structural point of view, we may call the network of sub-microscopic fibrils, as a whole, a supporting lattice of the ground substance but it is certainly valid to consider its units, the solitary fibrils, as extra-cellular protein structures that must be of high chemical activity, especially with respect to the surface groups. This aspect gives the network a significance that will justify further investigations using the electron microscope to study various pathologic conditions.

Our electron micrographs, including those of tissue from very young animals, do not permit definite statement about the formations of the fibrils, although in rapidly growing mice of about 10 days of age a great amount of collagen material will be produced. The fibrils are found in both young and old animals and are of even widths throughout their "indefinite" length. Free tapering ends, as described by Porter and Vanamee, in the fibrils in tissue culture were only very rarely found in our material and may not be genuine. How frequently free ends occur in tissue culture preparations cannot be seen from the electron micrographs reproduced by Porter and Vanamee. It also seems to be necessary to differentiate very carefully between fibrils, especially tapering ends of fibrils and cytoplasmic filaments that are seen in electron micrographs (Figure 5) and can be identified as extensions of cell processes as was shown in this author's previous report. If free ends of fibrils regularly occurred in tissue culture and were indicative of fiber formation there, it would still be a question whether the mode of fibril formation in tissue culture is the same as in the body. The uniformity of the fibrils, but for minor differences in width, and the scarcity of free ends in our material suggest that the mature fibril is preceded by a state that at present cannot be demonstrated with the electron microscope. The only, though important, observation that is likely to throw some light on the precursors of fibrils is that of fine gray filaments within the ground substance having widths estimated by this author as ranging between about 50 and 80 millimicrons (Figures 4 and 5). These findings were found corroborated by corresponding ones reported by Porter and Vanamee. Their description of a fine fibrous component, consisting of filaments of 50 to 100 Å, seen where parts of the supporting plastic membrane were exposed, fits the description of the filaments observed in the ground substance of the tissue itself by this author. According to the electron micrographs in Porter and Vanamee's paper tissue

culture does not seem to be superior to spreads in regard to the visibility of these "protofibrils." Provided these are genuine formations, they are certainly of interest and are likely to be protofibrils in the sense of Porter and Vanamee. The question of the formation of fibrils will be the subject of further investigations in close relationship to the examination of cells and cell processes.

ACKNOWLEDGEMENT

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Physiology

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ON THE NATURE OF CYSTEINE-INDUCED RADIORESISTANCE:
SULFHYDRYL LEVELS AND DISTRIBUTION OF CYSTEINE
SULFUR

by

H. M. Patt, R. L. Straube, Margaret E. Blackford, and D. E. Smith

ABSTRACT

Cysteine may increase radioresistance by 1) decreasing free radical production, 2) competing directly for oxidizing free radicals, 3) shifting the redox potential of critical cell elements, thus protecting them against oxidation, and 4) altering metabolic pathways. Assuming selective action on sulfhydryl groups by products of irradiated water, less than 1 per cent of nonprotein -SH (as cysteine) could theoretically be oxidized when a 200-g rat is exposed to 800 r.

X irradiation had no immediate observable effect on -SH levels in control and cysteine-treated rats, although cysteine per se (950 mg per kg I.V.) increased the oxidizable -SH content of TCA filtrates of serum, kidney, thymus, intestine, and liver. Rather identical retention of S^{35} was noted in blood of rats receiving labeled cystine, cysteine, or cysteine followed by X irradiation (60 mg cystine or cysteine, 5×10^5 counts per minute, I.V.). Uptake of S^{35} by liver and intestine was more rapid in cysteine-injected rats. X radiation appeared to decrease the rate of uptake and retention. The initial concentration of S^{35} in bone marrow was similar in cystine- and cysteine-treated animals but fell sharply during the first hour in the latter. Marrow uptake was decreased by irradiation. No clear relationship between S^{35} concentration in these tissues and the time course of cysteine protection was evident.

Our data do not support the concept of direct or indirect competition by cysteine for oxidizing radicals. If such a mechanism is operating, oxidation must be localized in specific sites where -SH or other essential substances are few and their inactivation critical.

Special Problems

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PRELIMINARY REPORT: THE EFFECT OF ACUTE ADRENAL
INSUFFICIENCY UPON THE RATE OF ABSORPTION AND THE
DISTRIBUTION OF PHOSPHORUS IN THE RAT

by
B. V. Hall

INTRODUCTION

Experiments have been initiated to study the effect of adrenalectomy in rats upon the rate of absorption of radiophosphorus, administered as Na_2HPO_4 , from the intestine and its retention by various organs and tissues during the first 40 minutes following administration.

A number of studies have been made of the absorption of P^{32} from the gut of the rat, ⁽¹⁾ mouse, dog, and man, and numerous studies of P^{32} retention and distribution have been made after parenteral administration, as those of Lawrence *et al.* ⁽²⁾ However, most of these studies have not considered the early changes in the radiophosphorus concentrations within an animal, and no published accounts are available detailing the effect of adrenal-cortical deficiency upon the absorption of P^{32} from the rat intestine.

METHODS

Twelve mature male rats of the Sprague-Dawley strain, weighing 275 to 380 g each, were separated into two equivalent groups on basis of weight. One group of 6 animals was totally adrenalectomized under Nembutal anesthesia. Both groups were then placed on a restricted diet for 5 days by limiting their food to drinking water to which 1 per cent of glucose had been added. On this diet the average loss in weight was 50.2 g for the normal and 46.7 g for the adrenalectomized rats.

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On the fifth day, the animals were again anesthetized with Nembutal and 1 cc of radiophosphorus solution was injected directly into the lumen of the duodenum of 3 normal and 3 adrenalectomized rats, the pylorus and the small intestine at the ileocolic valve were firmly ligated. The remaining 6 rats were given similar amounts of P^{32} intraperitoneally.

The radiophosphorus solution, obtained from Oak Ridge, was of high specific activity (0.025 mc P^{31} per mg) and was adjusted with 0.2 M NaOH to pH 7.0. Dilutions were made so that each rat received a dose of P^{32} that was determined to be about 2 μ c or 5000 counts per second.

The animals were sacrificed by heart puncture, and all the blood possible was withdrawn either at 15, 30, or 40 minutes after administering the P^{32} . Since one of the adrenalectomized animals died before time for sacrifice, it is not included in the data. After the blood was obtained, 4.5- to 6-g samples of liver, 3.5- to 6.5-g samples of skeletal muscle from the posterior border to femur, and 1.2- to 1.6-g samples of cerebral tissue were obtained and weighed in tared crucibles. In addition, the stomachs, small intestines with contents, and anterior lobes of the pituitary glands were carefully removed and weighed. The right femurs were freed of soft tissue by dissection and weighed.

All tissues were dried in a hood under infrared lamps and then ashed in a muffle furnace at 550°C.

The ashed tissues were transferred to Coors evaporating dishes with three 1- to 1.5-cc washings of 6 M HCl and dried as before. The radioactivity of each sample was obtained in a standard fashion by means of a Geiger-Mueller tube and amplifier counter.

RESULTS

The results are given in Table 9 and Figures 7 and 8 where the concentration of P^{32} in the tissues is given as disintegration counts per second per g of tissue at 15-, 30-, and 40-minute intervals following injection.

DISCUSSION

The data show that intraperitoneally injected P^{32} is absorbed more rapidly into the blood than that injected into the ligated small intestine. The blood P^{32} reaches a maximum before 15 minutes in the intraperitoneally-injected animals and only after 30 minutes in the animals which received the P^{32} by direct injection into the gut. The high value for the P^{32} content of the blood of the adrenalectomized animal, 40 minutes after injection, is surprising and perhaps should be viewed with suspicion. However, it must be noted

Table 9

EFFECT OF ACUTE ADRENAL INSUFFICIENCY UPON ABSORPTION
OF P^{32} FROM THE INTESTINE

Minutes after administration	Per cent absorbed from intestine	
	Normal	Adrenalectomized
15	30.0	19.5
30	46.7	11.0
40	49.4	44.2

in Table 9, that this same animal at 40 minutes had absorbed 44.2 per cent of the total P^{32} administered, which was only about 5 per cent less than the amount absorbed by the 40-minute normal control animal. This animal may not have been suffering from such acute lack of adrenal hormones as the other two.

Absorption from the gut is retarded by the acute effects of adrenalectomy and NaCl deprivation, although absorption from the body cavity is not depressed. In fact, the P^{32} activity in the blood of the adrenalectomized animals which were injected intraperitoneally is consistently 25 per cent higher than that in the nonadrenalectomized animals. The significance of this observation can be determined only by further experimentation.

The P^{32} activity of nearly all organs studied is directly related to the P^{32} activity in the blood. The level of P^{32} concentration per g of wet tissue decreases in the following order; 1) small intestine, 2) liver, 3) pituitary, 4) blood, 5) bone, 6) stomach, 7) skeletal muscle, and 8) cerebral tissue. The very high P^{32} activity in the small intestine is due to the retention of unabsorbed P^{32} . Direct contamination of the surface of the stomach, liver, and small intestine in the intraperitoneally-injected animals is a complicating factor that tends to increase the observed P^{32} level of these tissues. Experiments using intravenous injection are planned to avoid this complication.

The skeletal muscle did not take up P^{32} as rapidly as other well-vascularized organs. Sachs⁽³⁾ also found that heart muscle of the cat took up phosphate ions slowly and concluded that the diffusion process was inadequate to explain the observation. He suggested that a phosphorylating mechanism was involved. It is noteworthy that in our experiments the liver, at 15 minutes following the administration of the P^{32} , had 5 to 10 times more P^{32} per g than blood.

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- U-67-39
- 42
- 42
- ADRENALECTOMIZED P^{32} INJECTED VIA INTESTINE
 - ADRENALECTOMIZED P^{32} INJECTED INTRAPERITONEALLY
 -◐..... NORMAL P^{32} INJECTED VIA INTESTINE
 - NORMAL P^{32} INJECTED INTRAPERITONEALLY

Figure 7. Amount of P^{32} present in the small intestine and liver expressed as counts per second per gram of tissue at 15, 30, and 40 minutes after administration.

Figure 8. Amount of P^{32} present in bone, stomach, cerebrum, blood, anterior pituitary, and skeletal muscle expressed as counts per second per gram of tissue at 15, 30 and 40 minutes after administration.

21-67-40

It is surprising that skeletal muscle in normal animals absorbed more radiophosphorus per g than corresponding muscle in adrenalectomized animals in spite of the lower blood P^{32} level in the normal animals. This observation needs further study for it is well known that adrenal-cortical deficiency has a marked effect on the contractility of muscles, and it is possible that this change in phosphorus uptake by muscle may be related to the transfer of the muscular inefficiency of adrenalectomized animals.

Popjak⁽⁴⁾ has shown that the transfer of $^{32}PO_4$ ions from plasma to liver cells is severely depressed by NaN_3 and, like Sachs,⁽³⁾ concludes that the transfer of PO_4 ions across cell membranes is accomplished by a phosphorylating mechanism associated with cell surface. It is possible that the presence of adrenal cortical hormones is in some way essential for the efficient transfer of the PO_4 ions across the cell membrane.

In conclusion, although it must be emphasized that these experiments are preliminary, it appears that the syndrome of acute adrenal insufficiency in the rat, complicated by NaCl deprivation and starvation, retards the absorption of phosphorus as Na_2HPO_4 from the intestine during the first 40 minutes after administration and perhaps retards the uptake of the phosphate ion from blood by skeletal muscle.

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PRELIMINARY REPORT: EFFECTS OF HIGH OXYGEN
CONCENTRATIONS UPON THE GROWTH OF A TRANSPLANTABLE
MOUSE CARCINOMA

by
B. V. Hall and R. D. Boche

INTRODUCTION

Since Warburg's initial experiments⁽¹⁾ showing that the oxygen requirements of tumors differ from those of tissues in general, a number of attempts have been made to inhibit or depress the growth of tumors by varying the oxygen pressure of the atmosphere that tumor-bearing animals breathe. Warburg⁽²⁾ and Campbell and Cramer⁽³⁾ kept tumor-bearing rats and mice in atmospheres with 5 per cent and 9 per cent oxygen, respectively, with partial success in limiting the growth of tumors. Other attempts to inhibit growth of tumors in rats and mice were made by de Almeida⁽⁴⁾ and Campbell⁽⁵⁾ using high oxygen pressures, as high as 8 atmospheres of oxygen. de Almeida reported destruction and hemorrhage of rat sarcomas, 10 days after exposing animal to 6 atmospheres of oxygen. In some of de Almeida's experiments 23 per cent of the tumors regressed.

Campbell⁽⁵⁾ and Pollack et al.⁽⁶⁾ obtained negative results using partial oxygen pressures of 20 to 100 per cent and total pressures of 2 to 5 atmospheres of oxygen. In view of these and other contradictory reports, we decided to investigate the effect of increased oxygen pressure, in a range not yet explored, upon the in vivo growth of a transplantable, rapidly-growing mouse carcinoma. Since Campbell and Cramer⁽³⁾ reported negative results with 60 per cent oxygen at a total pressure of 1 atmosphere and Fischer et al.⁽⁷⁾ observed tumor regressions after keeping mice in 1.6 to 2.0 atmospheres of pure oxygen for 18 to 24 hours, we chose to test the effect of 100, 87, and 74 per cent oxygen at 1 atmosphere.

METHODS

A large glass desiccator, with a two-holed stopper in the lid, was used as an oxygen chamber. The inlet tube was connected to a tank of pure oxygen in the first experiment and to a tank of oxygen to which sufficient nitrogen was added to bring the partial pressure of the oxygen to 87 per cent in the second experiment and 74 per cent in the third.

The desiccator was fitted with a wire-screen basket to hold soda-lime to absorb the CO₂, and a wire floor was added to support the mice.

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The chamber was further equipped with a water bottle so the mice could drink ad libitum, and a dish of CaCl_2 was placed below the wire floor to aid in reducing the relative humidity of the oxygen chamber.

The stream of gas leaving the oxygen chamber was allowed to bubble through 3 to 4 mm of water at a rate of nearly one bubble per second. The flow of gas was maintained at a uniform rate throughout each experiment except for 15- to 30-minute periods at 24- or 48-hour intervals when it was necessary to add food or water and clean the chamber.

Twenty-four Swiss-Urbana mice of both sexes were used in each experiment. Twelve of these were given bilateral-axillary implants of the carcinoma by means of a trocar 4 or 5 days before beginning the oxygen treatment. These tumor-bearing mice were divided into 2 equivalent groups. The tumor-free mice were likewise separated into 2 groups. Six tumor-bearing mice and 6 tumor-free mice were placed in the oxygen at the beginning of each experiment, and the other 12 tumor-bearing and tumor-free mice were placed in a standard cage kept in the room atmosphere at 25°C.

Tumor measurements were made with vernier calipers, and weights of all mice were obtained at the beginning and end of the period spent in the oxygen chamber. The food consisted of Derwood pellets, and the mice were allowed to feed ad libitum in the first experiment. In the second and third experiments, each group of mice maintained in the oxygen chamber and in room-atmosphere was given 50 g of pellets at the beginning of the experiment, and 48 hours later each group was given 50 g more. The mice in the oxygen chamber did not consume as much food or use it as rapidly as the mice kept in the room atmosphere.

The carcinoma used and the method of measuring and calculating tumor volumes were described by Hall.⁽⁸⁾

RESULTS

The data obtained in this study are summarized in Tables 10 to 12. When the tumor-bearing mice were kept in 100 or 87 per cent oxygen an inhibition and depression of tumor growth was produced. Twenty-five per cent (3 of 12) of the implants failed to grow until the host mice were removed after 82 hours in the 100 per cent oxygen chamber. Two of these inhibited tumors later grew at somewhat less than a normal rate to cause the death of the host mouse 37 days later. One week after the beginning of 100 per cent oxygen treatment the treated tumors were on the average only 17 per cent as large as the nontreated tumors, and 120 hours after beginning 87 per cent oxygen treatment the treated tumors were only 35 per cent as large as the control tumors.

Table 10

EFFECT OF EXPOSURE TO 100 PER CENT OXYGEN, AT 1 ATMOSPHERE, FOR 82 HOURS UPON TUMOR SIZE, BODY WEIGHT, AND SURVIVAL. FOOD AND WATER AD LIBITUM.

	Animals with tumors		Animals without tumors	
	100% O ₂	20% O ₂	100% O ₂	20% O ₂
At beginning of experiment				
No. of animals	6	6	6	6
Av. weight	22.1 g	21.7 g	22.0 g	20.2 g
No. alive after 82 hours	5	6	6	6
No. alive after 7 days	3	5	0	6
Av. wt. of animals	16.2 g	19.0 g	--	19.7 g
Av. loss in wt.	5.9 g	2.7 g	--	0.5 g
No. of tumors	3	10	--	--
Av. vol. of tumors	61 mm ³	365 mm ³	--	--
No. alive after 14 days	2	4	--	6
Av. wt. of animals	20.0 g	15.0 g	--	19.8 g
No. of tumors	3	8	--	--
Av. vol. of tumors	195 mm ³	796 mm ³	--	--
No. alive after 21 days	2	1	--	6
Av. wt. of animals	19.8 g	15.0 g	--	20.2 g
No. of tumors	3	2	--	--
Av. vol. of tumors	413 mm ³	1545 mm ³	--	--
No. alive after 28 days	2*	0	--	6
Av. wt. of animals	18.0 g	--	--	21.3 g
No. of tumors	3	--	--	--
Av. vol. of tumors	1459 mm ³	--	--	--

*Last oxygen-treated animal died after 40 days with a tumor having a volume of 2704 mm³.

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Table 11

EFFECT OF EXPOSURE TO 87 PER CENT OXYGEN, AT 1 ATMOSPHERE, FOR
92 HOURS UPON TUMOR SIZE, BODY WEIGHT, AND SURVIVAL. FOOD
RESTRICTION: 100 g FOR 12 MICE.

	Animals with tumors		Animals without tumors	
At beginning of experiment	87% O ₂	20% O ₂	87% O ₂	20% O ₂
No. of animals	6	6	6	6
Av. weight	22.8 g	22.8 g	23.8 g	23.0 g
No. alive after 92 hours	3	6	5	6
No. alive after 120 hours	0	6	0	6
Av. weight	17.8 g*	19.3 g	19.8 g	20.5 g
Av. loss in wt.	5.0 g	3.5 g	4.0 g	2.5 g
No. of tumors	6	11	--	--
Av. tumor size	154 mm ³	443 mm ³	--	--

*Three dead animals.

Table 12

EFFECT OF EXPOSURE TO 74 PER CENT OXYGEN, AT 1 ATMOSPHERE, FOR
90 HOURS UPON TUMOR SIZE, BODY WEIGHT, AND SURVIVAL. FOOD
RESTRICTION: 100 g FOR 12 MICE.

	Animals with tumors		Animals without tumors	
At beginning of experiment	74% O ₂	20% O ₂	74% O ₂	20% O ₂
No. of animals	6	6	6	6
Av. weight	22.5 g	22.5 g	23.2 g	23.1 g
No. alive after 90 hours	6	6	6	6
Av. weight	19.3 g	20.4 g	21.2 g	21.5 g
Av. weight loss	3.2 g	2.1 g	2.0 g	1.6 g
No. of tumors	12	12	--	--
Av. tumor vol.	726 mm ³	431 mm ³	--	--
No. alive after 6 days*	0	4	3	5

*At 5 days 1 animal in each group was sacrificed for tissue samples.

Thirty-three of the 36 implants made into the mice kept in room atmosphere grew normally, killing all but one of the host mice in 11 to 27 days after transplantation. One implant failed to grow and one mouse survived after the regression of its tumors.

It must be emphasized that exposure of both tumor-bearing and tumor-free mice to 100, 87, and 74 per cent oxygen concentrations for 82, 92, and 90 hours was highly toxic and that it was the probable direct cause of death of 75 per cent of the mice treated in pure oxygen within the first 7 days. The 87 per cent oxygen treatment killed all 12 mice within 120 hours, and the 74 per cent oxygen exposure proved fatal to 70 per cent of the treated mice within 6 days following the initial exposure to increased oxygen pressure.

The animals that died from breathing the concentrated oxygen showed at autopsy excessive edema and hemorrhage of the lungs, which had a "liver-like" appearance. The damaged lungs were probably unable to supply sufficient oxygen to maintain life in the 30 animals which died within a week after entering the oxygen chamber. Only 6 animals out of 36 survived longer than a week after initial exposure to the increased oxygen pressure.

All animals maintained in the oxygen chamber lost weight. The survivors of one group lost nearly 30 per cent of their initial weight, while the minimum loss for any group surviving the oxygen treatment was 15 per cent. In the second and third experiments the limitation of 100 g of food to each group of 12 animals during the period of oxygen treatment resulted in an average of 7 to 17 per cent loss of weight in the animals kept in room atmosphere. Since the animals in the oxygen chamber did not use all their food, while the animals in room atmosphere rapidly consumed all their food, it appears that one effect of the high oxygen pressure is to produce perhaps indirectly a reduced appetite and much loss of weight.

DISCUSSION AND CONCLUSIONS

These results are in agreement with those of de Almeida,⁽⁴⁾ Campbell,⁽⁵⁾ and others; namely, that lengthy exposure to high oxygen tensions is lethal to mice and rats. The damage to the lung observed in our experiments may be the "pneumonia" mentioned by other authors as the cause of death of mice and rats kept under high oxygen pressures. The "oxygen poisoning" investigated by Stadie⁽⁹⁾ is of different origin since it causes death with convulsions, which develop in a matter of 30 to 60 minutes when animals are exposed to several atmospheres of oxygen. The extensive damage to the lung is the probable cause of the great debility, loss of weight, and anorexia, which lead to the death of 83.3 per cent of the animals exposed to high oxygen concentrations.

The influence of the nutritive state of host mice upon the growth of tumors can be a very great one as shown by Bischoff, Long, and Maxwell.⁽¹⁰⁾ Using sarcoma 180, they found that a 50 per cent reduction of caloric intake produced a ten-fold retardation of tumor growth 15 days after inoculation, although a 67 per cent reduction had little effect. These studies support our conclusion that the marked reduction in food consumption by the oxygen-treated animals was the cause of any retardation of tumor growth.

It is likely that the slight evidence for inhibition of the growth of the transplanted carcinomas in animals kept for 82 and 92 hours in 100 and 87 per cent oxygen is due to the resulting general debility of the host animal rather than to any direct effect of the increased oxygen tension upon the growth of the tumor. We agree with Campbell⁽⁵⁾ that variations in the partial pressure of oxygen, by themselves, are of no value in limiting the growth of tumors.

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PROGRESS REPORT: RENAL FUNCTION IN THE CHICKEN FOLLOWING X IRRADIATION

by

S. Phyllis Stearner, A. M. Brues, and Emily J. B. Christian

Blood uric acid has been shown to increase rapidly in the chicken following X irradiation (1000 r, 43 r per min.), indicating a possible early impairment of renal function. To explore irradiation effects on renal function, phenol red excretion has been measured on newly-hatched chicks following exposure to 1000 r at 43 r per min. Phenol red (0.2 mg per chick) in physiological saline was injected intraperitoneally and the excreta collected for 30 minutes following injection. The collected urine was made alkaline, diluted to 15 cc and the phenol red concentration was determined colorimetrically. The results obtained from control and irradiated chicks are shown in Table 13. The data indicate that there is almost complete inability to excrete the dye as early as 1 to 2 hours after irradiation. The loss of excretory function is incomplete 1/2 hour following irradiation, and it has been noted previously that blood uric acid correspondingly fails to rise at once after irradiation.⁽¹⁾ This strongly suggests that the uric acid increase is the result of renal failure rather than of an important alteration in nitrogen metabolism.

Production of radioactive uric acid (C^{14}) by biosynthesis is under way. Samples of low activity were obtained from chicks that had been fed C^{14} -containing alfalfa. However, chicks injected with C^{14} -sodium formate yielded uric acid of relatively high activity. With this tracer it will be possible to compare the rate of uric acid excretion by control and irradiated chicks, and, at the same time, to obtain quantitative information relating to uric acid metabolism and excretion in young chicks.

Table 13

EFFECT OF 1000 r X IRRADIATION (43 r PER MIN.) ON
PHENOL RED EXCRETION

Time between irradiation and phenol red injection	No. of animals	Per cent of dose excreted
		1/2 hour after injection
No irradiation	17	30.7
1/2 hour	2	8.8
1 hour	11	3.3
2 hours	4	3.5

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A PHASE CONTRAST MICROSCOPY STUDY OF X-RAY-INDUCED PYCNOSIS IN THE LIVING CELL

by

T. N. Tahmisian and Dorothy M. Adamson

ABSTRACT

The effect of X radiation ranging from 25,000 r to 200,000 r can be kept latent in Melanoplus differentialis embryos for six months by maintaining them at submetabolic temperatures. If diapause eggs are irradiated and left at 25°C, pycnosis of the nuclei takes place by eight days. When the eggs are irradiated and placed immediately at 0°C and kept at this temperature for six months no morphological or physiological changes are observed upon returning them to 25°C. However, pycnosis occurs by eight days at 25°C depending not only on the radiation dosage but also on the metabolic activity of the cell. Pycnosis in the living cell, as observed by phase contrast microscopy, resembles fixed material morphologically. Physiologically there is a difference in the physical state of the nuclei when observed in the living condition. Most nuclei show a drop in viscosity at the time of pycnosis as judged by the increase in Brownian movement. Vital staining with Janus green indicates that the mitochondria usually clump as a separate body whereas the chromatin material becomes pycnotic containing lipoid impregnated bodies.

PROGRESS REPORT: THE EFFECT OF ANOXIA ON X-RAY-INDUCED
INJURY IN MELANOPLUS DIFFERENTIALIS EMBRYOS

by

T. N. Tahmisian and Dorothy M. Adamson

X-ray-induced negative growth and the increment of some oxidative mechanisms were discussed in previous communications.^(1,2) Greatest negative growth and increment in an enzyme having the capacity to oxidize hydroquinone followed exposure to 25,000 r X radiation.

The purpose of our present investigations is to determine the effect of X irradiation upon anoxic embryos.

MATERIALS AND METHODS

Preliminary studies have shown that survival of diapause eggs of M. differentialis is not affected by a 24-hour exposure to an atmosphere of N_2/CO_2 (95 per cent/5 per cent).

Diapause eggs were dechorionated, sterilized, and placed in a special chamber (Figure 9). N_2/CO_2 gas (95 per cent/5 per cent) was passed over reduced copper shavings in a furnace to remove any traces of free O_2 . For 4 hours the gas was bubbled through distilled water, introduced into the chamber containing the embryos, and passed into another water trap. The stopcocks at both ends of the chamber were then closed, and, about 22 hours later, the embryos within the container were given 25,000 r X radiation. At the end of 24 hours at room temperature (25°C) the embryos were removed into room atmosphere.

Half of the anoxic eggs was placed at 0°C to break diapause and the other half was used to determine whether irradiation had produced an increase in hydroquinone oxidase.

The Warburg apparatus was used to determine oxygen utilization.

RESULTS

Diapause eggs exposed to 25,000 r X radiation in room atmosphere showed an increase of about 80 per cent in hydroquinone oxidase over a period of 14 days (Figure 10). Eggs exposed to the same dose but in N_2/CO_2 (95/5 per cent) showed a 20 per cent decrease as compared with controls.

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Figure 9. Nitrogen-carbon dioxide gas chamber.

Figure 10. Per cent oxygen uptake of eggs irradiated during anoxia and eggs irradiated in air.

24-67-54

Embryos that were maintained at 25°C following X irradiation in N_2/CO_2 did not undergo negative growth. However, negative growth was quite pronounced in the embryos that were irradiated in air and maintained at room temperature. The embryos kept at 0°C after irradiation in N_2/CO_2 atmosphere underwent blastokinesis and positive growth when returned to room temperature.

DISCUSSION

It is of interest that grasshopper embryos have the capacity to survive a 24-hour period of complete anoxia.

The diapause egg has a density that is higher than water, a volume that is slightly less than 5 mm³, and a weight of 5 mg. Therefore, if such an egg were totally saturated with oxygen at a partial pressure of 152 mm Hg, as found in air, with a solubility constant of 0.028 and an oxygen utilization rate of 0.25 mm³ at 25°C per egg per hour, it would require 40 minutes to use up the oxygen. When eggs with the above oxygen saturation are placed in an atmosphere of N_2/CO_2 (95 per cent/5 per cent), the environmental oxygen tension about the egg drops from 152 mm Hg to 0 mm Hg, and under these conditions of environmental oxygen deprivation it is possible that the dissolved oxygen within the egg is not completely used up, but an equilibrium is reached where no more oxygen can be metabolized, thus leaving some molecular oxygen in solution.

In these experiments X radiation was administered at a time of very low metabolic activity. It is unlikely that anaerobic glycolysis takes place in insect material.⁽³⁾ Therefore, metabolism cannot occur under anaerobic conditions. If molecular oxygen is necessary for the formation of free radicals that oxidize the components of a cell, some oxygen that is still available could go into the formation of X-ray-induced radicals. On the other hand, the lowered injury observed in material maintained under anoxic conditions may be due to a lowered metabolic rate rather than a lowered free radical formation.

More detailed cytological, cytochemical, and physiological studies will be carried out in the near future.

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PROGRESS REPORT

by
R. J. Flynn

A survey has been made to ascertain the cost of animal breeding and the value of the animals so obtained and utilized. As a result of this survey the breeding program has been curtailed, with the exception of C-58 mice; routine breeding is considered unnecessary, since equally good animals of all other strains and species are readily obtainable from other sources.

During this quarter a census was taken of all transplantable neoplasms that were being maintained in the Division. The results showed that ten types were being used by six staff members. In order to establish greater efficiency, the maintenance of seven of these neoplasms was centralized in our group, the other three tumors are readily available from outside sources. The seven now being maintained are given in Table 14.

A program of mechanization has been initiated. Portable, automatic faucets, like those that are used in restaurants to fill drinking glasses, have been installed. A hose connection is attached to the water supply and the faucets, which are mounted on a portable table, are wheeled up to the cage rack. This eliminates the tedious job of carrying hundreds of drinking bottles from cage to sink and back again.

A Kerrick Hydro-steam Kleaner has also been purchased. It is a small, steam-operated machine, which, when attached to a high-pressure steam line (100 lbs.), emits a pressure stream of properly concentrated cleaning solution. Dry steam picks up and heats a pre-mixed detergent, and wet steam furnishes velocity. The machine has proved to be extremely efficient for cleaning all types of cages and such large pieces as dog cages and broiler plants.

ANL STANDARD MOUSE CAGE

The mouse cage development program, initiated in 1948 by Dr. Miriam P. Finkel, was completed during this quarter with her assistance and with the aid of many other members of the staff. The new model (Figures 11 and 12) retained all the good features of the previously-used cages plus many new ones.

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Table 14
TRANSPLANTABLE NEOPLASMS MAINTAINED IN THE ANIMAL QUARTERS

Name	Host	Investigator	Approximate no. of transplant generations	When palpable (days)	Survival time (days)
Undifferentiated sarcoma (formerly designated as an osteogenic sarcoma)	CF#1	M. Finkel	24	10-14	30-60
Fibrosarcoma	SD	D. Gardiner	14	10	75-90
Lymphoma (localized)	CF#1	J. Passonneau	21	7-10	60
Lymphoma (localized)	CF#1	"	22	"	"
Lymphoma (localized)	C-57	"	19	"	"
Mammary carcinoma	C ₃ H	A. M. Brues	50	7	90-120
Anaplastic carcinoma	Swiss- Urbana	B. V. Hall	343	3	14-28

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PHOTOGRAPHED BY ATLEE S. TRACY

Figure 11. Two views of animal farm mouse cage.

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PHOTOGRAPHED BY ATLEE S. TRACY

Figure 12. Mouse cage with water bottle and food pellets in place.

Some of the features that were retained are listed below:

1. Dimensions: The cage is designed for 15 animals. The outside dimensions are $7 \frac{1}{4}$ " high, $8 \frac{5}{8}$ " wide, and 15" long.
2. Screen support: The method of bending in the sides and back so that the screen is supported on the outside of the cage is unchanged.
3. Collecting pan: The use of a collecting pan for easy daily cleaning is continued.

Many of the new ideas that were incorporated are as follows:

1. Material: For decontamination purposes and for durability, the cage is constructed entirely of number 304 stainless steel. This includes the floor screen, latch, latch bolt, cap nut, hinge, and hinge pin.
2. Ventilation: Instead of screening at the top, back, and sides, the new cage employs a total of $54 \frac{1}{4}$ " x $2 \frac{1}{2}$ " perforation at the top and back only for ventilation. Thus the cage is easier to clean, cheaper to build, and stronger. The side perforations were omitted in order to decrease the chances of spreading contamination or infection from cage to cage and also to provide a similar environment whether a cage be placed in the center of a row or at the end.
3. Floor screen: The .062" diameter, $2 \frac{1}{2}$ mesh per inch, intermediate crimp, stainless steel screening was chosen after checking many gauges, meshes, and types. It was shown to be sufficiently rigid and not too heavy, and the wires were found to be woven the proper distance apart so that the mice do not catch their ankles as they do with the old style 3-mesh per inch wire.
4. Door catch: The door catch is mounted on the outside of the cage so that proper closing of the cage can be determined by sight rather than by pull.
5. Water bottle: For ease of cleaning, the cage was designed to take a $\frac{1}{2}$ -pint, straight-sided, clear, glass water bottle, fitted with a neoprene stopper and a straight nipple. The bottle is mounted in the front of the cage, rather than the side, for easy accessibility and to conserve space.

6. Food container: The feeder is moved from the rear to the front of the cage so that it is more accessible and also to eliminate the cul-de-sac on either side of a feeder mounted in back. The cover prevents contamination of the food by stray rodents.
7. Cage door: The cage door contains the water bottle, the food container, and holder for the usual 3 x 5" identification card, plus a smaller holder for an added 1 1/2 x 5" clinical card. These features on the door permit a straight front when the door is closed, and large opening with the familiar cut back top, when it is opened.

PROGRESS REPORT: CHEMICAL DOSIMETRY

In the course of a study of growth hormone inactivation by ionizing radiation, it became necessary to evaluate the total energy absorbed by irregular glass volumes subjected to external X radiation. The lack of uniformity of the radiation, in conjunction with geometric and scatter considerations, frequently makes calculations of integral dose received both difficult and subject to error.

The auxin, indoleacetic acid (IAA), is smoothly inactivated by X radiation, the reaction proceeding with first order kinetics. Moreover, IAA can be assayed in rather low concentrations by the $\text{FeCl}_3\text{-HClO}_4$ method described below. Utilization of IAA as a quantitative chemical dosimeter thus seemed feasible.

Known volumes of IAA in water were irradiated by a 250-kv Maximar X-ray machine with a beam of $0.157 \text{ \AA}$ effective wavelength. Integral doses were calculated, correcting for absorption and scatter, with a calibrated Victoreen nylon thimble as a basic standard. In Figure 13, curve A, is shown the effect of the X-ray dose on the concentration of IAA remaining in a solution originally 0.05 millimolar. From these averaged data the amount of IAA inactivated as a function of dose is plotted in Figure 14.

In order to check the use of IAA for dosimetry against the Victoreen thimble, a cylindrical glass cell containing IAA in water was placed in a $0.133 \text{ \AA}$ beam. The total integral dose was determined colorimetrically, the dose rate at the incident surface then being calculated. It differed by less than 5 per cent from a direct nylon thimble determination.

The radiation sensitivity of IAA can be greatly increased by saturating the water with chloroform. This is indicated by the "B" curves in Figures 13 and 14. A dose of 100 r can be measured by this modification. It is possible that even smaller total energies can be evaluated by IAA in water-saturated chloroform; colorimetric assay could be carried out by extraction of the uninactivated IAA into an aqueous phase as a salt.

With suitable phantoms, the above methods should be applicable to the measurement of integral doses received by irregular volumes, or the absorption of heterogeneous radiation. The suitability for measurement of radiation energy absorbed from beta emitters in solution is being investigated.

LOG CONCENTRATION
 $\mu \text{ mol} \times 10^3 / \text{cc}$

Figure 13. Residual concentration of indoleacetic acid as a function of X-ray dose.

- A. In water
- B. In water saturated with CHCl_3

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Figure 14. Micromols indoleacetic acid inactivated as a function of X-ray dose.

A. In water

B. In water saturated with CHCl_3

COLORIMETRIC ESTIMATION OF INDOLEACETIC ACID

by
S. A. Gordon and R. P. Weber

ABSTRACT

Existing methods for the colorimetric assay of indoleacetic acid (IAA) lack specificity, sensitivity, and stability of the color complex formed. The utility of a colorimetric method in our inactivation studies, and the wide use of IAA in physiological and biochemical experiments, made elimination of the above-mentioned faults desirable.

The following points were investigated:

1. Nature of the complexing metal (Sr, Ti, Sn, Cu, Ce, Cs, Ni, U, Cd, Hg, Co, and Fe).
2. Acid medium (H_2SO_4 , HCl , and HClO_4)
3. Ratio of reagents
4. Wavelength of maximum absorption
5. Time at which maximum color is reached and stability of the color
6. Effect of added H_2O_2
7. Stability of reagent
8. Specificity to other 3-substituted indoles (indole, skatole, indole-aldehyde, indole carboxylate, indole propionate, indole butyrate, and tryptophan)

The procedure given below has been adopted as the most sensitive and specific of the methods studied. The stability of the color formed allows a large number of determinations to be made simultaneously without adherence to a fixed time schedule:

1. To a 1.0-ml aqueous aliquot add 2.0 ml of reagent
2. Read after 25 minutes at $530 \text{ m}\mu$.

Range: 0.1 - 0.2 to $45 \mu\text{g}$ per ml.

Reagent: 1.0 ml 0.5 M FeCl_3 , 50 ml 35 per cent HClO_4 .

A MODIFIED DIGESTION-DISTILLATION APPARATUS FOR WET CARBON PROCEDURES

by
R. E. Stutz and S. A. Gordon

ABSTRACT

Contamination of BaCO_3 by BaSO_4 may be prevented in a wet combustion procedure if the modifications suggested by Steele and Sfortunato⁽¹⁾ are followed. They make use of an intermediate trapping and regeneration stage after combustion and before formation of BaCO_3 , which frees the CO_2 of SO_3 .

The apparatus here described (Figure 15) has combined Steele's two stages into a single unit. It is thus unnecessary to transfer the first stage receiver to a second unit for regeneration of the CO_2 , reducing the possibility of contamination from atmospheric CO_2 . The use of hot and cold water baths to facilitate final diffusion of CO_2 can be eliminated in the present design without excessive time being required for CO_2 diffusion. The capacity of the system has also been increased to permit larger sample sizes, thereby reducing the sampling error. The reagents used are the same as those described by Steele.

Aqueous samples of plant extracts may be either dried in the oxidation flasks or taken nearly to dryness. Plant tissue extracts should be near neutrality before drying to minimize the loss of any volatile acidic or basic components.

Approximately 200 to 300 mg of KIO_3 is added to the sample in the oxidation flask, the neck is lubricated with syrupy H_3PO_4 and securely attached to the oxidation side. The oblique bore capillary stopcock (#1) is also lubricated with H_3PO_4 and held in place with a rubber band.

Approximately 4 to 5 ml of 6 N H_2SO_4 is introduced into the side arm of the first stage receiver flask, the side arm being secured by rubber bands. This receiver flask is then evacuated and flushed with nitrogen several times on the apparatus shown in Figure 16, before the introduction of about 10 ml of 0.8 N NaOH containing 2 per cent hydrazine sulfate. The flask is then inserted directly into the apparatus.

The large stopcock (#4) is closed between the first stage receiver and the second stage receiver which contains the Ba(OH)_2 , permitting the first stage to be flushed with CO_2 -free air. The second stage receiver is also flushed with nitrogen before the introduction of about 10 ml of the $\text{Ba(OH)}_2 \cdot \text{BaCl}_2$ reagent; 1 to 2 ml of 0.5 per cent aerosol solution (Fisher)

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Figure 15. Wet combustion apparatus.

U-67-67

Figure 16. Flask flushing apparatus.

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is added to improve filterability of the carbonate at the time the flask is put on the apparatus. The whole system is then flushed several times with CO_2 -free air, evacuated, and both the second stage receiver and the evacuation stopcocks (#3 and 4) are closed off. This leaves the oxidation flask and the first stage receiver connected.

Three to 20 ml of the Van Slyke Folch oxidizing mixture (roughly 0.5 ml per 1 mg of carbon) is introduced into the oxidation flask from the measuring tube, leaving a solid column of reagent in the capillary. The mixture is gently heated without boiling until the gas evolution ceases (about 2 minutes). It is then boiled gently for 2 minutes. The first stage receiver flask is immersed in liquid air, and the oxidation mixture boiled vigorously for 2 additional minutes. The oblique bore stopcock (#2), between the oxidation flask and the chilled first stage receiver, is closed, and the contents of the receiver are thawed, and then shaken for 15 minutes to permit the absorption of all the CO_2 , SO_3 , and any oxides of nitrogen that may have entered. The stopcock (#4) to the second stage receiver is opened, the side arm inverted, and the apparatus shaken for 2 hours at the lowest speed on a Burrell shaker to permit diffusion of the CO_2 into the $\text{Ba}(\text{OH})_2$.

The apparatus will handle 15 mg of carbon. It is suggested that samples containing more than this quantity of carbon be avoided because of excessive gas pressure.

The BaCO_3 may then be quantitatively filtered off on an "M" porosity Pyrex fritted disk (as is suggested by Steele), washed, dried, and weighed. Specific activity may be determined on the same carbonate samples.

The recoveries obtained with the above apparatus for the oxidation of several samples of sucrose are given in Table 15.

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Table 15

CARBON RECOVERIES FROM SUCROSE OXIDATION

Mg carbon (theoretical)	Mg sucrose oxidized	Mg BaCO ₃ (theoretical)	Mg BaCO ₃ recovered *	% Recovery
1.0	2.38	16.2	16.5	102
1.0	2.38	16.2	17.5	108
2.0	4.76	32.4	31.3	97
5.0	11.9	80.9	82.8	102
5.0	11.9	80.9	79.0 †	98 †
10.0	23.8	162	161	99
15.1	35.9	244	260	106

*By direct weighing.

†Shaken 1.5 hours instead of 2 hours.

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Microbiology

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PRELIMINARY REPORT: AUTOAGGLUTINATION IN PARAMECIUM
BURSARIA FOLLOWING EXPOSURE TO ULTRAVIOLET AND TO
X RADIATION

by
C. F. Ehret

Investigation of the effects of electromagnetic radiations upon the mating reaction of Paramecium bursaria has been extended to the far ultraviolet region. In previous reports from this laboratory, the effects of visible light^(1,2) and X rays⁽³⁾ on the mating reaction have been indicated.

Autoagglutination may be defined as the sticking together of animals of the same mating type. When this process leads to the production of conjugants, the entire process is more specifically termed "selfing." In normal clones of P. bursaria either process is very rarely encountered, and, prior to this study, selfing had never been detected in the stocks of this laboratory.

Autoagglutination was recently observed in populations of P. bursaria following exposure to ultraviolet radiations, and some of the results of subsequent investigation of the phenomenon are the subject of this report.

The ultraviolet source consisted of a battery of 2 GE T-8, 30-watt germicidal lamps (principal output at λ 2537 Å) mounted in parallel beneath an aluminum reflector, and at a distance of 20 inches from the exposure position. The approximate incident energy density at this position was 150 ergs per mm² per sec. This value is considerably higher than the average energies incident upon the paramecia because of absorption by the medium, some surface reflection (< 4 per cent), and scattering by the animals themselves.⁽⁴⁾ The absorption of 99.9 per cent of the incident energies by 10 mm of animal-free medium at 225 mμ was determined spectrophotometrically. In most experiments, fluid-depth approximated 1 mm, and, by applying Lambert's law, the midplane intensity (at 0.5 mm) was calculated to be 69 per cent of the incident radiant energy. No attempts were made to measure the energy absorbed by the animal population for quantum efficiency determinations in this series. Post-ultraviolet autoagglutination proved to be a dose-dependent and highly reproducible phenomenon, grossly indistinguishable from the early stages of the normal mating reaction.

It was, with the exception of one experiment,* never followed by selfing. Immediately after exposure, there was no tendency to stick, despite animal velocities and concentrations high enough to insure an adequate collision frequency; the earliest autoagglutinations appeared at about 5 minutes, and the reaction peak occurred between 15 and 30 minutes following exposure to sublethal doses. Concomitant physiological damage, as evidenced by vesiculation, rotation, bleb-formation, immobilization of body cilia, some cytolysis at higher doses, resembled that described in detail in the early literature.^(4,5)

A sigmoid dose response curve was obtained, with weak autoagglutination reactions (<1 per cent) after exposures of 60 seconds, and strongest reactions (50 to 75 per cent) after exposure periods of 120 to 150 seconds, followed by a sharp decrease in autoagglutination after exposures above 150 seconds. The peak reactions were not a consequence of increased collision frequency, since after exposures longer than 60 seconds, there was a decrease in animal velocity. However, after exposure-times in excess of 150 seconds, animal velocity approached zero and became an appreciable factor. In such cases, the animals were agitated by shaking the spot plate, collisions were observed microscopically, and any tendencies toward agglutination were noted. By this method, an unmistakable decrease in agglutination was measured at the higher dosages. Reduction in stickiness was further verified by introduction of lively animals, previously exposed to ultraviolet for 90 to 120 seconds. These indicator animals showed relatively weak tendencies to agglutinate with immobilized, high-dose animals, despite frequent collisions with them.

The possible role of the fluid in postirradiation autoagglutination was tested in several ways with animals of stock So6-C (*P. bursaria*). Animal-free fluid was irradiated, and nonirradiated animals were added to it. Also, animals were irradiated in fluid, and allowed to remain together for periods of 30 seconds, 15 minutes, and 30 minutes before filtration. Immediately after filtration (through Whatman No. 24 filter paper), the animal-free filtrate was tested with nonirradiated animals. In none of these cases did the fluid have any effect upon the tendency of animals to stick together, and no autoagglutination was observed. Irradiated animals transferred to fresh fluid immediately after irradiation underwent typical autoagglutination. It is therefore probable that the effect is a direct one upon the animal itself.

It was of interest to determine whether certain categories of animals, which do not participate in the preliminary agglutinations or mating reactions of unmated sexually mature animals, would undergo autoagglutinations following ultraviolet irradiation. These categories include 1) conjugants, 2) normal animals during their dark period, and 3) immature (recently

*The animals were irradiated for 150 seconds 6 hours after the start of the dark period and retained in darkness for 8 hours.

exconjugant) clones throughout their "infancy." Each of these three categories responded by autoagglutination following exposure to ultraviolet. The dose response of the unmated categories 2) and 3), was indistinguishable from that of the normal animals autoagglutinating in their light period. Conjugants were more resistant by a dosage-time factor of about 30 seconds, with a reaction peak occurring after 180 seconds exposure.

Chlorella-less animals as well as animals representing three other species of Paramecium were also tested to determine the universality of the reaction. The reaction with the chlorella-less P. bursaria was qualitatively identical with that of the normal parent clones. The other species harvested and tested at the "ring-culture" (and presumably more sexually-reactive) stage in no case showed any autoagglutination in dosage ranges extending from 30 seconds to the vegetative lethal dose (cytolysis at 240 to 300 seconds).

Stocks tested were P. aurelia (Sonneborn, Variety IV 51.8K), P. caudatum (Gilman, L,6), and P. multimicronucleatum (Genl. Biol. Supply).

P. multimicronucleatum after irradiation showed a greater tendency to stick to bacterial debris when debris was present in the fluid. However, there was no autoagglutination in debris-free fluid, despite a high collision frequency, and intense crowding of animals peripherally at the surface at higher doses (120 to 210 seconds). It has also been observed that X rays induce the same phenomenon.

Populations of P. bursaria were X-irradiated with a 50-kv, 40-ma machine (no filters), at a distance of 10 mm from the source. At dosages of approximately 5×10^5 r, and 7.5×10^5 r, a maximum amount (between 5 to 10 per cent) of autoagglutination occurred, approximating the degree of autoagglutination in controls exposed to ultraviolet for seconds. At 10^6 r, animals were almost completely immobilized and exhibited no tendency to stick to each other even after being closely aggregated by shaking. This is in contrast to the observation of Wichterman⁽⁵⁾ who reported no autoagglutination, but instead a phenomenon "strongly suggestive of the mating reaction" in which living animals clumped around intact dead and disintegrating animals. He concluded "this appears to be a simple food-reaction...." Whether or not this constitutes a stock difference remains to be tested. Aggregation on "protoplasmic-islands"⁽⁵⁾ has also been observed in these studies subsequent to ultraviolet- and X-ray-induced autoagglutination in many reactions, and in nearly all reactions in which some animals are lysed. There is little difficulty in distinguishing this phenomenon from autoagglutination if 1) the medium is free of debris, and 2) observations are made between 15 and not later than 30 minutes after irradiation.

In summary, the autoagglutination that has been observed following ultraviolet and X irradiation in P. bursaria is grossly similar to the mating reaction agglutination observed between opposite mating types of

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sexually reactive normal animals during their light period. Unlike the mating reaction, post-ultraviolet autoagglutination occurred between animals of the same mating type in their dark as well as in their light period, between animals of sexually immature clones, and between conjugant pairs. Representative stocks (sexually non-photoperiodic) of three other species of Paramecium showed no post-ultraviolet autoagglutination.

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ANL-4488
(EXCERPT)BiochemistryDr. E. S. Guzman Barron
Mrs. Veronica Bonzell
Mrs. Betty Redfield
Miss Louise SekiPROGRESS REPORT: STUDIES ON THE MECHANISM OF ACTION OF
IRRADIATED WATER ON CELL METABOLISM

by

E. S. G. Barron and Betty Redfield

It was shown previously⁽¹⁾ that the respiration of sea urchin sperm was inhibited when the cells were suspended in X-irradiated sea water or artificial sea water. The mechanism of this inhibition is still unknown. The effect is due to the formation of stable substances, since inhibition has been observed even 50 minutes after irradiation.

ClOH formation: The possibility that the toxic substance might be ClOH, formed by combination of OH radicals with Cl^- , has been studied. For this purpose, a very sensitive test for the detection of ClOH was used based on the so-called Wolter's test.⁽²⁾ Freshly prepared artificial sea water was irradiated with 100,000 r, 200,000 r, and 500,000 r, at a dose rate of 7200 r per minute. At the end of irradiation the water was tested for ClOH. None was detected.

The influence of electrolytes on H_2O_2 formation in X-irradiated water: Bonet-Maury and Lefort⁽³⁾ have recently studied the influence of the H^+ ion concentration on the production of H_2O_2 by X irradiation of water. They found that H_2O_2 formation depended on the pH value of the solution, on the amount of dissolved oxygen, and on the temperature. We have found that the concentration of electrolytes has also an effect on H_2O_2 formation. This was first observed in irradiated sea water, artificial sea water, and freshly distilled water. Irradiation of distilled water in the presence of TiSO_4 (trapping reagent for H_2O_2) and H_2SO_4 gave the highest values; filtered sea water the lowest (Table 16).

Addition of electrolytes to water depressed H_2O_2 formation to different degrees. In these experiments the X-ray dose was kept constant (100,000 r) while the concentration of electrolyte was varied. NaNO_3 had the greatest inhibiting effect. (This is probably due to slight contamination of the sample with NaNO_2 .) CaCl_2 was next in inhibitory action. The depressing effect of NaCl and KCl was about the same (Figure 17). K_2SO_4 had no effect.

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Table 16

H₂O₂ FORMATION ON X IRRADIATION OF WATER
(150,000 r)

Experimental Conditions	H ₂ O ₂ , μ g in 3 cc	Inhibition per cent
H ₂ O + TiSO ₄ + H ₂ SO ₄	24.8	
H ₂ O + H ₂ SO ₄	23	7.3
H ₂ O	19	23.4
Artificial sea water	7.1	71.2
Filtered sea water	5.8	76.7

Figure 17. Effect of salts on the formation of H_2O_2 by X irradiation of distilled water.

Effect of X-irradiated water on sperm respiration: To determine the influence of salts on the inhibition of respiration of sea urchin sperm produced by irradiated sea water, freshly distilled water was irradiated with 200,000 r. At the end of irradiation the necessary amounts of the different salts of sea water were added, and the solution was left standing for one-half hour. At the end of this time sperm were added. The respiration of sea urchin sperm suspended in this irradiated water was 90 per cent inhibited. These experiments demonstrate that the inhibiting effect is independent of the salt content and is due exclusively to the formation of stable products in distilled water.

Whether this inhibition is due to the H_2O_2 produced on irradiation of distilled water is not yet certain. In one experiment, distilled water was irradiated with 200,000 r and was divided into two portions; to one of which a piece of platinized platinum was added. The two flasks containing the water were kept at 3°C for 50 minutes, the flasks being shaken occasionally. At the end of this time sperm were added to the two flasks and the respiration was measured for two hours. The platinum had destroyed 57 per cent of the H_2O_2 present in the irradiated water. The respiration of sperm in irradiated water (with salts added after irradiation) was 75 per cent inhibited; the respiration of sperm in irradiated water which had contained platinum was 55 per cent inhibited. These experiments are being continued in order to determine definitely whether the H_2O_2 produced on irradiation of water is responsible for the inhibition of respiration.

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Radiochemistry

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PROGRESS REPORT: RADIOCHEMISTRY

by
John H. Pomeroy

During this period, construction of the organic laboratory was completed, and installation of equipment and supplies has proceeded. In addition, work on the following problems has been carried out.

Reduction of cystine-S³⁵ to cysteine-S³⁵ hydrochloride: Cystine-S³⁵ was reduced to cysteine-S³⁵ by the use of palladium black and hydrogen, using the general procedure of Bergmann and Michelis.⁽¹⁾ A 15-ml centrifuge tube was used as the reaction vessel, in which was placed 100 to 400 mg cystine, dissolved in 2 to 5 ml N HCl and 100 mg palladium black. At room temperature under 50 psi hydrogen in the Parr Pressure Reactor, type 3911, the reaction was complete in 12 hours. It was found necessary to "age" the catalyst by using it on a number of preliminary "cold" runs before using it on a sample containing S³⁵. Fresh catalyst gave a persistent yellow color to the reduced cysteine which could not be removed by recrystallization. The cysteine was recovered as the hydrochloride, dried in vacuo, and stored under nitrogen. Yields of 95 per cent were obtained of material with a melting point of 184 to 185° (literature value, 184°).

Kavanagh's procedure, using a colloidal palladium catalyst suspended on polyvinyl alcohol,⁽²⁾ was tried, but separation of the product from the catalyst was found to be difficult on a micro scale.

The cystine-S³⁵ was obtained from Dr. H. Tarver, at Berkeley, California. The cysteine-S³⁵ hydrochloride has been given to Dr. Harvey M. Patt, who will use it in further studies of the mechanism of radiation protection by cysteine.

Irradiation of solutions containing stable free radicals: It is believed that the primary effect of radiation on solutions is that of producing molecular fragments known as free radicals. These are usually highly

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reactive and exist for only a short time in solution. However, certain organic compounds that are stabilized by their molecular structure can exist in the free radical form and can serve as convenient model compounds for further study of free radical behavior. One of the reactions that free radicals can undergo is that of direct combination, $A^{\cdot} + B^{\cdot} = A:B$. This reaction can also take place with DPPH in combining with the radicals formed by irradiation; in this process the intense purple color of the DPPH disappears. This decolorization is being investigated for the purpose of seeing if it may be used in radiation dosimetry, and also as a method of studying the chemical effects of radiation on solutions; since DPPH may be considered to be a trap for reactive radicals.

Recrystallized DPPH was dissolved in repurified chloroform; the solution contained 26.6 μg per ml. Pyrex capsules containing 5-ml portions of this solution were exposed to successive doses of 100 kv X radiation up to 16,000 r, at the rate of 490 r per min. The optical density of the irradiated samples at 650 $m\mu$ dropped from 0.281 at zero dosage to 0.024 at 2000 r. When plotted on semi-log paper, this gave a straight line passing through six points. There was an abrupt break at 2000 r and further irradiation diminished the optical density only slightly. The values of optical density are not stable. Upon standing, the irradiated samples between 0 to 2000 r become still lighter in tint. Investigations on the cause of this fading are under way. It is believed that dissolved oxygen may be responsible, or that other secondary effects may be taking place.

Attempts to prepare a radiation-sensitive gel containing the hydrazyl were not successful. Chloroform solutions of DPPH were dispersed in gelatin or agar to give stable rigid gels upon cooling, but radiation up to 20,000 r did not cause appreciable change in the color of the dye.

Further studies are intended with porphyrindin, which is a water-soluble free radical, and may give results more applicable to biochemical systems.

Preparation of p-chloromercuribenzoic acid: For use as a sulfhydryl group reagent in enzyme studies by Drs. T. N. Tahmisian, H. M. Patt, and S. A. Gordon, p-chloromercuribenzoic acid and its sodium salt have been prepared, using a modified procedure based on that of Whitmore and Woodward.(3).

Testing of materials for construction of laboratory benchtops: At the request of the Building Committee, a collection of various possible materials for use as laboratory benchtops has been assembled and tests to determine their suitability for use in the new Biology Building 202 are under way. These materials include Masonite Die Stock, treated with Die Stock Hardener, Panelyte on plywood, stainless steel types 304 and 316, stainless steel type 302 on plywood, Shelstone, Micarta, and Transite.

Chemical resistance tests have been performed on Masonite Die Stock, showing that it is resistant to organic solvents and 25 per cent acids except nitric. Steam, boiling water, temperatures above 160°C, and 10 per cent sodium hydroxide break down the surface upon continued exposure.

Pressure reactor with temperature control: A pressure reactor has been designed and constructed for use by Dr. James Arnold. This will enable him to embed bone samples at pressures up to 50 psi, at a controlled temperature of 60°.

Under these conditions, the bones are completely impregnated with the embedding plastic, making it possible to section the bones more satisfactorily for making radioautographs to show the distribution of the radioisotopes. By using industrial Pyrex pipe, 1 1/2" inside diameter, as a reaction vessel surrounded by a separately-mounted Nichrome helix as a resistance heater, the course of reaction may be followed. Safety glass windows are used to protect the observer. Stainless steel flanges and fittings permit the use of a variety of reagents and reaction conditions. A Weston thermometer internally mounted gives the temperature of the system, and mountings have been provided for carrying out the reaction with shaking.

The device has been designed in this manner to be usable not only for tissue embedding, but also to carry out a variety of reactions, such as hydrogenation, on a scale of from 100 g to a few mg.

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PROGRESS REPORT: PREPARATION OF DETAILED RADIOAUTOGRAPHS OF BONE

by
J. S. Arnold

The development of a satisfactory method for preparing detailed radioautographs of α -emitting substances in bone is progressing.

As outlined in a previous report⁽¹⁾ the problem consists of four parts: 1) fixation techniques, 2) bone sectioning, 3) autograph preparation, and 4) improved microscopic detail in prepared radioautographs.

At present absolute acetone is used for fixation since there seems to be little or no leaching out of plutonium. Other isotopes will be tested in this respect.

Nuclear as well as protoplasmic fixation appears to be satisfactory for cellular recognition but cellular detail in the final autograph is impossible at the present time. An investigation of the amount of distortion and shrinkage is underway.

The actual sectioning of bone has been successfully accomplished using an embedding medium of nitrocellulose plasticized with diamylphthalate, using acetone as a solvent. Embedding is accomplished for open bones in 24 to 48 hours under a pressure of 3 atmospheres of nitrogen and a temperature of 60°C. A more prolonged period may be necessary in the case of very thick or dense structures. After 1 to 2 weeks of drying, the tissue is ready for sectioning. Sections as thin as 6 to 8 μ are obtainable, using a heavy sliding microtome and a dilute aerosol solution for wet sectioning. Dense cortical bone of adult rats and dogs may be sectioned without any covering substance.

Using the above technique tissues that have been fixed by freeze drying will be embedded and sectioned during the next quarter.

Autographs are now being made of rat and dog bones (long bones and vertebrae) containing Pu. The individual α tracks can be traced to the cell or area from which they originated. Preliminary studies indicate that calculations of regional dosage will be predictable. Further studies using uranium isotopes and radium and its daughters are being initiated. At present, we are using NTA nuclear track plates, 25 μ thick.

The autograph and section are stained after development and fixing are completed. Mayer's hematoxylin has proved to be a successful nuclear

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stain despite its uptake by the gelatin of the photographic plate. This uptake is not a problem since observation is carried out microscopically under very bright illumination.

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Radiobiochemistry

Dr. Jack Schubert
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RATE OF CITRIC ACID ACCUMULATION IN TISSUES INDUCED BY FLUOROACETATE ADMINISTRATION

by

J. Schubert, A. Lindenbaum, and Marcia R. White

We are investigating the in vivo accumulation of citric acid, CA,⁽¹⁻³⁾ induced by relatively nontoxic amounts of sodium fluoroacetate (NaFA). Female Sprague-Dawley rats weighing 220 to 260 g were injected intravenously or intraperitoneally with freshly prepared NaFA¹⁴C solutions and sacrificed at intervals by cervical fracture. Several tissues were analyzed⁽⁴⁾ for CA.

After an induction period of 20 to 40 minutes the CA in most tissues rises rapidly for 4 to 6 hours and then decreases (Figure 18). The rise in CA levels is proportional to the dose of NaFA up to about 1.5 mg per kg but not at the higher more toxic doses. As little as 0.2 mg per kg of NaFA significantly increases CA above the control levels. No rise in tissue CA levels is observed when rats have developed a tolerance⁽⁵⁾ to repeated injections of NaFA.

The over-all accumulation and disappearance of CA with time were postulated to occur as consecutive reactions of the first order:

where A is the precursor⁽⁶⁾ forming CA at rate k_1 , the CA in turn forming C at rate k_2 . According to hypothesis^(2,7,8) the step $[CA] \rightarrow C$ is blocked by NaFA because of the formation of fluorocitric acid.

It is noted that the limiting values of the slopes representing CA accumulation are very nearly the same for spleen and heart (Figure 18), as well as for kidney, as time approaches zero. It is possible that the common value obtained may be equal to the unimolecular rate constant, k_1 , and may represent the normal rate of production of CA in unpoisoned tissues. This is being tested by measuring the accumulation of CA at various concentrations of NaFA in the range 0.2 to 1 mg per kg.

*Supplied by the Monsanto Chemical Company, St. Louis, Mo.

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Figure 18. In vivo formation of citric acid in the spleen and heart of the rat as a function of time after a single intravenous injection of 0.48 mg per kg of NaFA. Zero time is taken as the time at the end of the induction period (40 min. for the spleen and heart, respectively). The CA levels are the net amounts after subtracting normal control levels (60 μ g per g for spleen, 20 μ g per g for heart).

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PRELIMINARY REPORT: ACTH AND EXPERIMENTAL BERYLLIUM POISONING

by

Marcia R. White, A. J. Finkel, and J. Schubert

The possible use of pituitary adrenocorticotrophic hormone (ACTH) in beryllium poisoning was studied by investigating its effect 1) on the tissue distribution of previously injected radioberyllium (Be^7) and 2) on experimental beryllium poisoning in CF #1 mice.

Tracer doses of Be^7 as the sulfate were injected intravenously into six mice. Two of these received no further treatment; two were immediately given 0.25 mg ACTH* intraperitoneally, and two were given the same amount of ACTH 42 hours later. All were killed three days after Be^7 was given, and tissues were weighed, ashed and counted; excreta were not collected. Table 17 gives the average Be^7 content of several tissues in terms of per cent of the injected dose. It is evident that ACTH as administered here had no appreciable effect on the distribution or excretion of previously injected tracer levels of Be^7 .

BeSO_4 was given intravenously to 4 groups of mice of 10 each at approximately LD_{70} strength, i.e., 0.021 mg Be in 0.12 ml water per mouse (= 0.74 mg Be per kg). ACTH (0.5 mg in 0.1 ml water) was given intraperitoneally to one group 24 hours before injection with beryllium. The same amount of ACTH was given to a second group of mice immediately after injection of beryllium, and to a third group 24 hours later; a fourth group received only BeSO_4 . Animals were weighed daily. The mortality and weight curves are practically superimposable, and most of the mice were dead by the fourth day. Again no appreciable effect was found which could be attributed to ACTH.

Although the pathology of chronic pulmonary granulomatosis due to beryllium has not been reproduced in experimental animals, from the results presented here it seems plausible to conclude that the reported therapeutic effect of ACTH on the clinical course of berylliosis in the human is not likely a result of elimination or translocation of beryllium from or within the body.

*ACTH (Lot #83ASI) kindly supplied by Dr. John R. Mote, Armour Laboratories.

Table 17DISTRIBUTION OF Be⁷ IN MOUSE TISSUES

Tissue	Per cent of injected dose		
	Controls	Be plus ACTH immediately	Be plus ACTH in 42 hours
Liver	44.6	40.7	48.0
Lungs	4.17	9.76	6.11
Spleen	2.01	2.08	1.99
Femur	0.96	0.83	0.93
Kidney	0.47	0.40	0.53
Carcass	18.6	18.1	15.5
Total tissue	70.81	71.87	73.06

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MiscellaneousTHE ABSENCE OF INHIBITION OF -SH ENZYMES BY MODERATE
AMOUNTS OF X RADIATION

by
Marjorie Le May

ABSTRACT

This experiment was designed to determine whether the reduced oxygen uptake by rat tissues following irradiation is due to (1) inhibition of -SH enzymes and not to inhibition of other enzymes concerned with complete oxidation of substrates or (2) failure of the substrate to diffuse as easily in irradiated tissue as in normal tissue.

The succinoxidase system, including its component enzymes (succinoxidase, succinic dehydrogenase, and cytochrome oxidase), was determined in rat kidney homogenates within two hours after the administration of 400 r or 800 r total-body X radiation. There was no evidence of inhibition of any of the enzymes.

The metabolism of kidney slices from rats exposed to the same doses was studied manometrically with sodium succinate and lithium pyruvate substrates. No statistically significant differences could be found between the values for the control and the X-irradiated animals.

Results of this experiment indicate that X-ray doses that are high enough to cause hematological changes and death in rats do not cause measurable effects in the metabolism of rat kidney slices nor in the enzyme systems studied, including succinic dehydrogenase. A discussion of the lack of correlation between radiation-induced in vivo and in vitro effects is given.

PRELIMINARY REPORT: THE EFFECT OF X IRRADIATION ON FIGHTING BEHAVIOR IN MALE MICE

by
H. H. Vogel, Jr.

There have been few investigations on the effects of ionizing radiations on the social behavior of mammals. These experiments were carried out in order to test the effects of chronic X irradiation upon fighting behavior in male mice.

MATERIALS AND METHODS

Since Scott^(1,2) has shown that there are clearcut genetic differences in aggressiveness between different inbred lines of mice, three different strains were used in these experiments: C-57 black, C₃H agouti, and CF #1. The mice were placed individually in wire-mesh cages, each 8" x 8" x 7" in size, which were housed on a rack in one of the air-conditioned rooms of the animal farm. Temperature was kept at approximately 74°F. Water and Derwood food pellets were constantly available.

The mice were trained to fight in a multiple-escape pen, which contains interconnecting runways in the shape of a square with two diagonals, one cage being located at each intersection (Figure 19). Mice can roam through all five pens or can be restricted to one pen or any other combination of pens.

Training the mice to fight: The usual technique was to introduce a pair of mice into isolated sections of the multiple escape pen and allow them to investigate thoroughly for several minutes. Then, by appropriately opening and closing gates, the two mice were brought together into a single pen and their mutual behavior carefully recorded during the next five-minute period. When five-week-old mice were introduced in small groups, usually in pairs, no definite fighting behavior was observed in any of the males during their first several encounters. Within a week, however, several displayed aggressive behavior. Ginsburg and Allee⁽³⁾ have shown that individual mice may be conditioned to become more aggressive as a result of continuous victories over submissive mice. This type of social conditioning was employed in these experiments in order to develop more aggressive fighters. Only those mice showing fighting behavior were selected for training as fighters. Every day the "fighters" were introduced into the multiple escape pen with other male mice of their strain which had not shown fighting behavior. The result was that the fighters became more and more aggressive, as they defeated their submissive opponents, until they would attack any other male mouse immediately upon sight.

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PHOTOGRAPHED BY JANE K. GLASER

PHOTOGRAPHED BY JANE K. GLASER

Figure 19. Two views of multiple escape pen in which male mice were trained to fight.

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As an example of the training period, a summary is given in Table 18, for one of the mice, CF #1, no. 1, before irradiation. In this case, the fighter had defeated eleven other CF #1 males before any X irradiation. Six of the three dozen males were similarly trained as fighters in these experiments: four of these were CF #1 mice; one was of the C-57 black strain, and one was a C₃H.

Fighting behavior in mice: Although different mice developed individual fighting techniques, there was a general pattern of activity which constituted a typical fight. There was almost invariably at first some mutual sniffing. The dominant mouse would approach its partner, sniff its head, face, and particularly its ears, often mount the other mouse with its forepaws and sniff or groom its back. When a pair of mice met for the first few times, this type of investigatory behavior might last for several minutes.

An aggressive mouse would then try to get at the posterior region of its adversary and sniff at the genital area, a stimulus that would often set off an attack by the fighting mouse. A submissive mouse might raise its hind leg and allow the aggressor to "nose under" its posterior region.

One pattern of fighting behavior that was often seen was a lashing or rattling of the tail, ⁽⁴⁾ a characteristic of certain pugnacious mice, and used as a warning usually preceding and often following an attack. Those mice that exhibited tail lashing used it in most of their fights, but some fighters rarely rattled their tails when attacking. Fluffed hair and increased respiration were usually observed before a fight.

After the mice were conditioned to fight, the aggressive member of the pair usually attacked his opponent directly and without preliminaries. Attacks varied in length from a single nip to a vigorous and continuous biting and chasing fight that could last for as long as a minute and be followed shortly by another fight. In some cases a fighter spent most of the five-minute period in aggressive behavior. In between attacks there was often much independent investigation of the pen. Individual mice developed definite habits of attacking which became stronger with conditioning: one mouse always biting in the mid-back region of its opponents, another biting tail and rump region, a third nosing under and lifting up its adversary's posterior before biting.

Once a mouse was submissive to an attacker, a characteristic "defense reaction" occurred: the subordinate mouse reared up on its hind legs, pulled its forelegs up close to its body, remained motionless, and often squealed whenever the attacking mouse approached or touched him. In some mice this reaction became greatly intensified so that the mere sight of another male would produce squealing and a submissive stance. A few mice would actually roll over almost on their backs and hold all four feet up, trying to ward off an attacking male.

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Table 18

HISTORY OF FIGHTING BEHAVIOR IN MALE MOUSE, CF #1, NO. 1

Date	Daily training
4-19-50	CF #1 mice received, 5 weeks of age; into multiple pen for investigation; met other CF #1 males; no fighting
4-21-50	1 vs 2: no fight
4-24-50	1 vs 2: no fight
4-25-50	1 vs 4: no fight; submissive to 4
4-25-50	1 vs 12: no fight
4-26-50	1 vs 6: no fight; 1 dominant
4-26-50	3 attacks 1
5- 1-50	2 dominant over 1, no fight
5- 1-50	1 dominant over 12, no fight
5- 4-50	1 defeats 10
5- 8-50	1 defeats 6
5- 9-50	1 defeats 2, 4, and 11
5- 9-50	1 defeats 3
5-10-50	1 defeats 7
5-11-50	1 defeats 8
5-12-50	1 defeats 5
5-12-50	1 defeats 9
5-15-50	1 defeats 12
5-15-50	1 defeats 5
5-16-50	1 defeats 10
5-17-50	1 defeats 7
5-17-50	1 defeats 2
5-18-50	1 defeats 11
5-18-50	1 defeats 7
5-19-50	1 defeats 8
5-19-50	1 defeats 4
5-19-50	1st X ray, 50 r

Note: After this conditioning period, each fighting mouse was irradiated with 50 r daily, 5 days a week, usually in the afternoon. Each mouse was also fought daily, usually meeting one or two opposing males each day.

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There seems to be a similarity between fighting and sexual behavior in mice. Beeman⁽⁵⁾ has shown that the male sex hormone is a definite factor in causing fighting in mice in the absence of competition. If two males and a female mouse are introduced together in the same pen, one is struck by the similar patterns of behavior when 1) a male mounts and bites the back of a female (sexual) and 2) a male mounts and bites the back of a male (aggressive). It is not inconceivable that fighting in male mice is a type of homosexual behavior. Occasionally fights have been observed between females.

An excellent review article on the causes of fighting in mice and rats by Scott and Fredericson⁽⁶⁾ is now in press.

Nonfighting mice: Ginsburg and Allee⁽³⁾ have shown that it is possible to condition male mice to become less aggressive as a result of repeated defeats, and they have used this technique to change the position of individual mice in the social hierarchy. The type of training used in this experiment prevented many of the mice from becoming aggressive. They were matched against males that already had demonstrated fighting ability, and it did not take many defeats to inhibit completely any aggressive tendencies in submissive mice when they opposed trained fighters in the multiple escape pen.

In both the C-57 black and the C₃H strain of mice only one male was trained as a fighter. The other eleven mice of each strain were fought, usually in turn, against the fighter, both during the conditioning and after the X irradiation was started. Only one of these twenty-two mice showed any aggressive behavior under the conditions of the experiments. Most of them showed complete submission to the dominant fighter, assuming a defensive stance, squealing, and running when possible. Thus, whereas in the fighting experiments designed by Ginsburg and Allee, Scott, Beeman, and others, there were usually attacks and counter-attacks between fighting mice, in these experiments the attacking was completely one-sided. In more than 500 fights observed during the course of these experiments, only a very few showed fighting by both mice. These few cases were in preliminary encounters when two fighters met. Whenever possible, such combats were not allowed.

Irradiation data: After the six male mice had been trained to be fighters and had defeated all other males of their strain, they were irradiated daily with 50 r X rays, 5 days a week. The mice were exposed in individual lusteroid containers, 4" by 1 1/8" diameter, and placed on a rotating turntable.

Two different X-ray machines were used. One was a 200-kv, 15-ma machine, and the other a 250-kv machine. Filtration consisted of 0.5 mm Cu and 3.0 mm Bakelite; half-value layer, 0.98 mm Cu. The target distance was 26 inches.

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The mice were irradiated at a dose rate of approximately 20 r per min. so that the beam time was approximately 2.5 min.

A Victoreen chamber was used for dosimetry. Measurements were made in air, 4.0" off center and 1.0" off table top.

RESULTS

Figures 20, 21, and 22 indicate the body weights of the irradiated fighting mice as compared with those of the nonirradiated controls over a period of approximately fifteen weeks. Although there is some variation among the individual mice, the general picture is fairly constant. Approximately one week after X irradiation was started, most of the experimental mice showed a significant decrease in body weight, not seen in the control animals. Although the experimental mice regained this loss, they generally did not exceed their pre-irradiation weights by more than a gram or two and usually showed a precipitous weight loss before death. In most of the cases studied to date all fighting behavior ceased immediately before this terminal weight loss.

In Table 19 the data are summarized that show the accumulated X-ray dosage given to each of the six experimental mice at the time that all fighting behavior ceased, and at the time of death. Two of the animals are still living (8-15-50) after having received accumulated doses in excess of 2000 r. However, they are no longer fighting.

The effects of X irradiation upon fighting behavior in male mice were cumulative. Each fighting mouse retained its characteristic method of attack up to the day when all fighting ceased. There was a definite threshold after which an individual mouse did not fight at all. However, before this threshold was reached, the male fighters defeated their opponents daily. The aggressiveness of the mice definitely decreased as the cumulative X-ray dose increased. This was seen clearly by the increase of the period previous to actual attacks, the length of attacks, and the fierceness of the fights. The conditioning that the mice had received was strong and it took a large dose of X rays to eliminate it completely.

It can be seen from Table 19 that all signs of fighting behavior in these six male mice were not completely suppressed until shortly before the animal died. By this time the accumulated dosage had produced such severe radiation sickness that the animal could no longer fight. The usual reaction of all the males tested, after their aggressive reactions had been completely eliminated, was to "freeze." The mouse would remain motionless, close to the ground, ears back, eyes closed, and submit to grooming by his opponent.

Figure 20. Weight values of CF #1 male mice. Four "fighters" irradiated with 50 r daily, 5 days a week.

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WEIGHT IN GRAMS

Figure 21. Weight values of C_3H male mice. Experimental "fighter" irradiated with 50 r daily, 5 days a week.

Figure 22. Weight values of C-57 black male mice. Experimental "fighter" irradiated with 50 r daily, 5 days a week.

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Table 19

X-RAY DOSAGES DELIVERED TO FIGHTING MICE

Fighting mice	Total accumulated X-ray dose at 50 r daily before all aggressive behavior ceased	Total accumulated X-ray dose at 50 r daily before death
CF #1, no. 1	1800 r	1950 r
CF #1, no. 3	1450 r	1750 r
CF #1, no. 5	1200 r	1550 r
CF #1, no. 9	1950 r	still living (8-15-50)
C ₃ H, no. 9	1650 r	1750 r
C-57, no. 6	1200 r	still living (8-15-50)

Beeman and Allee⁽⁷⁾ have reported a series of experiments in which vitamin B₁ (thiamin) deficiency was used as an experimental factor in a study of fighting behavior in mice. They found that once dominance had been established the dominant animal could maintain his position almost to the point of death from avitaminosis, the subordinate animal still responding to the threatening attitude of the feeble dominant male. These results resemble the effects of X irradiation on fighting in male mice.

There was no noticeable immediate effect of irradiation on the fighting behavior. Such an effect might possibly be expected from the work of McKeen *et al.*⁽⁸⁾ on physical endurance of rats following total-body X irradiation. In these animals a shortened swimming endurance time was noted after a single total-body dosage of 50 r X irradiation.

More recent work on rats by Kimeldorf, Jones, and Fishler⁽⁹⁾ has indicated that the lethality of roentgen rays at the doses studied (600 r, 700 r, and 860 r) was increased by the repeated performance of vigorous exercise following irradiation. It is suggested that the increased mortality observed in animals exercised after irradiation provides additional evidence of the relation between radiosensitivity and metabolic level. Fighting might well be used in future exercise experiments.

Once a mouse had "slowed up" in his fighting, it was occasionally difficult to have him initiate an attack. A moving mouse seemed to be one stimulus for aggression. Thus, attacks were often stimulated by opening the gate from a pen to a runway and allowing the aggressor to follow his running opponent. The smaller space of the alley and the movement of another mouse served to stimulate attacks when mice would not fight in the main pens.

One interesting observation was that even after a mouse had succumbed to the severe effects of radiation sickness and would no longer fight at all, he still would show interest in female mice, following them around the pen and sniffing them. This seemed to indicate that some sexual behavior still is evident after all aggressive actions are gone.

Because of the small numbers of animals used in these experiments, no finality is claimed for these results. However, the similarity of reactions in the three different strains of mice used suggests a certain amount of universality.

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PRELIMINARY REPORT: A COMPARATIVE STUDY OF
RADIATION LETHALITY IN SEVERAL SPECIES OF EXPERIMENTAL
ANIMALS IRRADIATED FOR THE DURATION-OF-LIFE

by
George A. Sacher

INTRODUCTION

In this paper several species of experimental animals are compared with respect to their survival under duration-of-life exposure to various qualities of externally administered ionizing radiations. The approach used is one developed in a previous paper⁽¹⁾ that may be consulted for a more extended discussion of some of the questions raised by this treatment. The fundamental hypothesis of this approach states that exposure to radiations initiates in organisms a lethal process that is a function of the many forms of physiological injury produced. The hypothesis is developed under the simplifying assumption that the organism responds to radiations in linear fashion. This would imply that 1) following a short pulse of radiation the lethal process (or any weighted sum of responses of the constituent physiological mechanisms) would follow a characteristic time course, 2) if the quantity of radiation delivered in the same short period of time were altered, the lethal process would be unaltered in form but its amplitude at every moment would alter proportionally and, 3) the response to a sequence of like impulses would be given by a summation of the responses to the impulses delivered separately.

For arbitrary time patterns of exposure the lethal process is deduced from lethality data by the solution of an integral equation. In the special case considered here, of exposure at a constant average rate throughout life, the simple result is found that the lethal process at a time t^* is inversely proportional to that dose rate for which the mean survival time is t^* . The added complication that some of the radiation survival data extend over considerable fractions of the natural span of life makes necessary the introduction of a term to account for the change of physiological state with age. The nature of the correction of aging is discussed in a previous paper.⁽¹⁾

The cumulant lethality functions deduced for the several species have characteristics in common. This, with other evidence, suggests that there is a generic mammalian radiation-injury process and that the cumulant lethality functions provide at least a rough measure thereof.

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THEORY

We consider a model system that is a linear system in n variables. By linear we mean here that in the differential equations describing the motion of the system, 1) the dependent variables and their derivatives are of the first degree with constant coefficients, and 2) the dependent variables are linear in their dependence on dose rate. The dose rate may be a function of time.

We assume that the chances of survival of an organism depend on a single variable that is denoted by the symbol M . The value of M is found by summing the values of all the physiological variables, giving to each process a weight according to its importance for survival:

$$M = \sum_{i=1}^n m_i x_i \quad (1)$$

Following a brief exposure to ionizing radiations (or to any toxic agent) delivered at time $\tau = 0$, each physiological variable undergoes a characteristic injury process that at time t has the value

$$x_i(t) = k_i \phi(\tau) I_0 d\tau \quad (2)$$

where $\phi(\tau)$ is the time-response to unit dose for the i^{th} variable

$I_0 d\tau$ is the quantity of irradiation delivered at intensity I in time interval $d\tau$

k_i is the dose-sensitivity constant for the i^{th} variable

The injury to the i^{th} variable at time t after a brief exposure at time $t > 0$ is

$$x_i(t, \tau) = k_i \phi_i(t - \tau) I_{\tau} d\tau \quad (3)$$

The value of the lethal process is therefore

$$M(t, \tau) = \sum_{i=1}^n m_i k_i \phi_i(t - \tau) I_{\tau} d\tau \quad (4)$$

By setting

$$\sum_{i=1}^n m_i k_i \phi_i(t - \tau) = \phi(t - \tau) \quad (5)$$

We may rewrite equation 4 as

$$M(t, \tau) = \phi(t - \tau) I_{\tau} d\tau \quad (6)$$

On the hypothesis of a linear system the response to a sequence of like impulses is given by the sum of the responses to the impulses delivered

separately. In the limiting case of continuous exposure this sum becomes an integral and we obtain

$$M(t) = \int_0^t \phi(t-\tau) I(\tau) d\tau \quad (7)$$

The function $\phi(t-\tau)$ is proportional to the impulse lethality function that we wish to evaluate. The impulse lethality function is defined below by equations 13 and 17. The function $I(\tau)$ gives the radiation intensity as a function of time.

Equation 7 is a Volterra integral equation of the first kind. We shall not discuss the general solution at the present time,* for we will be concerned here only with the mathematically trivial case

$$I(\tau) = \text{constant} = I: \tau \gg 0 \quad (8)$$

In this case there is only one variable under the integral, and equation 7 reduced to

$$M(t) = I \int_0^t \phi(\tau) d\tau \quad (9)$$

Before applying equation 9 however, it is necessary to take into account the progressive change in the lethal process due to aging. Adhering to the linear assumption, we consider that the effects of aging and irradiation cumulate independently and are additive, so that the aging process may be taken into account by an additive term:

$$M(t) = I \int_0^t \phi(\tau) d\tau + A(t) \quad (10)$$

The aging function can be shown to be approximately of the form⁽¹⁾

$$A(t) = \beta t \quad (\beta = \text{constant}) \quad (11)$$

so that we obtain finally

$$M(t) = \beta t + I \int_0^t \phi(\tau) d\tau \quad (12)$$

Equation 12 is applied in the following manner. For a given I the function $M(t)$ becomes equal at time t^* to a value L , called the lethal bound

*Cases in which $I(t-\tau)$ is not constant (retained radioisotope) and will be treated in future communications.

of injury (which is here assumed to be constant). We define a cumulant lethality function, C_L

$$C_L(t^*) = \frac{1}{L} \int_0^{t^*} \phi(\tau) d\tau \quad (13)$$

Solving equation 12 for C_L , we obtain

$$C_L(t^*) = \frac{1}{I} (1 - \frac{\beta t^*}{L}) \quad (14)$$

Now let t_0 be the survival time of nonirradiated animals under the same condition. We have

$$\beta t_0 = L \quad (15)$$

so that equation 14 becomes:

$$C_L = \frac{1}{I} (1 - \frac{t^*}{t_0}) \quad (16)$$

The C_L has dimensions of $(r \text{ per day})^{-1}$. In the case $t^* \ll t_0$, C_L is equal to $\frac{1}{I}$. The impulse lethality function, S_L , is defined as the time derivative of C_L

$$S_L = \frac{d C_L}{dt} \quad (17)$$

In the following sections we shall discuss the responses of various species to duration-of-life exposures at constant daily dose. For this purpose the cumulant is more suitable than the impulse function, since in most cases the data available are not sufficient for the estimation of the S_L .

In applying the theory developed here we calculate the cumulant for a representative animal, which is taken to be the mean or median animal, depending on the data. The survival time of exposed populations is actually distributed about the mean with coefficient of variation (CV) ranging from 8 to 40 per cent, and the magnitude of CV is related to the behavior of the cumulant function in the time neighborhood of t^* .⁽¹⁾ As a consequence, the values of C_L and S_L are slightly biased.[†] This does not affect their utility for our present purpose of species comparison.

[†]The bias exists because $M(t)$ has a random component. If the variance of survival times were assignable all to distributed population parameters, and none to random variance, C_L would be estimated without bias.

DATA AND COMPUTATIONS

The data on which this study is based were gathered from the literature, supplemented by original records of studies carried out in this Laboratory. The relevant information to assist the reader in weighting the values which appear in the graphs is given in Table 20.

In all cases except one, which is noted, the t^* values are mean or median after-survival times as published, or as estimated from published curves. The after-survival of controls, t_0 , presented more difficulty. In some cases (dog, monkey, and rabbit) no satisfactory experimental data are available. In other cases it was found that the actuarial functions of controls and treatment groups showed differences that cannot be accounted for in terms of any simple concept of the effect of radiations on survival. The alternatives presented by these latter cases are either 1) at certain dose rates radiation decreases death rates in the early stages to such a degree that life expectation (but not life span) is increased, or 2) the differences in the actuarial functions of controls and experimentals are due to differences in experimental conditions other than the radiation factor. In any case the analysis of threshold effects by means of the present schema is not valid, and we have omitted from consideration all data at 0.1 r per day and some data at higher dose rates (see Table 20).

One of four courses was followed in estimating control survival:

1. Accept control data obtained by investigator
2. Combine control survival and survival at nonsignificant treatment level to estimate a pooled control
3. Take survival at nonsignificant treatment level as an estimate of control survival
4. Use data from other sources in estimating control survival (where experimenters give no control data).

In Table 20 the procedure for estimating control survival is indicated and the numerical values used are tabulated. The estimated C_L values at the lowest dose rates may have large uncertainties in some cases, but the effect on C_L of the control value diminishes as the ratio t^*/t_0 decreases, and for $t^*/t_0 < 0.5$ the uncertainty from this source is negligible compared with that from other sources. The radiation quality, amount of protraction, and treatment pattern are also indicated in Table 20. These variables affect the cumulant lethality, and in following sections adjustments for some of these factors are discussed.

Survival times and cumulant values range over several orders of magnitude, so they are plotted on a log-log grid.

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Table 20
SUMMARY OF DURATION-OF-LIFE STUDIES

Investigator	Strain, Sex	Quality	Protraction	Exposure pattern	Dose rate range used	No. in group	No. of points	Estimate of control	
								Source*	Value (days)
MOUSE									
Evans (2)	Swiss ♂, ♀	X	Brief	5 day/wk	11-80	50	2	Control	475
Evans (2)	Swiss ♂, ♀	n	Brief	5 day/wk	.07-10.0	50	4	Control	475
Evans (2)	CF#1 ♂, ♀	n	Brief	5 day/wk	.07-1.4	50	3	Control	420
Henshaw (3)	C ₃ H ♂, ♀	X	Brief	5 day/wk	10-80	10-15	8	Control	322
Lorenz (4)	LAF ₁ ♂, ♀	γ	8 hr/day	7 day/wk	2.2-8.8	50	3	Control	703
Sacher (1)	ABC ♂, ♀	X	Brief	6 day/wk	20-1000	5-133	12	Control	538
Sacher (1)	CF#1 ♂, ♀	X	Brief	6 day/wk	20-200	20-54	6	Control	425
Sacher (5)	CF#1 ♂, ♀	X	Brief	5 day/wk	5	109	1	Control	480
Thompson et al. (6)	CF#1 ♂, ♀	γ	24 hr/day	7 day/wk	2	?	2	Est.	400
RAT									
Boche (7)	Wistar ♂, ♀	X	Brief	6 day/wk	1-10	100-200	2	Control	601
Hagen (8)	Sprague-Dawley ♂, ♀	X	Brief	6 day/wk	12.5-500	10-20	7	Est.	500
Patt (9)	Sprague-Dawley ♂, ♀	X	Continuous	-	10 ⁵	1	1	-	-
Thompson et al. (6)	Sprague-Dawley ♂, ♀	γ	24 hr/day	7 day/wk	60-120	?	3	Est.	500
RABBIT									
Boche (7)	Wistar ♂, ♀	X	Brief	6 day/wk	10	20	1	Control +0.1	400
Lorenz (4)	- ♂, ♀	γ	8 hr/day	7 day/wk	8.8	6	1	1.1+2.2	1450
Sacher (5)	Swift ♂, ♀	X	Brief	6, 7 day/wk	40-325	2-6	8	Est.	1000
Tang (10)	- ♂, ♀	X	Brief	7 day/wk	10-50	2-4	4	Est.	1000
DOG									
Boche (7)	Mongrel ♂, ♀	X	Brief	6 day/wk	3-10	10-20	3	Est.	1800
Ducuing (11,12)	Mongrel ♂, ♀	X	Brief	6 day/wk	25-100	1-2	2	Est.	1800
Prosser (13)	Mongrel ♂, ♀	X	Brief	6 day/wk	12.5-50	2-4	4	Est.	1800
GUINEA PIG									
Lorenz (4)	Hybrid ♂, ♀	γ	8 hr/day	7 day/wk	1.1-8.8	6	4	Control +0.11	1320
Sacher (5)	- ♂, ♀	X	Brief	3 exp/wk	30	38	1	Est.	1000
Thompson (6)	- ♂, ♀	γ	24 hr/day	7 day/wk	60-120	---	3	Est.	1000
MONKEY									
Boche (7)	Rhesus ♂, ♀	X	Brief	6 day/wk	0.5-10	14-30	3	0.1	350

*Treatment group or groups used in estimating control survival time "est." signifies estimate from other sources; used only for short survival times.

RESULTS

A synopsis of most of the information at present available is given in Figure 23, in which we plot $\log C_L$ vs. $\log t^*$. In appraising this graph the four points should be borne in mind that determine that it is a valid comparative representation:

1. The abscissa is a physical variable, time from the beginning of treatment, that has the same significance for all species.
2. The ordinate is also a physical variable with dimensions $(r \text{ per day})^{-1}$ (so that C_L is the injury per unit dose rate).
3. Direct comparison of radiation specific effects is made possible by the correction for natural aging in the various species. The order observed in the forms of the cumulant functions of different species would not be apparent if raw survival data were used.
4. The cumulant function is an empirical function; no arbitrary constants or functions are introduced into its estimation.

It should also be remembered that approximations have been made in the theoretical development, and that insufficient control data and certain experimental difficulties introduce error which becomes more serious as t^* approaches t_0 .

General characteristics of the cumulant function: Those species for which the cumulant is adequately mapped show in the plot of $\log C_L$ vs. $\log t^*$: a steep linear rise at the shortest survival times followed by a plateau period in which the cumulant is almost independent of time. This horizontal branch is succeeded at later times by a linearly increasing branch. The greatest differentiation between species is observed in the plateau branch.

The initial ascending branch: The initial steep branch of the cumulant function is so nearly alike for all species that a single trend line adequately fits most of the values on this branch. The trend line drawn on Figure 23 has the equation

$$\log C_L = 1.8 \log t^* - 4.056 \quad (18)$$

or, in absolute units

$$C_L = 8.8 \times 10^{-5} (t^*)^{1.8} \quad (19)$$

—○— A. Rabbit, X-ray exposure	—△— E. Dog, X-ray exposure
—⊙— B. Rat, X- and γ-ray exposures	+●+ F. Guinea pig, X- and γ-ray exposures
—●— C. Mouse, X- and γ-ray exposures	---●--- G. Monkey, X-ray exposure
---▲--- D. Mouse, neutron exposure	----- H. Common asymptote

Figure 23. Cumulants for mouse, rat, rabbit, guinea pig, dog, and monkey; X-ray, γ-ray, and neutron exposures. Data from sources indicated in Table 20.

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In the previous communication⁽¹⁾ it was noted that for the first 8 days the relation between dose rate and survival time in the mouse and rat is given by

$$It^2 = 15,500 \text{ roentgen days (mouse)} \quad (20)$$

$$It^2 = 16,200 \text{ roentgen days (rat)} \quad (21)$$

The survival behavior in this region was attributed to the accumulation of radiation injury almost unretarded by recovery. The fact that the initial branches of the cumulant function of five species lie close to a single line having logarithmic slope close to two suggests the three following working hypotheses:

1. The over-all trend of the impulse lethality function, S_L , for all species is linear through the origin (to account for the cumulant increasing as t^2).
2. In the absence of recovery, the amount of physiological injury produced per unit dose of ionizing radiations is roughly constant for mammalian species.
3. The lethal level of acute injury (L) is roughly constant for mammalian species.

Hypothesis 1 is independent of hypotheses 2 and 3, but 2 and 3 are interdependent and both are necessary if we are to make a reasonable physiological interpretation of the observed facts.

The rabbit, rat, and mouse cumulant curves bend away from the branch of unretarded accumulation at times between 10 and 15 days, while the dog and guinea pig cumulants continue close to this branch for 30 days or longer. This does not imply that no recovery has taken place. If, for example, the trend line were taken to pass through the mouse and rat points and to have a slope of 2.0, the dog and guinea pig cumulants would lie considerably below this trend line at 30 days. It is evident, however, that some very important process (or processes) has a much lower recovery rate in these species than in mouse, rat, or rabbit. In a previous investigation it was established that in dogs which received daily exposures of 12.5, 25, and 50 r, the red count, hemoglobin, hematocrit, heterophil, eosinophil, monocyte, and sedimentation values showed little or no evidence of recovery within the survival spans of the animals.⁽¹³⁾ (Dogs treated with 12.5 r per day lived 121 days on the average.) This is evidenced by the fact that the depression of these variables could be expressed as a function of accumulated dose at all three dose rates. Lymphocytes on the other hand, showed definite equilibration, with a time constant between 10 and 20 days. It has also been shown, however, that relative radiosensitivity

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of several species is correlated with the sensitivity of the myeloid and erythroid elements and is not correlated with the sensitivity of the lymphoid elements (which is almost constant for these species).⁽¹⁴⁾ On the whole then, the behavior of the cumulant lethality function of the dog is consistent with the physiological behavior of those systems known to be significant for survival. Other species should be investigated in similar fashion.

We next consider the consistency between species differences in the cumulant functions and in single LD_{50} . It is evident that at almost all times the cumulant functions are in the rank order of LD_{50} 's (Table 21). Actually only a small portion of the cumulant function bears a close relation to single-dose killing, namely the portion extending over the period of single-dose killing, from about 5 to 30 days.

Table 21

MEDIAN LETHAL DOSES, MEAN SURVIVAL TIMES AT LESS THAN LD_{50} 'S AND RANK ORDER OF CUMULANT FUNCTION IN 10- TO 20-DAY REGION

	30-day LD_{50}	30-day MST	Rank order of CL
Rabbit (17)	800 r	11 days	5
Hamster (18)	700	10	-
Rat (8)	600	12	4
Mouse (19)	550	12	3
Monkey (18)	450	16	-
Goat (20)	350	17	-
Dog (18)	335	18	-
Dog (20)	315	17	2
Guinea pig (19)	225	17	1

Estimation of the cumulant values at the time of peak single-dose killing is not feasible because the cumulants are so poorly determined in this region for dog and guinea pig. It can be seen, however, that the rank order correlation persists in this region and, more important, there is a factor of about 4 between the lowest and highest cumulant values at these times, which agrees well with the factor of 3 to 4 between the highest and lowest LD_{50} values. We should expect that the impulse lethality function, S_L , have a maximum at the time of peak single-dose killing and this has been shown to be true for the ABC male mouse, which is to date the only strain studied with closely spaced dose rates.⁽¹⁾

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Comparisons have been made between daily dose survival and the survival following 100 per cent lethal single doses⁽¹⁾ and a discrepancy of almost 40 per cent was found, indicating that the linear assumptions break down when massive doses are administered. This point will be discussed in a future communication.

The mean survival times of decedents from less than the LD₅₀'s for the various species, are negatively correlated with LD₅₀ (Table 21). This is in agreement with the forms of the cumulant functions of sensitive and resistant species, for if physical sensitivity is constant and the recovery time is increased, the maximum intensity of injury following a single dose should increase, and the maximum should be reached at a later time.

The point at 0.11 days is based on a single rat which was irradiated till death in the X-ray beam.⁽⁹⁾ Other data gathered under similar conditions but with terminated exposures are consistent with this.⁽¹⁶⁾ Critical investigation of the region from 0.1 to 10 days will be difficult but should be rewarding. We anticipate some interesting and paradoxical behavior in this region. Such has already been reported, e.g., the almost discontinuous relation between size of single dose and survival at high dosage time described by Quastler⁽¹⁶⁾ and Bishop.⁽¹⁵⁾

The plateau branch: Since it has been possible to demonstrate consistency between the cumulant functions and other kinds of information on the rising branch, we can venture out on the horizontal limb with more confidence. The single most striking feature of this branch is the great spread between the most sensitive and the least sensitive species. At about 200 days the guinea pig harbors about 13 times more injury than the rat per unit dose rate. There is little doubt that this inference is quantitatively correct within a small error factor. The situation of the monkey is even more striking (see p. 119).

Let us for the moment ignore the details of the cumulant functions and regard them as being simple linear processes of the form

$$\frac{dC_L}{dt} = K - q C_L \quad (22)$$

where K is the mean dosage sensitivity constant and q is the recovery rate constant. Then the steady state value, $\tilde{C}_L$ (at $dC_L/dt = 0$), is given by

$$\tilde{C}_L = \frac{K}{q} \quad (23)$$

However, we (p. 111) found justification for the hypothesis that K has the same value for all species. Therefore, we have approximately

$$\tilde{C}_{Lj} \propto 1/q_j = T_j \quad (24)$$

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where the subscript j denotes the j^{th} species, and T_j is a mean recovery time in the j^{th} species. The value of the cumulant function for a given species in the plateau region is proportional to an average recovery time of a constellation of most vital (not necessarily most sensitive) systems. This offers a valuable key to the problem of species sensitivity in the acute and subacute range. On the question of whether this mean recovery constant is related to taxonomic position of the species or to variables such as body size, physiological tempo, or life span, the evidence so far seems to point in all (or none) of these directions. Need for a well-conceived program of comparative study is indicated.

The determination of species recovery constants may prove to be an exacting task, but meanwhile much can be learned from quantitative investigations of the displacement of physiological steady states per unit dose rate. The fact that dramatic differences have not already been observed is somewhat discouraging.

It is too early to inquire into the detailed characteristics of the S_L and C_L functions. The S_L function of mouse shows two and perhaps three distinct maxima before the final ascending branch is reached. The existence of three maxima of the S_L function of rabbit can be inferred from the form of the C_L function. One would like to know whether homologies can be established between the processes corresponding to maxima in the different species. Isolation of distinct maxima may prove difficult in dog and guinea pig because of the swamping effect of the large general sensitivity factor.

The late ascending branch: This branch is perhaps the most important for society and, for reasons brought out earlier (p.109) it is the least well-established. Some tentative impressions may be formulated.

1. In a species for which the late branch is fairly well established (mouse) the trend is clearly linear with unit slope. In other words, late injury accumulates in proportion to time or to accumulated dose. This is the behavior to be expected if low levels of radiation cumulate with the natural aging process, accelerating pathological tendencies but introducing no qualitatively new pathology. From what little is known, this appears to be the case. In contrast to the plateau region, we should expect to find no displacements of the physiological steady states of greater magnitude than those that accompany aging.

2. There is a slight indication that species differences decrease when the late branch is fully attained. One notes that the dog and guinea pig have appreciably reduced their disadvantage over the mouse by 1000 days, while the rat on the other hand apparently lost its advantage over the mouse at 500 days. This behavior of the rat, like the anomalous monkey behavior, is open to another interpretation (see p.118).

Although the late branch of the cumulant functions of the experimental animals shows no recovery within the life spans of these animals, it cannot be inferred that no recovery would be shown in the comparable component of the longer-lived species. For the time being, however, the safest and most reasonable working assumption is that the chronic branch is non-recovering in all species (in the absence of medical intervention).

Cumulant functions for mouse and rat; the effect of radiation quality and protraction: In Figure 24 is plotted the cumulant functions for mouse and rat on a somewhat larger scale, and with the contributions of the different investigators identified. Until about 30 days the cumulants for the two species almost superimpose, but by 50 days the mouse cumulant has moved up by a factor of 1.7 and remains above that of rat thereafter until overtaken at about 500 days.

It seems reasonably certain that the mouse cumulant does increase abruptly at 40 days, for this is clearly seen in the ABC series⁽¹⁾ and is not contradicted by the results of other investigators. The rat data are too sparse to permit any decision as to whether a similar transition is present there. The rabbit cumulant shows two such breaks, one at about 25 and the second at about 50 days (Figure 23).

The cumulant values for the γ -ray data could be made to superimpose reasonably well on those for X rays by multiplying the γ -ray cumulants by a factor of 2. This factor is a correction both for radiation quality and for protraction, since all γ -ray exposures were given over periods of 8 or 24 hours per day. It appears then that X and γ rays have about the same relative effectiveness from the acute through the chronic range, and it is therefore probable that their mechanisms of action are very similar.

In the case of daily neutron exposure it is seen that the effectiveness ratio changes markedly from the acute through the chronic range. This was pointed out by Evans⁽²⁾ in his report of this work.

All except one of the guinea pig cumulant values are based on γ -ray exposures and the same factor of 2 was employed with the justification that it operated for mouse and rat. There is, however, no direct evidence that the guinea pig cumulant values for X-ray exposure are as indicated in Figure 23. Studies of guinea pig survival under daily exposure to X rays in the dose rate range from 10 r per day to 1 r per day are badly needed.

Monkey and rat; possible effect of intercurrent disease: The highest cumulant values observed for any species are those for the monkey.*

*In obtaining the cumulant values used here, deaths in the first 10 days of treatment were excluded from the averages and the 0.1 r per day level was taken as the control group.

Mouse - X-ray exposure

- A. Evans⁽²⁾, Swiss
- B. Henshaw⁽³⁾, C₃H
- C. Sacher⁽¹⁾, ABC
- ⊙ D. Sacher^(1,5), CF #1

Mouse - γ-ray exposure

- E. Lorenz⁽⁴⁾, LAF₁
- F. Thompson⁽⁶⁾, CF #1

Mouse - Neutron exposure

- △ G. Evans⁽²⁾, Swiss
- ▲ H. Evans⁽²⁾, CF #1

Rat - X-ray exposure

- ◇ I. Boche⁽⁷⁾, Wistar
- ◆ J. Hagen⁽⁸⁾, Sprague-Dawley

Rat - γ-ray exposure

- ◆ K. Thompson⁽⁶⁾, Sprague-Dawley

Figure 24. Cumulants for mouse and rat; X-ray, γ-ray, and neutron exposures. The lines are drawn merely for visual guidance.

Furthermore, if we take the monkey data at face value, there is no evidence that the monkey cumulant is approaching a plateau as late as 250 days.

These observations are most disquieting, but there are other factors to take into account. Tuberculosis was enzoötic among these animals and the death rate from this cause was exceedingly high. Also, analysis of the survival data shows that in all the treatment groups, including the 0.1 r per day level, the dying out was exponential from the beginning of treatment. Thus the mode of action of radiations must have been entirely different here than in other species, which show an entirely different order of death as described above (p. 108).

These observations taken together suggest the possibility that when morbidity from other injury or disease processes reaches a high level, the radiation lethality process is radically altered, both quantitatively and qualitatively.

The behavior of the rat cumulant function after 300 days is perhaps an example of a similar situation, for the cumulant rises very abruptly in this period. This is also an age at which respiratory disease becomes enzoötic or epizoötic in some colonies and strains of rats. We have no knowledge of the status in this respect of the particular colony from which these data were gathered.⁽⁷⁾

CONCLUSION

An analysis of radiation lethality in metazoan forms has been developed on the basis that (1) many or all aspects of physiological function are affected by radiations, (2) the responses of physiological systems to irradiation, and the interactions between systems are linear, (3) a lethal process arises which is a weighted sum of the injuries to the constituent systems, (4) death occurs when this process intersects with a lethal bound of injury, and (5) when aging is a significant factor, the effects of the aging process and the radiation process combine additively. The further approximation is made that the behavior of a population is described by a representative animal, taken to be the mean or median animal. This latter approximation permits the interpretation of data from small groups of animals, which is the burden of the present paper.

The data analyzed were mean survival times at various daily dosages. Data for several species were available. By using the above hypothesis, it is possible to deduce for each species a cumulant lethality function and an impulse lethality function which describe the course physiological injury would follow consequent to continuous and to single exposures, respectively, if the species behaved according to hypothesis. No internal test of consistency is possible, but one can determine whether the derived lethality functions are consistent with other kinds of information. At least semi-quantitative consistency was obtained for several types of comparison. The results

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obtained thus far justify the conclusion that the essential assumptions are realistic and that the generalizations that may be necessary for a more exact treatment will not make the problem intractable.

When the cumulant lethality functions of several species are compared, an orderly picture emerges. The generalizations made should not be regarded as established facts, but rather as plausible working hypotheses. Outstanding are the following:

1. The dosage sensitivity of the lethal processes in mammalian species is essentially constant.

As a consequence it follows that

2. The steady state level of recoverable injury per unit dose rate for the various species is proportional to a mean recovery time for a constellation of most vital physiological processes.

The determination of this recovery time for man and the economically important animals becomes a matter of importance.

The most sensitive and the least sensitive species investigated differ by more than a factor of 10 in the plateau (steady state) values of their cumulant functions, and there is about a factor of 4 between the LD_{50} values of the same species.

The form of the cumulant function suggests unambiguous definitions of acute, subacute, and chronic injury. Chronic injury in a given species is manifested in relatively pure form only when the final branch, on which accumulated injury is proportional to accumulated dose, becomes predominant over the horizontal branch of equilibrated recoverable injury.

It would appear that the chronic branch of the cumulant function shows considerably less species variation than the horizontal branch. It will be a matter of considerable difficulty to assay this, for we have the experimental difficulty of investigating chronic survival, and the further problem of species differences in aging pattern.

The problems of the relative biological effectiveness of different radiation qualities and of different protraction can be clarified insofar as it is possible to attribute the altered effectiveness to a specific phase of the lethal process. Thus, the increased relative effectiveness of neutron exposure at low dose rates over that at high dose rates is perhaps attributable to a specifically increased effectiveness of neutrons on the branch of chronic nonrecovering injury. On the other hand the relative effectiveness of X rays and γ rays seems to be constant at all dose rates.

A problem that needs further investigation is that of the interactions between a radiation-induced lethal process and co-existing lethal processes of other kinds, especially those arising from disease. The case is of practical importance, and also affords crucial tests of the fundamental postulate of linearity of mechanism.

The theory as developed may be applied to any type of process or function where the datum recorded is the relation of time and dosage to reach an unequivocal end-state. The approach is best suited for the preliminary investigation of the more complex performances of organisms since it accomplishes a reduction of the original data to a form in which the contributions of specific mechanisms of lower order can be more easily recognized.

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Background Analytical Group

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PROGRESS REPORT

by
W. H. Johnston

INTRODUCTION

Work during this quarter has been devoted to the construction and installation of a Libby screen-wall counter, with anti-coincidence and shield, for low level beta counting. In addition, a method has been outlined for the accurate determination of small amounts of radon in human breath. As a part of this program, a proportional alpha gas counter with a plutonium "gun" has been developed. This permits an easy means of checking the efficiency of the counter gas.

SCREEN-WALL COUNTER PROGRAM

A screen-wall counter, as developed by Libby and co-workers, consists of a large brass cylinder (3" in diameter), a copper screen cylinder of smaller diameter and occupying roughly the middle third of the large cylinder, and a movable sample cylinder fitting snugly in the large cylinder and roughly two-thirds of its length. The screen cylinder acts as a cathode of a Geiger counter whose anode is a wire in the center of this screen-wall cylinder. The sample to be counted is mounted on one-half of the sample cylinder which can be shaken to one end of the large cylinder, bringing the sample around the screen-wall Geiger cylinder. To increase the active volume of the counter, the outer cylinder is maintained at 90 volts negative with respect to the screen at ground potential. In use, the entire assembly is evacuated and filled with a self-quenching counting gas, e.g., ethylene-argon. Following a count of the sample, the sample cylinder may be shaken to the opposite end of the outer cylinder, thereby bringing the bare half around the screen cylinder. The background count is taken in this position.

With the above arrangement the natural cosmic ray background of 400 or 450 counts per minute places a severe limitation upon the accuracy of low level counting. To remedy this high background, the soft components of the cosmic rays are removed by an eight-inch iron shield. With such a shield the residual background, comprised almost wholly of cosmic ray mesons, is usually of the order of 100 counts per minute. Finally, the

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mesons are removed from the background count by a bank of anti-coincidence counters, which are large Geiger counters that surround the screen-wall counter. Whenever a count occurs essentially simultaneously in the screen-wall counter and one of the A-C counters, the count is electronically cancelled. Thus, the high energy mesons that pass through the central counter must also pass through an anti-coincidence counter and be cancelled. With this arrangement the background is between 5 and 10 counts per minute. Since the sample area is approximately 400 square centimeters and since there is no window between the sample and counter, extremely low level and low energy beta samples may be counted accurately.

The screen-wall counter assembly is shown in Figures 25 - 32. The iron shield for removing the soft component of the cosmic rays is shown in Figures 25 and 26. The door, which is partly open in Figure 26, is operated by the crank in the upper right hand corner of the frame. Each half of the door weighs approximately one-half ton and counterweights the other. A gear train and ratchet in the center prevent the lowering of the upper door unless the ratchet is manually disengaged. This provides an automatic independent safety device against accidental closing of the doors.

Figure 26 also is illustrative of the electronics, consisting of the mixing circuit for operation of the A-C's, the stabilized high voltage supply for the screen-wall counter, the scaling circuit and A-C high voltage, and the oscilloscope.

The arrangement of the anti-coincidence counters before installation in the iron shield is shown in Figure 27. This special frame allows the removal of one A-C at a time for test or replacement.

The A-C's and screen-wall counter installed in the iron shield are illustrated in Figure 28. The arrangement of the present A-C's and the present electronics allows the simultaneous and independent operation of two screen-wall counters.

Tests on this equipment show a normal unshielded background of 430 counts per minute. With the iron shield the background is approximately 155 counts per minute and with anti-coincidence, approximately 17 counts per minute.

Although these values indicate that the apparatus is ready for general use, further tests are planned to determine the source of the somewhat high shielded and A-C backgrounds. It is suggested that the following sources of extra counts should be investigated:

1. Loose contamination on the outside of the screen-wall counter. In the future white cotton gloves (mortician's type) should be worn in handling the counters

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Figure 25. Iron shield.

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Figure 26. Iron shield partly open.

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PHOTOGRAPHED BY ATLEE S. TRACY

Figure 27. Anti-coincidence counter arrangement.

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Figure 28. Anti-coincidence counters and screen-wall counter in place in iron shield.

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Figure 29. Radon gas counter.

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Figure 30. Radon gas counter in position in coffin.

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Figure 31. Covered coffin and modified PC circuit.

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Figure 32. Vacuum and argon-ethylene lines.

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2. The aluminum paint on the inside of the iron shield may be somewhat "hot"
3. Several of the iron plates in the shield may be hot with gamma activity that could not be removed by the A-C's
4. The inside of the screen-wall counter may have been slightly contaminated

These points may be adequately checked by running a background on our screen-wall counter in one of the iron shields belonging to Dr. Libby and carefully checking the background of one of his counters in our shield. Permission has been obtained from Dr. Libby to check our counter in the shield in Jones Laboratory of the University of Chicago.

RADON DETERMINATIONS

The proposed method for the determination of radon in human breath consists of the adsorption of radon in charcoal, the de-adsorption into an argon-methane gas mixture at 200 to 300°C, the introduction of this gas into a special gas counter, and the counting of alpha particles in the proportional region. The standardization is accomplished with a 10^{-10} curie radon generator, and each gas mixture in the counter is checked with a plutonium "gun" mounted on the end of the counter. This gun may be fired into the counter or cut off for counting a sample.

The radon gas counter is shown in Figure 29. The plutonium gun, consisting of a ball joint, a short length of glass tubing, and plutonium source on platinum at the end of the tubing, is shown at the left. The gun is not in position to fire into the counter (Figure 29). By rotating the ball joint, the alpha's from the plutonium may be fired into the counter for checking the plateau and efficiency of the gas mixture in the counter.

Figure 30 shows the counter in position for counting inside the tin "coffin" that serves as the electrostatic shield to prevent stray counts from electrical pick-up. On the front of the coffin is mounted the pre-amplifier (two 6AH-6's and one 6-J-7) from a Nuclear Measurements Corporation PC-1 circuit. In Figure 31 the covered coffin and modified PC circuit are shown together.

The vacuum line for manipulation of the charcoal traps and gas counter and the separate line for filling the screen-wall counter with argon and ethylene are presented in Figure 32.

Although various data have been obtained with this apparatus and "milkings" of radon from the 1.00×10^{-10} curie of radium in the radon generator, no detailed summary nor graphs are given in this report since a

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complete paper is in preparation and will include the results of tests now in progress. Briefly, the radon generator has been milked with the argon-methane mixture through the charcoal trap after a two- and eight-day growth of radon having a half-life of 3.825 days from radium. After desorption into the gas counter, the measured activities followed the theoretical decay curve and extrapolated to approximately 80 per cent of the theoretical activities at the time of milking. The theoretical counts were calculated assuming 100 per cent geometry for the radon alphas, 50 per cent geometry for the radium A alphas, and 50 per cent geometry for the radium C' alphas.

Upon pumping out a sample of radon, the solid decay products of the radon remaining in the counter decayed with the theoretical half-life of 27 minutes. Thus, after several hours a counter is ready for a new sample. The natural background of this counter, whose cathode is painted with silver paint, is only 0.5 counts per minute.

Current studies are directed toward obtaining data:

1. to show that CO_2 and O_2 do not interfere with the adsorption-desorption cycle (for these tests the radon generator is milked with human breath from a rubber balloon)
2. to show that adsorbed CO_2 or O_2 does not de-gas into the counter and destroy the counting characteristics of the argon-methane mixture (preliminary tests suggest that this effect is not occurring)
3. to obtain statistics on the standardization of the method and thereby calculate its precision
4. to choose the best design and to manufacture charcoal traps and accessory equipment for the program of measurement at Elgin

Radiological Physics Research

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PROGRESS REPORT

by

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BETA-RAY DOSIMETRY

The work on beta-ray dosimetry continues to be concerned with the problem of the distribution in air of the ionization due to a point source of β particles. The experimental work is being done in a room large enough to exclude β radiation, (P^{32}) scattered by walls, floor, and ceiling, from reaching the ionization chambers (Figure 33). The relative positions of the source and ion chamber are determined with the aid of a surveyor's transit. Minimal scattering effects from the source and its mount are obtained by drying an active solution containing minimum solids on a small area at the center of a very thin aluminum foil (approximately 1μ) that is mounted on a thin washer-like aluminum disk (Figure 34). This is then suspended by means of fine wires from a large tower that is shaped like an inverted "L." There have been two ion chambers used so far, a 1-liter and a 10-liter. These chambers are covered with aluminum foil 0.0003" thick (Figure 35). The physical setup is shown in Figure 36.

Several potential sources of error have been evaluated. These include the earth's magnetic field, which has been shown to be negligible both theoretically and experimentally; the effect of asymmetrical response of the ion chambers, for which correction factors have been obtained; and the effect of the stem that supports the chambers, for which correction factors have been obtained by a modified extrapolation technique.

The principal source of error that remains to be evaluated is the effect of the skin of the chamber. A new ion chamber is being built in an attempt to determine the magnitude of this effect. This is a parallel plate chamber consisting of grids having about 99 per cent transmission for light, and constructed in a way that minimizes the scattering and absorption effects of the members supporting the chamber.

The results obtained with the two aluminum covered chambers are summarized in Figure 37. The "inverse square law deviation" referred to in this figure arises when the chamber to source distance becomes

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PHYSICAL SETUP

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HORIZONTAL SECTION

VERTICAL SECTION

I.C. = IONIZ. CHAMBER

S = SOURCE

R = RANGE OF β PARTICLE ~ 680 cm

E = ENVELOPE OF ELLIPSES

H = EARTH'S FIELD

T = TRANSIT

Figure 33. Plan of room used for studies of the distribution aid of ionization due to a point source of β particles.

BETA-RAY SOURCE : DETAILS

Figure 34. Beta-ray source.

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β -RAY IONIZATION CHAMBER

= INSULATOR

SUPPORTING METAL BRASS

WALLS: 7.5×10^{-3} mm. AL.

$a = b = 23.0$ or 11.0 cm

Figure 35. Beta-ray ionization chamber.

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PHOTOGRAPHED BY ATLEE S. TRACY

Figure 36. Photograph of room used for studies of the distribution in air of ionization due to a point source of β particles.

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Figure 37. Beta-ray absorption.

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comparable to the linear dimensions of the chamber. This correction is a purely geometrical one, and was computed with the aid of the Biometrics Section of the Division of Biological and Medical Research. Aside from the possible effects of the skin of the chamber, the least reliable points (at the two extremes) are probably good to about five per cent.

In connection with the construction of the new ion chamber, the density of ionization from a point source in air is being calculated under the assumption that the scattering of the particles in the medium is negligible. This is being done numerically, using theoretical stopping powers and β -ray spectra of widely different energies.

RADIUM TOXICITY

Work on the more accurate evaluation of Ra toxicity in man is continuing. No effort is being spared in tracing patients injected with known quantities of Ra twenty years ago at Elgin State Hospital. The instrumentation necessary to assess their present Ra content is being prepared.

PROGRESS REPORT: AIR CONTAMINANTS

by

H. A. Schultz and R. E. Rowland

Further investigations have been made of the factors involved in the measurement of traces of freon in the air. A new, more flexible detector unit based on the principle of the G. E. leak detector has made possible a more detailed study of some of the parameters affecting the performance of the instrument. The results indicate that a further modification of design will be necessary in order to achieve the type of regulation desirable for stable long-time field use, and work along this line has been started.

Development of a dynamic gas handling system has been developed so that the composition of the mixture entering the unit may be modified at will. This makes it relatively simple to check the calibration of the instrument at any time. Certain modifications of the circuits have also been made to improve the over-all operation of the equipment.

and Studies of the spectroscopic method of freon detection, ^{are briefly mentioned/END} indicated that while considerable sensitivity and selectivity are available, the principle would be more adaptable to laboratory work than to field operation.

In the presence of the background from the arc source, it appears that the measurement of the characteristic spectrum arising from freon in trace quantities would require spectral resolution and amplifier stability that would not be easily attained in a rugged, portable, field unit. In view of the great sensitivity of the ionization method, and of the progress made toward attaining long term stability, the work on the spectroscopic method has been interrupted and effort has been concentrated on the ionization method.

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